

Templated Self-Assembly of Polymer Particles into Mesoscopic Clusters with well-defined Configurations

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We report on the fabrication of colloidal clusters through the combination of spherical particles. Polystyrene latex particles bearing amino groups on their surface were used as building blocks of the clusters. Packing of these particles with diameters of 91 and 154 nm into assemblies with defined configurations was accomplished using narrow dispersed emulsion droplets as templates. The building blocks of the clusters adhered to the oil-water interphase due to the Pickering effect. Subsequent evaporation of the dispersed phase forced them to pack into small clusters. Addition of the particles *via* the dispersed phase led to higher yields of clusters than if the building blocks were added *via* the continuous phase. All clusters had well-defined configurations. Because the dimensions of these clusters were below 400 nm, the colloidal assemblies underlay Brownian motion which resulted in stable suspensions. The number and yields of different species could be controlled *via* the concentration of the building blocks and surfactant within the emulsions. Moreover, the nature of the dispersed phase itself had a strong impact on the cluster formation. When cyclohexane was used as the dispersed phase, predominately particle doublets and triplets were obtained. The use of toluene-in-water emulsions resulted into a broader spectrum of clusters of up to 12 constituents. Such clusters could satisfy the demand for particles with complex but defined shapes and special symmetries for the fabrication of novel hierarchically organized materials.

Colloidal clusters, latex particles, Pickering emulsion, self-assembly, ultrasonication, disk centrifugation

Introduction: Preparation of particle clusters

In recent years, the combination of spherical particles into complex hierarchically ordered architectures has evolved into an emerging field in modern colloid science

[1, 2]. Within this area, colloidal crystals have developed into a class of its own. Colloidal crystals present arrays of particles which are ordered over a wide range in length. Under appropriate conditions, colloidal particles can spontaneously self-assemble into crystals which are kept together by van der Waals interactions [3-5]. One can also prepare colloidal crystals layer-by-layer on planar surfaces [6]. Furthermore, ionic colloidal crystals could be prepared by the combination of positively and negatively charged particles in a solvent that moderates the interactions among the particles [7, 8]. Applications of colloidal crystals include photonic bandgap materials [3, 9-13] and templates for the fabrication of membranes with defined pore sizes [14, 15].

Besides rather extended structures, assemblies from spherical particles of finite size have received considerable interest because of their potential to open new avenues for complex particulate systems with unique rheological, optical, magnetic, or electric properties [1]. Colloidosomes, *i.e.* hollow capsules whose wall consists of colloidal particles, present one example for such aggregates [16-18]. Photonic supraballs present a further class of aggregates of finite size. They are assemblies from a large number of particles. The ordered arrangement of the constituents at their surface gives them photonic properties [19, 20].

Clusters consisting of a small number of either the same or different constituent spheres present the most intriguing class because they can be considered as colloidal analogues to small molecules [21-24]. Hence, alternative terms such as “colloidal molecules” or “patchy particles” were used to emphasize the fascinating potential of particle clusters [25].

Atoms form covalent bonds when they join together into molecules. Unlike atoms, colloidal particles usually do not undergo directed interactions. Therefore Granick and co-workers prepared microspheres having oppositely charged hemispheres. These particles spontaneously self-assemble into clusters of defined configurations because of their directional interactions [26]. However, such clusters present intermediates during formation of larger assemblies because there is no limitation to the size of clusters formed through electrostatic interactions.

Velev and co-workers developed an approach which allows the fabrication of assemblies of finite sizes from microspheres [23]. This method is based on the agglomeration of particles while adsorbed onto emulsion droplets. Solid particles adhere to the surface of emulsion droplets because the adsorption lowers the

interfacial energy due to the Pickering effect [27-29]. Subsequent evaporation of the dispersed phase causes capillary forces which pack the particles together. Pine and co-workers prepared micron-sized clusters by this technique [22]. These clusters have well-defined configurations which are believed to result from an arrangement of the particles in regular polyhedra already at the droplet surface due to long-ranged dipole-dipole repulsion through the oil droplet and Coulomb interactions [30, 31].

An emulsion droplet carrying N particles on the surface results in a cluster of N constituent spheres. Hence, the statistical distribution of the particles onto the droplets is crucial to the dispersity of the resulting clusters. A broad size distribution of the droplets gives rise to a broad range of different clusters. The use of monodisperse emulsion droplets would limit the variance in number of particles per droplet. Nonetheless, this would not result in a single type of clusters because the distribution of the particles on the droplets is statistical, but it would significantly reduce the number of resulting species and thus increase the yield of distinct clusters.

Only few studies have been devoted to the preparation of particle assemblies from monodisperse droplets up to now. One approach is to prepare the droplets one by one using a micropipette [19] or a microfluidic device [32]. Droplets made by this method have diameters of several tens of micrometers. Therefore assemblies consisting of a large number of particles such as the photonic supraballs described above can be prepared from droplets of these dimensions [19].

Bibette and co-workers presented a first approach towards small clusters from narrow dispersed Pickering emulsion droplets. Shearing of a macroemulsion of polydisperse droplets bearing 1.2 μm silica particles at their surface in a Couette apparatus allowed the preparation of narrow dispersed droplets carrying a small number of particles. Hence, this led to large yields of particle doublets, triplets and quadruplets [24].

Recently, we developed a novel route towards small clusters which is based on the miniemulsion technique [33]. Narrow dispersed toluene droplets with 1.9 μm in diameter, onto which a small number of 154 nm sized cross-linked polystyrene particles was bound, were obtained by emulsification through high-power ultrasonication. Clusters of less than six constituent spheres and overall dimensions below 400 nm could be prepared. Hence, this approach which

combines small emulsion droplets with smaller spherical particles allows the fabrication of clusters with overall dimensions that are much smaller than the clusters described so far. Because their dimensions are in the colloidal regime, such clusters are stabilized by Brownian motion which prevails over sedimentation [34].

Here we present a detailed study of the formation of colloidal clusters along the basic principle given in Ref. [33]. The paper is organized as follows: The first part is devoted to the influence of the concentrations of emulsifier and constituent spheres on the total yield of clusters as well as on the yields of the individual cluster species together with their configurations. In the second part, experiments in which the constituents were either added *via* the dispersed oil phase or the continuous aqueous phase are compared in terms of cluster yields. Whereas the previous sections highlight the preparation of clusters from toluene-in-water emulsions, the last section describes the fabrication of colloidal clusters of less than four constituents from cyclohexane-in-water emulsions.

Experimental Section

Chemicals

Styrene (Sigma-Aldrich) was purified by washing with 10 wt% NaOH solution, drying over CaCl₂, and distillation under reduced pressure. Divinylbenzene (DVB, 80%, Merck) was passed through an inhibitor remover (Aldrich) column before use. Aminoethylmethacrylate hydrochloride (AEMH, Polysciences), cetyltrimethylammonium bromide (CTAB, Merck), α,α' -azodiisobutyramidine dihydrochloride (V-50, Fluka), toluene (Riedel-de Haën), cyclohexane (Riedel-de Haën), Pluronic[®] F-68 (Sigma), Tween[®] 80 (Fluka), Nile red (Fluka), sucrose (Fluka), and glycerol (Merck) were of analytical grade and used as received. Deionized water obtained from a reverse osmosis water purification system (Millipore Academic A10) was used throughout the experiments.

Amino-modified polystyrene latex particles

The polymer latex particles (L1 and L2) used as building blocks of the clusters were prepared by emulsion polymerization of styrene with DVB (5 mol% relative

to styrene) as the cross-linking agent, AEMH (3 mol% relative to styrene) as the comonomer, CTAB as the emulsifier, and V-50 as the initiator (Table 1). The reaction was carried out at 80 °C under a nitrogen atmosphere and continuous stirring at 320 rpm for 6 h. Purification of the latex particles was accomplished by exhaustive ultrafiltration against water. The size and the size distribution of the spherical particles were determined by dynamic light scattering (DLS), transmission electron microscopy (TEM), and differential centrifugal sedimentation (DCS). The particles can be regarded as monodisperse because their polydispersity index given as the weight-average diameter divided by the number-average diameter is less than 1.01. The zeta potential of the particles bearing amino groups on their surface is above + 60 mV (Table 1).

Cluster fabrication from oil-in-water emulsions

The combination of the amino-modified polystyrene spheres into clusters was accomplished through the agglomeration of the latex spheres adsorbed onto toluene or cyclohexane droplets. The building blocks of the clusters were either added *via* the oil or the aqueous phase.

Addition of the building blocks via the dispersed (toluene) phase

The preparation was performed along the lines given in Ref. [33]. Briefly, a part of the aqueous suspension of the cross-linked polystyrene particles was freeze-dried and resuspended in toluene at concentrations of 1 to 4 wt% by ultrasonic homogenization. 3 ml of these suspensions were added to 27 ml aqueous solutions of Pluronic[®] F-68 of different concentrations (0.25, 0.5, and 1 wt%). Emulsification was accomplished in a rosette cell (Bandelin RZ 3) through ultrasonication under ice-cooling using a high-shear homogenizer (Bandelin Sonoplus HD 3200, 200 W, probe KE 76). Three sonication steps, each for 5 min with 2 min rest in between, were performed at 30 % amplitude and a frequency of 20 kHz. Packing of the particles into clusters was induced by evaporation of the toluene using a rotary evaporator (Heidolph Laborota 4000 efficient).

Addition of the building blocks via the continuous (water) phase

0.4, 0.8, and 1.2 wt% suspensions of L2 particles in 0.5 wt% aqueous solution of Pluronic[®] F-68 were prepared. To 27 ml of these suspensions, 3 ml toluene was added. Emulsification and evaporation of toluene were performed as mentioned above.

Clusters from cyclohexane-in-water emulsions

Suspensions of 108 mg and 324 mg L2 particles in 1 wt% Tween[®] 80 solution were prepared. 9.1 ml cyclohexane was added to 27 ml of each suspension. Right after sonication for 10 min at 30 % amplitude, the dispersed phase was removed under reduced pressure.

Cluster separation

Fractionation of the suspensions into clusters of the same number of constituents was accomplished through density gradient centrifugation [22]. Concentration gradients were built from 9 wt% and 20 wt% aqueous glycerol solutions using a gradient maker. The clusters of different sizes were separated by their sedimentation velocity during centrifugation at 12,000 rpm for 75 min (Kontron Instruments Centrikon T-1080, Sorvall Surespin 630 rotor). The cluster fractions were isolated through a piercing unit (Kontron Instruments). Glycerol was removed by exhaustive dialysis against water. Field emission scanning electron microscopy (FESEM) was used to specify size and configuration of the clusters within each individual fraction.

Laser scanning confocal microscopy (LSCM)

The emulsion droplets were stained with the hydrophobic dye Nile red. LSCM micrographs were recorded on an inverted confocal laser scanning microscope (Zeiss LSM 710, 63x oil objective) equipped with an argon gas laser (excitation wavelength 514 nm). Scans at a resolution of 1204 × 1204 pixels were taken in the line-averaging mode. Micrographs were analyzed by the LSM software (Zeiss ZEN 2008).

Field emission scanning electron microscopy (FESEM)

FESEM specimen were prepared by drying one drop of a highly diluted suspension on a clean silicon wafer (CrysTec) at room temperature and coating with a platinum layer of 2 nm thickness using a sputter coater (Cressington 208HR) to make the specimen conductive. Micrographs were recorded on a LEO Gemini microscope (Zeiss) equipped with a field emission cathode operating at 3 - 5 kV which corresponds to a lateral resolution of 2 nm.

Differential centrifugal sedimentation (DCS)

The content of the clusters of the same number of constituents was determined by DCS using a disc centrifuge (CPS Instruments CPS-24000). A detailed description of this analytical technique is given in Ref. [35]. Within a rotating hollow disc (24,000 rpm) a gradient was prepared by layering eight sucrose solutions of decreasing density (8 to 2 wt%) upon one another. 0.1 ml of a 0.5 wt% cluster suspension was placed on top of the gradient. The distribution of the clusters is obtained by measuring the time required for the different species to reach a known position within the gradient. The concentration at this position and time was measured by light absorption at 405 nm. DCS has an excellent resolution which allowed resolving the peaks that stem from clusters of one size individually. Integration of the peaks gave the content of the different cluster species within the suspension.

Further methods

Dynamic light scattering (DLS) measurements were performed at 25 °C on an ALV-4000 (Peters-ALV) light scattering goniometer equipped with a He-Ne laser which provides a wavelength of 632.8 nm, and an ALV-5000 Multiple Tau Digital Correlator (Peters-ALV). The results were not depended on the scattering angle because of the low dispersity of the droplets [33]. Hence, the autocorrelation functions could be recorded at a fixed scattering angle of 90°C. Hydrodynamic radii were obtained from a cumulant analysis of the correlation functions [36].

Electrophoretic mobilities (u) of the building blocks of the clusters were measured on a Malvern Zetasizer Nano ZS and converted into zeta potentials (ζ) via the Smoluchowski equation ($\zeta = u\eta / \epsilon_0 \epsilon$, where η denotes the viscosity and $\epsilon_0 \epsilon$ the permittivity of the suspension).

Results and Discussion

Preparation of Colloidal Clusters

The fabrication of the clusters was carried out along the lines given in Figure 1. Two different routes were used to investigate how addition of the building blocks *via* either the dispersed oil phase or the continuous aqueous phase affects the formation of the clusters from oil-in-water emulsions.

Emulsification by ultrasound

Route A in Figure 1 is based on the approach described in Ref. [33]. Here the spherical building blocks of the clusters were suspended in toluene. Because of the hydrophilic surface of the building blocks, ultrasonication was used to obtain homogeneous suspensions. An aqueous solution of the emulsifier Pluronic[®] F-68 was overlaid with a suspension of the particles in toluene. Emulsification was accomplished through ultrasonication. An ultrasonic horn was dipped into the sample so that the end of the sonotrode was touching the oil-water interphase. This procedure in combination with the geometry of the rosette vessel used ensured efficient mixing of the two phases immediately after sonication. Several mechanisms of droplet formation and droplet rupture by ultrasound were described in the literature [37, 38]. Among those, acoustic cavitation is considered as the most important one. Ultrasonication of the emulsion causes cavities within the emulsion. These cavities collapse when they gain a critical size. This causes pressure waves in the vicinity of the emulsion droplets which rupture the droplets into smaller ones until a steady state of fusion and fission processes is attained [37]. The steady state is characterized by narrow dispersed emulsion droplets [33]. In route B in Figure 1, the particles were suspended in the aqueous solution of the emulsifier. The aqueous suspension was then overlaid with toluene. The emulsification through ultrasound was performed as mentioned above.

Size of the templating emulsion droplets

The average size of the emulsion droplets presents a crucial parameter in the preparation of particle clusters. Given a fixed volume of the oil phase, a reduction of the average droplet size raises the number of droplets in the emulsion. This in

turn reduces the average number of building blocks per droplet and thus the size of the resulting clusters if the total number of particles is kept the same.

Measuring the droplet size by light scattering or microscopic techniques is difficult because dilution of the emulsions could alter the droplet size [38]. In order to minimize this effect, the emulsions were diluted with oil-saturated water. However, even in this case slight changes of the droplet size cannot be excluded [39]. Moreover, the droplets might grow with time due to coalescence or Ostwald ripening [37]. Fusion of the droplets after sonication was suppressed by the addition of the emulsifier which gives the droplets steric stabilization against collision growth. Ostwald ripening, *i.e.* droplet growth *via* oil diffusion and subsequent dissolution of small droplets due to their high Laplace pressure, could be suppressed by the addition of a hydrophobe such as dodecane. The hydrophobe cannot diffuse through the water phase from one droplet to another. Hence, the osmotic pressure of the hydrophobe within the droplet would increase upon droplet shrinkage. This keeps the droplet size stable. Recent measurements of the turbidity of emulsions revealed that adding dodecane is an efficient way to prevent Ostwald ripening of the droplets used in this study [33]. However, we did not add dodecane to the emulsions used for cluster preparation because dodecane cannot be removed during evaporation of the oil phase. In order to keep the time in which the emulsion can undergo Ostwald ripening as short as possible, the evaporation of the oil phase was performed right after sonication. To get a rough estimate of the droplet size, emulsions stabilized by dodecane and without the hydrophobe were prepared. Table 2 shows the droplet sizes obtained by DLS. The formation of narrow dispersed droplets through ultrasonication was corroborated by CONTIN analysis of the autocorrelation functions [40]. The polydispersity given as the weight-average diameter divided by the number-average droplet diameter is less than 1.01. This is in full accord with previous results by LSCM [33]. Hence, all further DLS measurements could be performed at a fixed scattering angle of 90 °C.

The effect of the low quantity of dodecane on the droplet diameter should be negligible. Hence, the size of the droplets stabilized against Ostwald ripening should be characteristic to the droplets during ultrasonication. Increasing the emulsifier concentration allows stabilization of a larger oil-water interphase and gives thus rise to smaller droplets [41]. The formation of more but smaller

droplets should decrease the average number of constituents of the resulting clusters. This will be discussed below.

The size of the emulsions without dodecane was determined two minutes after sonication. Already in this short time, a significant increase of the droplet size is observed due to Ostwald ripening (Table 2). This is in accord with the observed increase of the turbidity of the emulsions [33]. Because of the short delay time between sonication and evaporation of the oil phase, the size of the unstabilized droplets might be more appropriate to estimate the size of the droplets just before the assembly into clusters.

The LSCM micrographs shown in Figures 1 and 7 give complementary information on the droplet size. Micrographs of the droplets could be only taken if no dodecane was added to the emulsion. In the presence of dodecane, the droplets diffused too fast to map them because they are smaller than the unstabilized droplets (Table 2). The size of the unstabilized droplets measured by DLS was lower but of the same order than the droplet size of 1860 nm derived from the LSCM micrographs. This is because DLS measures the size of the droplets in the bulk, whereas in LSCM one records the less mobile fraction of droplets near the microscope slide. These droplets might be larger than droplets in the bulk because larger droplets have a higher tendency to float towards the cover slide.

Morphologies of the clusters

An ordered arrangement of the particles at the droplet surface due to Coulomb and dipole-dipole repulsions results in clusters with well-defined configurations after evaporation of the dispersed oil phase [31]. Clusters made up from 2 to 12 constituents were found in the suspensions. However, only few clusters of 8 and more building blocks were found by FESEM. The low quantity of large clusters was corroborated by the DCS analyses which are discussed below.

Figure 2 gives an overview of the individual cluster morphologies obtained from toluene-in-water emulsions. Most of them are based on regular polyhedra. This is in accord with earlier observations on micron-sized clusters [22]. Clusters of two to four constituents can only assume one specific configuration when fabricated from a spherical template. In fact, solely particle doublets, triplets and tetrahedrons were found. For this reason and because such clusters are obtained in large amounts, they could be used as model systems to study the dynamics of

particles with complex shapes [34]. Clusters of five constituents had two different configurations, *i.e.* the triangular dipyramid and the square pyramid. In addition to the octahedron, a second configuration was frequently found for clusters of six constituents. This species with C_{2v} symmetry in Schönflies notation [42] has the same shape as the pentagonal dipyramidal clusters (D_{5h}) made from seven particles except that one building block in the horizontal plane is absent (“flower-minus-one” configuration in Fig. 2). Two specific configurations were found again for clusters of eight constituent spheres. The formation of different compact morphologies in specific cases ($N = 5, 6,$ and 8 , where N is the number of constituents) must result from similar energetic arrangements of five, six, and eight particles at the droplet surface.

Except for $N = 5, 6$ and 8 , the morphologies of the micron-sized clusters prepared by Pine and co-workers were identical to those shown in Figure 2. These configurations based on regular polyhedra present favourable packings of spheres because they minimize the second moment of the mass distribution [22]. For $N = 5, 6$ and 8 , we observed compact morphologies, *i.e.* square pyramid, “flower-minus-one”, and twisted square configurations, in addition to assemblies based on regular polyhedra. Yang and co-workers could self-assemble silica particles into square pyramids and twisted square structures as well [43]. Brenner and co-workers predicted both the octahedral and the “flower-minus-one” configuration as ground states in the packing of six hard spheres with short range attractions, whereas square pyramids ($N = 5$) and twisted squares ($N = 8$) are not minimal energy clusters of hard spheres [44]. Remarkably, besides the present study there is only one other study that gives experimental evidence on the “flower-minus-one” configuration so far. Granick and co-workers found clusters with this configuration as intermediates during the electrostatically driven aggregation of microspheres having oppositely charged hemispheres [26].

The clusters could serve as building blocks with complex shape and special symmetries to build up novel hierarchical organized materials. For this purpose, the mixture of different species has to be separated into fractions of uniform clusters. This can be accomplished through density gradient centrifugation [33]. In this technique, the different clusters are separated by their sedimentation velocity which depends on the cluster size. Figure 3 shows FESEM micrographs of the cluster fractions. The clusters were prepared from the L1 particles with a diameter

of 91 nm. The small size of the particles results in small absolute differences in the mass between clusters of N and $N + 1$ constituents. However, this did not present an obstacle in the preparation of suspensions of clusters with the same number of constituents (Fig. 3).

Clusters obtained from toluene-in-water emulsions

All Pickering emulsions described in this section were prepared from 3 ml toluene and 27 ml aqueous solution of Pluronic[®] F-68. The amounts of particles added to the emulsions refer to these quantities.

Effect of the concentration of the building blocks

The concentration of the spherical building blocks within the Pickering emulsion has a decisive impact on the average number of constituents of the clusters. If N particles are bound to a specific droplet, a cluster of N constituents will result from this droplet, provided that droplet fusion by collision is suppressed by the protective layer of the emulsifier on the droplet surface. To ensure the latter point, all experiments in this section were performed at the highest Pluronic[®] F-68 concentration of 1 % used in this study. Because DLS measurements showed that within the limits of experimental errors the sizes of toluene droplets loaded with and without particles did not differ, the experimental observations discussed below depend solely on the concentration of particles within the Pickering emulsion.

Figure 4 displays differential size distributions of the clusters obtained by DCS at different amounts of building blocks added *via* the toluene phase. DCS provides an excellent resolution because the clusters made up of the same number of constituents could be resolved as individual peaks. At the lowest amount of particles used, *i.e.* 26 mg, only clusters consisting of up to five constituent spheres were observed. Integration of the individual peaks gives the contents of the clusters of the same N (Table 3). A large fraction of single particles was found in the cluster suspension. The yield of clusters is only 29 wt% in this case. Hence, a large number of droplets confined only one single particle at their interface because of the low concentration of particles within the emulsion.

Doubling the amount of building blocks to 53 mg increases the average number of particles per droplet. In this case clusters made up of six constituents were found in addition to smaller clusters. Moreover, a larger total yield of clusters of 46.5 wt% as compared to single particles was obtained. The yield of clusters could be further enhanced to 49.8 wt% when the amount of building blocks was raised to 108 mg. Clusters of up to 9 constituents could be resolved by DCS (Fig. 4).

DLS measurements showed that the diameter of the L2 particles increases from 154 nm to 240 nm when transferred from water to toluene. The swelling of the cross-linked polystyrene particles leads to high volume fractions even at moderate weight fractions. Thus it was not possible to get homogeneous suspensions of concentrations higher than 4 wt% (108 mg L2 particles dispersed in 3 ml toluene) which corresponds to 13 vol%. Hence, the cluster yield could not be improved further as long as the average size of the droplets is kept the same.

Effect of the emulsifier concentration

Besides the concentration of the building blocks, the emulsifier concentration presents a second important parameter in cluster fabrication. Formation of larger toluene droplets, while the total volume of toluene and the number of particles are kept constant, increases the average number of confined particles per droplet. Table 2 shows that the droplet size can be increased by reducing the Pluronic[®] F-68 concentration. This in turn might be used to further decrease the number of remaining single particles in favor of higher yields of clusters. Clusters were prepared at Pluronic[®] F-68 concentrations of 0.25, 0.5, and 1 wt%. In each experiment, 108 mg L2 particles were added to the toluene phase. Figure 5 shows the differential size distributions of the resulting clusters. The total yield of clusters was significantly higher at low emulsifier concentration. For example, lowering the Pluronic[®] F-68 concentration from 1 wt% to 0.25 wt% allowed raising the total cluster yield from 49.8 wt% to 73.7 wt% (Table 3).

Lowering the emulsifier concentrations has two different effects: 1) The total oil-water interphase is reduced; 2) The stability of the emulsion droplets against collision growth is decreased. Both effects result in larger droplets. This enhances not only the total yield of clusters but also broadens the spectrum of clusters (Fig. 5). In this case considerable amounts of clusters made from up to 9 constituent

spheres were observed. Hence, a trade-off between the total cluster yield and the yields of small clusters ($N < 3$) is crucial in order to get high yields of clusters of one specific size (Table 3).

Addition of the constituents via the dispersed or the continuous phase

As mentioned above, the upper limit of building blocks that can be added to the Pickering emulsion *via* the 3 ml of toluene is 108 mg. However, provided that the particles have a strong affinity to the oil-water interphase, they could be also added through the continuous water phase.

For this purpose, two different Pickering emulsions were prepared through emulsification of 3 ml toluene and 27 ml 0.5 wt% Pluronic[®] F-68 solution. 108 mg L2 particles were used in each experiment. In the first experiment, the particles were dispersed in toluene as done before, whereas in the second experiment a suspension of L2 particles in Pluronic[®] F-68 solution was prepared. After removal of toluene, the size distributions of the clusters were recorded by DCS. Regardless of the phase in which the building blocks were dispersed, clusters of up to nine constituents were observed (Table 3). Hence, despite the hydrophilic surface of the particles, they have a marked affinity to adhere at the oil-water interphase, which is the prerequisite to obtain clusters. For the particles used, this affinity is not solely due to the Pickering effect. At the toluene-water interphase the cross-linked polystyrene particles are partially swollen, which should enhance binding to toluene droplets.

Migration of the particles between the dispersed and the continuous phase would lead to the same yields of clusters, which is not the case. If the particles are added *via* the continuous water phase instead of the dispersed toluene phase, the yields of all cluster species were significantly lower. In total, 46.8 wt% clusters were obtained, whereas addition of the particles *via* toluene resulted in 70.8 wt% clusters (Table 3). All particles confined to droplets, regardless if bound to the surface or dispersed within the droplets, become part of clusters except if they are the only particle confined by a droplet. Moreover, the average size of the droplets should not differ much in both experiments. Swelling of the droplets due to the confinement of a small number of particles does not result in a drastic increase of the droplet size. Hence, if the particles are added *via* the water phase, a certain

fraction of the particles (at least 24 wt%, as calculated from the data in Table 3) remains in the continuous aqueous phase. This in turn would argue that equilibrium distributions among particles in both phases and at the interphase were not yet adjusted. The reason might be related to the swelling of the polystyrene particles with toluene. The hydrophilic surface of the L2 particles retards swelling with toluene and thus binding to the droplets when dispersed in water. On the other hand, if already swollen with toluene, the L2 particles can hardly escape to the continuous water phase.

Variation of the concentration of the particles within the continuous aqueous phase shows that the cluster yield can be increased stepwise up to 66.7 wt% when the number of particles is doubled or tripled (Table 3). Thus, the fraction of particles that is confined to droplets can be augmented by increasing the concentration of particles in the continuous aqueous phase.

Clusters from cyclohexane-in-water emulsions

In the previous sections toluene droplets templated packing into clusters. In this section cyclohexane was used as the dispersed phase within the Pickering emulsions. Tween[®] 80 was chosen as oil-in-water emulsifier following the hydrophilic–lipophilic balance (HLB) concept by Griffin [45]. Figure 7 displays a LSCM micrograph of cyclohexane droplets bearing L2 particles on their surface. DLS measurements revealed that growth of cyclohexane droplets proceeds much slower than in the case of toluene droplets. This observation can be qualitatively understood as the HLB numbers of cyclohexane and Tween[®] 80 exactly match (15 in both cases), which indicates a stable oil-in-water emulsion. Toluene (15) and Pluronic[®] F-68 (> 24) differ in their HLB numbers suggesting a lower stability of the droplets [45]. For this reason, the better stability of the cyclohexane droplets slows down growth processes which results in smaller droplets during cluster preparation (Table 2). Although the total volume of the dispersed phase in the cyclohexane-in-water emulsions is higher, the smaller droplets sizes result in a larger number of droplets as compared to the toluene-in-water emulsions described above. Hence, the 108 mg L2 particles can be distributed over a larger number of droplets which should lead to smaller clusters. In fact, only single particles, doublets, triplets, and few tetrahedral clusters were

formed (Fig. 7). Increasing the number of L2 particles by a factor of three, allowed doubling the total yield of clusters. However, this increase did not yield clusters of more than four constituents. Hence, particularly small clusters can be prepared in this way.

Conclusion

In summary, colloidal clusters were prepared through the agglomeration of particles at the surface of emulsion droplets while the dispersed phase is evaporated. Emulsification by powerful ultrasonication allowed limiting the dispersity of the templating emulsion droplets. This together with the small average diameters of the droplets and the constituents helped to keep the dimensions of the clusters below 400 nm. The present study showed how decisive experimental parameters such as the concentration of building blocks and the emulsifier concentration have to be chosen in order to improve the yields of clusters of specific configurations. Clusters could be made both if the constituents were initially suspended in the dispersed or the continuous phase of the emulsion. This approach could be used to assemble particles *via* different phases to hybrid clusters even in cases where mixtures of the constituents would not form stable suspensions. Such clusters which combine complexity in shape and composition open new perspectives for designer particles and hierarchically organized materials.

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References

- [1] Edwards EW, Wang D, Möhwald H (2007) Hierarchical organization of colloidal particles: From colloidal crystallization to supraparticle chemistry. *Macromol. Chem. Phys.* 208:439-445

- [2] Yang S-M, Kim S-H, Lim J-M, Yi G-R (2008) Synthesis and assembly of structured colloidal particles. *J. Mater. Chem.* 18:2177-2190
- [3] Lange B, Metz N, Tahir MN, Fleischhaker F, Theato P, Schroeder H-C, Mueller WEG, Tremel W, Zentel R (2007) Functional polymer-opals from core-shell colloids. *Macromol. Rapid Commun.* 28:1987-1994
- [4] Velev OD, Lenhoff AM (2000) Colloidal crystals as templates for porous materials. *Curr. Opin. Colloid Interface Sci.* 5:56-63
- [5] Zeng F, Wu S, Tang T, Sun Z, Wang C, Liu X, Tong Z (2004) Preparation of colloidal crystals with polyhedral building blocks through post-polymerization. *Colloid Polym. Sci.* 282:651-655
- [6] Velikov KP, Christova CG, Dullens RPA, van Blaaderen A (2002) Layer-by-layer growth of binary colloidal crystals. *Science* 296:106-109
- [7] Hynninen AP, Christova CG, van Roij R, van Blaaderen A, Dijkstra M (2006) Prediction and observation of crystal structures of oppositely charged colloids. *Phys. Rev. Lett.* 96:138308/138301-138308/138304
- [8] Leunissen ME, Christova CG, Hynninen A-P, Royall CP, Campbell AI, Imhof A, Dijkstra M, van Roij R, van Blaaderen A (2005) Ionic colloidal crystals of oppositely charged particles. *Nature* 437:235-240
- [9] Fleischhaker F, Arsenault AC, Kitaev V, Peiris FC, von Freymann G, Manners I, Zentel R, Ozin GA (2005) Photochemically and thermally tunable planar defects in colloidal photonic crystals. *J. Am. Chem. Soc.* 127:9318-9319
- [10] Hellweg T (2009) Towards large-scale photonic crystals with tuneable bandgaps. *Angew. Chem. Int. Ed.* 48:6777-6778
- [11] Hynninen A-P, Thijssen JHJ, Vermolen ECM, Dijkstra M, van Blaaderen A (2007) Self-assembly route for photonic crystals with a bandgap in the visible region. *Nat. Mater.* 6:202-205
- [12] Huang J, Hu X, Zhang W, Zhang Y, Li G (2008) Ph and ionic strength responsive photonic polymers fabricated by using colloidal crystal templating. *Colloid Polym. Sci.* 286:113-118
- [13] Li J, Xue L, Wang Z, Han Y (2007) Colloidal photonic crystals with a graded lattice-constant distribution. *Colloid Polym. Sci.* 285:1037-1041
- [14] Thomas A, Goettmann F, Antonietti M (2008) Hard templates for soft materials: Creating nanostructured organic materials. *Chem. Mater.* 20:738-755
- [15] Yan F, Goedel WA (2004) A simple and effective method for the preparation of porous membranes with three-dimensionally arranged pores. *Adv. Mater.* 16:911-915
- [16] Dinsmore AD, Hsu MF, Nikolaides MG, Marquez M, Bausch AR, Weitz DA (2002) Colloidosomes: Selectively permeable capsules composed of colloidal particles. *Science* 298:1006-1009
- [17] Velev OD, Furusawa K, Nagayama K (1996) Assembly of latex particles by using emulsion droplets as templates. 1. Microstructured hollow spheres. *Langmuir* 12:2374-2384
- [18] Texter J (2009) Templating hydrogels. *Colloid Polym. Sci.* 287:313-321
- [19] Kim S-H, Lee SY, Yi G-R, Pine DJ, Yang S-M (2006) Microwave-assisted self-organization of colloidal particles in confining aqueous droplets. *J. Am. Chem. Soc.* 128:10897-10904
- [20] Moon JH, Yi G-R, Yang S-M, Pine DJ, Park SB (2004) Electrospray-assisted fabrication of uniform photonic balls. *Adv. Mater.* 16:605-609
- [21] Cho Y-S, Yi G-R, Kim S-H, Elsesser MT, Breed DR, Yang S-M (2008) Homogeneous and heterogeneous binary colloidal clusters formed by evaporation-induced self-assembly inside droplets. *J. Colloid Interface Sci.* 318:124-133
- [22] Manoharan VN, Elsesser MT, Pine DJ (2003) Dense packing and symmetry in small clusters of microspheres. *Science* 301:483-487
- [23] Velev OD, Furusawa K, Nagayama K (1996) Assembly of latex particles by using emulsion droplets as templates. 2. Ball-like and composite aggregates. *Langmuir* 12:2385-2391
- [24] Zerrouki D, Rotenberg B, Abramson S, Baudry J, Goubault C, Leal-Calderon F, Pine DJ, Bibette J (2006) Preparation of doublet, triangular, and tetrahedral colloidal clusters by controlled emulsification. *Langmuir* 22:57-62
- [25] van Blaaderen A (2006) Materials science: Colloids get complex. *Nature* 439:545-546
- [26] Hong L, Cacciuto A, Luijten E, Granick S (2006) Clusters of charged janus spheres. *Nano Lett.* 6:2510-2514
- [27] Binks BP, Horozov T (2006) Colloidal particles at liquid interfaces. Cambridge University Press, Cambridge
- [28] Boneva MP, Christov NC, Danov KD, Kralchevsky PA (2007) Effect of electric-field-induced capillary attraction on the motion of particles at an oil-water interface. *Phys. Chem. Chem. Phys.* 9:6371-6384

- [29] Tarimala S, Dai LL (2004) Structure of microparticles in solid-stabilized emulsions. *Langmuir* 20:3492-3494
- [30] Horozov TS, Aveyard R, Clint JH, Binks BP (2003) Order-disorder transition in monolayers of modified monodisperse silica particles at the octane-water interface. *Langmuir* 19:2822-2829
- [31] Manoharan VN (2006) Colloidal spheres confined by liquid droplets: Geometry, physics, and physical chemistry. *Solid State Commun.* 139:557-561
- [32] Yi G-R, Thorsen T, Manoharan VN, Hwang M-J, Jeon S-J, Pine DJ, Quake SR, Yang S-M (2003) Generation of uniform colloidal assemblies in soft microfluidic devices. *Adv. Mater.* 15:1300-1304
- [33] Wagner CS, Lu Y, Wittemann A (2008) Preparation of submicrometer-sized clusters from polymer spheres using ultrasonication. *Langmuir* 24:12126-12128
- [34] Hoffmann M, Wagner CS, Harnau L, Wittemann A (2009) 3d brownian diffusion of submicron-sized particle clusters. *ACS Nano* 3:3326-3334
- [35] Laidlaw I, Steinmetz M (2005) Introduction to differential sedimentation. In: Scott DJ, Harding SE, Rowe AJ (eds) *Analytical ultracentrifugation*. The Royal Society of Chemistry, Cambridge, pp 270-290
- [36] Frisken BJ (2001) Revisiting the method of cumulants for the analysis of dynamic light-scattering data *Applied Optics* 40:4087-4091
- [37] Antonietti M, Landfester K (2002) Polyreactions in miniemulsions. *Prog. Polym. Sci.* 27:689-757
- [38] Landfester K (2001) Polyreactions in miniemulsions. *Macromol. Rapid Commun.* 22:896-936
- [39] Wang ST, Schork FJ, Poehlein GW, Gooch JW (1996) Emulsion and miniemulsion copolymerization of acrylic monomers in the presence of alkyd resin. *J. Appl. Polym. Sci.* 60:2069-2076
- [40] Ju RTC, Frank CW, Gast AP (1992) Contin analysis of colloidal aggregates. *Langmuir* 8:2165-2171
- [41] Abismail B, Canselier JP, Wilhelm AM, Delmas H, Gourdon C (2000) Emulsification processes: On-line study by multiple light scattering measurements. *Ultrason. Sonochem.* 7:187-192
- [42] Dresselhaus MS, Dresselhaus G, Jorio A (2008) *Group theory: Application to the physics of condensed matter*. Springer-Verlag, Heidelberg
- [43] Cho Y-S, Yi G-R, Chung YS, Park SB, Yang S-M (2007) Complex colloidal microclusters from aerosol droplets. *Langmuir* 23:12079-12085
- [44] Arkus N, Manoharan VN, Brenner MP (2009) Minimal energy clusters of hard spheres with short range attractions. *Phys. Rev. Lett.* 103:118303/118301-118303/118304
- [45] Tadros TF (2009) Emulsion science and technology: A general introduction. In: Tadros TF (ed) *Emulsion science and technology*. Wiley-VCH, Weinheim, pp 1-56

Figure captions

Fig. 1 Preparation of particle clusters using emulsion droplets as templates: In route A, the building blocks of the clusters were suspended in toluene and added to an aqueous solution of an emulsifier, whereas in route B the constituent spheres were added *via* the aqueous phase. In both cases, the samples were emulsified through powerful ultrasonication using a high-shear device. This resulted in narrow dispersed emulsion droplets carrying the particles on their surface (I: LSCM micrograph of the Pickering droplets). Packing of the particles into clusters was achieved by gentle evaporation of the oil (toluene or cyclohexane) phase (II: FESEM micrograph of colloidal clusters).

Fig. 2 Synopsis of all configurations observed for clusters which were prepared from toluene-in-water emulsions: N denotes the number of constituent spheres (L2 particles) of the clusters. For $N \leq 4$ and $N = 7$, clusters of the same N showed one specific configuration, whereas for $N = 5, 6$, and 8 two different configurations were observed. Only few clusters were composed of more than eight constituents.

Fig. 3 Fractionation into clusters of the same N was accomplished through centrifugation in a density gradient: a) particle doublets ($N = 2$), b) triplets ($N = 3$), c) tetrahedrons ($N = 4$), d) pentagonal dipyrramids and square pyramids ($N = 5$), e) octahedrons and “flower-minus-one” assemblies ($N = 6$), and e) pentagonal dipyrramids ($N = 7$). The clusters were built up from L1 particles. Scale bars are 200 nm.

Fig. 4 Effect of the amount of building blocks added *via* the toluene phase on the distribution of the resulting clusters: 26 mg (bold), 53 mg (semi-bold), and 108 mg L2 particles (thin line). Each of the well-separated peaks corresponds to clusters of the same N . The peak areas give the content of the different cluster species within the suspension. Raising the number of building blocks particularly favors the formation of clusters.

Fig. 5 Effect of the emulsifier concentration on the preparation of clusters from toluene-in-water emulsions and L2 particles: 0.5 wt% (bold), and 1 wt% (semi-bold line). Increasing the concentration emulsifier enhances the yield of clusters while the amount of remaining single particles is significantly decreased.

Fig. 6 Addition of the building blocks *via* the continuous water phase during cluster preparation: 108 mg (bold), 216 mg (semi-bold), and 324 mg L2 particles (thin line). Clusters were also obtained when the particles were pre-dispersed in the water phase instead of the toluene phase, which confirms that the particles tend to adhere at the toluene-water interphase regardless in which phase they were initially suspended.

Fig. 7 Cluster formation from cyclohexane-in-water emulsions: The LSCM micrograph (I) displays cyclohexane droplets which templated packing of L2 particles at their interface into small clusters such as particle doublets, triplets, and few tetrahedrons (II: FESEM micrograph; III: cluster size distribution).

Tables

Table 1 Preparation and important parameters of polystyrene latex particles

	L1	L2
styrene	357.3 g	250.3 g
AEMH	17.1 g	12.0 g
DVB (80%)	27.9 g	19.5 g
CTAB	6.25 g	1.21 g
V-50	1.41 g	0.98 g
water	1437 g	1031 g
d_n^a	91 nm	154 nm
d_w / d_n^b	1.002	1.004
zeta potential	+ 73 mV	+ 66 mV

^a number-average diameter as derived from TEM micrographs by counting more than 500 particles

^b polydispersity given as the weight-average diameter d_w divided by the number-average diameter d_n

Table 2 Hydrodynamic diameters of the oil droplets prepared in the presence and absence of dodecane as obtained by DLS

oil phase	continuous aqueous phase	droplet diameter	
		with dodecane	without dodecane
toluene	0.25 wt% Pluronic [®] F-68	824 nm	1290 nm
toluene	0.5 wt% Pluronic [®] F-68	455 nm	1274 nm
toluene	1 wt% Pluronic [®] F-68	325 nm	1083 nm
cyclohexane	1 wt% Tween [®] 80	437 nm	532 nm

Table 3 Weight percentages of the clusters made up of N constituents within the suspensions: The clusters were assembled from L2 particles at the surface of toluene droplets. Experimental parameters are the concentrations of the building blocks and the emulsifier (Pluronic[®] F-68). Moreover, the constituents were added either through the dispersed toluene or the continuous water phase.

	particles in toluene			particles in toluene			particles in water		
F-68 (wt%)	1.0			0.25	0.5	1.0	0.5		
L2 (mg)	26	53	108	108			108	216	324
$N = 1$	71.0	53.5	50.2	26.3	29.2	50.2	53.2	40.6	33.3
= 2	16.4	23.9	22.5	17.7	19.0	22.5	18.7	20.5	20.2
= 3	5.0	11.9	10.0	12.3	13.7	10.0	8.2	10.2	11.2
= 4	2.3	4.4	4.6	7.1	8.2	4.6	4.1	5.6	6.3
= 5	1.1	1.9	2.4	5.3	5.6	2.4	2.7	3.6	4.1
= 6	-	1.3	1.7	4.0	3.8	1.7	2.0	2.6	2.9
= 7	-	-	1.3	2.9	2.6	1.3	1.3	2.1	2.3
= 8	-	-	1.0	1.9	2.1	1.0	1.2	1.6	1.8
= 9	-	-	-	1.8	1.5	-	1.0	1.3	1.5
= 10	-	-	-	-	-	-	-	0.9	1.2
R^a	4.2	3.1	6.3	20.7	14.3	6.3	7.6	11.0	15.2
C^b	29.0	46.5	49.8	73.7	70.8	49.8	46.8	59.4	66.7

^aR denotes all clusters which cannot be resolved as individual peaks in the DCS measurements

^bC is the total yield of clusters in respect to the number of constituents added