Assembly of Latex Particles by Using Emulsion Droplets as Templates. 1. Microstructured Hollow Spheres

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We describe an emulsion-based technique that allows the assembly of colloid particles into microstructured or multicomponent clusters ("supraparticles"). The particles are gathered, assembled, and fixed together in the restricted, colloid-size 2D or 3D space provided by emulsion droplets. The process is carried out by multiple modification of the colloid interactions within the particle/droplet system—"interaction-tailored colloid assembly". In the first paper of the series we provide a general description of the method. Then we present the data on the assembly of negatively charged (sulfate) or positively charged (amidine) latexes into ordered hollow spherical supraparticles. The following steps are included in the assembly schemes: (1) modification of latex surface charge and properties, so the microspheres are able to adsorb on the droplet surfaces but without homocoagulation; (2) adsorption and structure formation around the emulsion drops (at this stage the interfacial mobility of the particles is of crucial importance); (3) sterical protection of the particle/droplet complexes against coagulation or flocculation; (4) "binding" of particles within the assembled microspheres by strong coagulant; (5) extraction of the supraparticles by dissolving the carrier droplets in the surrounding environment. To characterize the electrostatic interactions throughout the assembly stages, we used electrophoretic mobility measurements. The electrophoretic data are in good qualitative agreement with the interaction-tailored assembly scheme. Clues on the other interactions involved are provided by altering the assembly process and using different modifying agents.

1. Introduction

The recent introduction of high-technology processes and products in practice has put forward new requirements for colloid suspensions of unique and strictly defined properties. Particular interest is shown in the fabrication of particles that display structured or inhomogeneous morphology.[1]

One typical example of such colloid species is the core−shell latex particles, prepared by two-stage emulsion polymerization.[2] Other examples are the encapsulated or microcrystalline particles.[3] Closest to the present work are the publications on the fabrication of composite particles based on a core of one big particle, onto whose surface a shell of smaller particles is attached.[4−6] On a few special occasions, the outer shell of the composites exhibits good 2D ordering.[5]

While significant progress has recently been achieved in the fabrication of composites and particles of special properties, some aspects still remain to be covered. All of the above references basically deal with composite assemblies from particles of dissimilar size. The heterocoagulation as well as the other techniques for coating with particles produces poor results when the core particle is of a size comparable to the one of the shell particles.[6]

For some applications it might be of interest to obtain ordered aggregates of defined size and shape out of similar colloid particles. The simple coagulation of suspensions of colloid particles produces aggregates of dissimilar size and fractal appearance[7,8] (similar clusters are shown in Figure 4 of this paper). A few techniques allow the coagulation into clusters of approximately the same size,[9] but these clusters could not be ordered and their shape is uneven and fractal-like. Therefore, the fabrication of colloid particle aggregates that display ordering and have a symmetric and defined shape is still a challenge to the colloid investigator. In an attempt to tackle the above problems, we have applied a novel technique that allows the assembly of different types of colloid particles into ordered or multicomponent clusters (these we sometimes denote as "supraparticles"). The size and the composition of the supraparticles during the assembly are controlled by gathering and confining their components in a restricted, colloid-size 2D or 3D space. Emulsion droplets are used to provide this restricted space. After the particles are assembled and fixed, the emulsion droplets are dissolved in the environment and the supraparticles are extracted as a colloid suspension.

In this paper, first a general description of the emulsion assembly method is given (section 2.3). Then we present data on the supraparticles obtained from a negatively charged sulfate latex (section 3.1). In section 3.2 we present the results on supraparticles assembled from positively charged amidine microspheres. In the Discussion part of the paper we first discuss the data on the

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sulfate latex from the viewpoint of the colloid interactions involved in the assembly scheme (section 4.1). The data on the assembly of amidine supraparticles are discussed in section 4.2. A few possibilities for the further improvement and development of the method are listed in section 4.3 and the Concluding Remarks.

2. Experimental Section

2.1. Materials. The suspensions of latex microspheres of 1 μm in diameter were supplied from IDC Corporation. A summary of the surface properties of these microspheres is supplied in the Supporting Information of this paper. Prior to being used, the contents of the bottles were redispersed and sonicated for a few minutes.

The 1-octanol was purchased from Aldrich and was not subjected to further purification. Hexadecyltrimethylammonium bromide (HTAB) and β-casein from bovine milk were obtained from Sigma. L-Lysine monohydrochloride and L-glutamic acid were purchased from Chameleon, Japan. The solution of the latter was converted to the monobasic salt by addition of NaOH solution until pH = 7 is reached. Tween 20 was supplied by Bio-Rad, USA. Glutaraldehyde, electron microscopy grade, was purchased from TAAB Laboratories, USA. The other substances not listed above were supplied by Wako Pure Chemicals, Japan. The water phase for the experiments was purified by a Millipore Milli-Q system.

2.2. Methods. The latex supraparticle suspensions were prepared in volumes of 2−10 mL, handled, and stored in tightly capped disposable test tubes of inner diameter ≈14 mm. The homogenization of the emulsions was carried out on a Janke and Kunkel Ultra-Turrax T25 rotor-stator type device, equipped with a S-25N 10G dispersing tool and control of the speed of rotation. Whenever centrifugation was necessary, a RS-20IV (Tomy Seiko Co., Japan) centrifuge was used.

The obtained supraparticle dispersions were examined by optical microscopy in transmitted illumination. Samples for microscopic observation were prepared by placing a few small drops of the emulsion or suspension on microscope slide glasses and covering them by cover glasses. To minimize evaporation and convection during the observation, slide glasses with shallow pits (Toshinriko Co., Japan) and wide cover glasses (24 × 32 mm) were preferred. Most of the pictures presented hereafter were obtained on an Olympus Vanox microscope. A few of the samples were also observed on a Zeiss Axioplan FL microscope. The choice of the objective lens is important for the resolution of the supraparticle structure. We found that the best results are obtained at not very high numerical aperture and medium working distance. Out of the few objectives available, a Plan 40/0.65 was empirically found as most suitable. To capture the observed data, the microscope was equipped with a high-resolution digital CCD camera (Hamamatsu C4742) and a photocamera. The digitized images were directly stored on a Macintosh computer. Before the images were saved and printed out, we routinely applied level optimization, cropping, contrast enhancement (if appropriate), and data compression.

The electrophoretic mobility measurements were carried out by using a Zeecom 1P-120B ζ-potential analyzer (Japan). The apparatus performs automatic tracking of the particles in the center of a 10 cm long electrophoretic cell. The ζ-potentials are calculated from the obtained electrophoretic mobility by using the Smoluchowski equation.[10] The particle velocities were measured by frequently changing the direction of the field to minimize possible errors from cell leakage. At least 50 different particles were counted in each measurement. All particle trajectories were followed both automatically and manually on screen to ensure the correctness of the automatic tracking. Emulsion samples for the measurements were prepared by dispersing a few droplets of oil in 50 mL of water by the homogenizer, taking all possible care to avoid contamination with ionic surfactants.

2.3. General Description of the Method. The different types of supraparticle suspensions reported in this study were assembled from monodisperse latex spheres of micrometer size. The colloid interactions between the latex particles (electrostatic, van der Waals, steric, hydration, hydrophobic, etc.) are principally known.[7,11] These interactions may involve many particles, and they are unidirectional. Therefore it seems highly unlikely that by controlling these interactions alone one would be able to directly assemble microstructured aggregates of definite size and shape.

In this study, emulsion drops are used as 2D or 3D colloid "templates" whose size and shape control the overall size and shape of the obtained supraparticles. The basic principles of the "assembly-and-dissolution" scheme(s) used in our study are illustrated in Figure 1. The process starts with a suspension of latex microspheres in water. Emulsion drops of the oily phase are introduced after that in the system. The particles could be adsorbed either on the droplet surfaces or in their bulk. The obtained ordered shells or balls of densely packed particles are fixed together by an appropriate agent. The carrier emulsion droplets are later dissolved by addition of a mediator phase or solubilizing agent. Thus the final product is supraparticle clusters resuspended in the water phase. The emulsion template scheme has the ability to assemble composite supraparticles comprising different types of colloid species, if a second type of particles is adsorbed on the droplets. In the study presented hereafter we concentrate on the formation of spherical supraparticles from one type of latex beads only—the left branch in Figure 1. Experimental data on the formation of ball-like and composite supraparticles (middle and right branches in Figure 1) are presented in the second paper of this series.

The initial investigation as to how the assembly and dissolution scheme could be experimentally implemented encountered a number of difficulties and even controversies in the required properties of the particles, the oil, and the dissolving agents. Being convinced that it will be close to impossible to find particles and phases that would *a priori* possess the proper surface properties, we made use of a number of agents that by physical or chemical means modify the colloid interactions within the particle/droplet system. Each of the steps of the multistage assembly process is carried out under specified and controllable conditions.

As the second phase for the oil/water emulsions, we chose octanol. This oil does not dissolve or cause swelling of the polystyrene microspheres. The mutual solubility of octanol and water is small, and a clearly defined interface is formed. The mutual solubility however drastically increases upon the addition of ethanol to the system, and we found that a 1:1 water/ethanol mixture can dissolve about 15 vol % octanol.

The practical assembly schemes and the experimental data for different types of latex are presented below.

3. Results

3.1. Sulfate Latex Assemblies. These supraparticles were assembled from the negatively charged "surfactant-free" sulfate polystyrene latex. The stability of the original latex suspension is based only on the electrostatic repulsion between the microspheres.[12−14] The charge is provided by a mixture of sulfate and hydroxyl groups on the latex surface.[12−15] The biggest part of the latex/water interface remains hydrophobic.[13,14] The success of the assembly scheme heavily depends on the surface properties of the used latex samples. The available data of the used microspheres from the producer's specifications and from the literature are summarized in the Supporting Information to this paper.

After experimenting with different substances and conditions, we formulated the assembly flowchart, pre-

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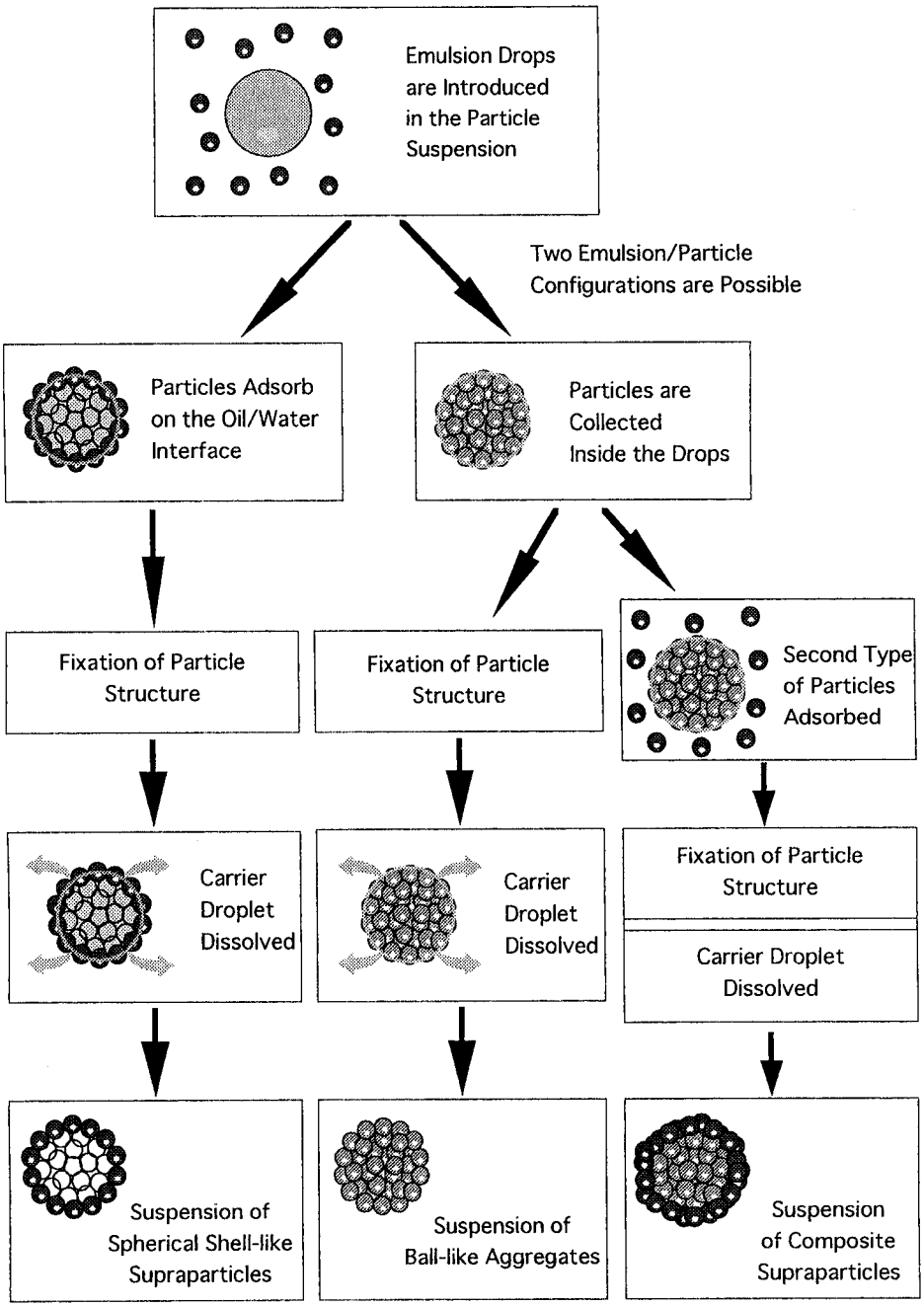

Figure 1. Possible modifications of the "emulsion template" method for supraparticle assembly. This paper describes the experimental data on spherical shell-like supraparticles. The ball-like and composite assemblies are described in the second paper of the series.

sented in Figure 2. The latex particles in their original state do not adsorb on the oil droplets. Therefore, the first step required is to modify their surface properties (electrostatic charge and hydrophilicity) in such a way so that the microspheres become adsorbable on the oil/water interface. This process is denoted hereafter as "sensitization". Note, that the ideal modification of the latex surfaces should incite neither the flocculation of the beads in the suspension nor 2D coagulation after their adsorption on the interface, as both of these are disastrous for the 2D ordering. Out of the substances tried as sensitizers, the amino acid lysine worked best (a few others are briefly discussed further below). Lysine was used in the form of its monohydrochloride salt at a concentration of 0.017 M. The pH of the thus prepared solution is neutral (5.5−6.0). The used latex treated with lysine under these conditions did not coagulate. It was however able to slowly adsorb on the octanol/water interface and to form 2D ordered structures. This process was greatly enhanced and quickened by the homogenizer.

The homogenization step of the assembly process was carried out by first introducing 0.3 vol % sulfate latex in aqueous solution of lysine hydrochloride. Five minutes after that 1−1.5 vol % octanol was added. The system was then homogenized on the Ultra Turrax device. The adsorption of the latex onto the dispersed oil droplets during the homogenization process is noticeable by the change of the appearance of the white suspension from milky to opalescent and the buildup of oil/latex deposits on the wall of the test tube. We have tried both a continuous homogenization for 5−8 min and a cyclic scheme that combines the same time of treatment but split and prolonged into cycles of 60 s of homogenization and 30 s of rest. The adopted cyclic scheme worked better,

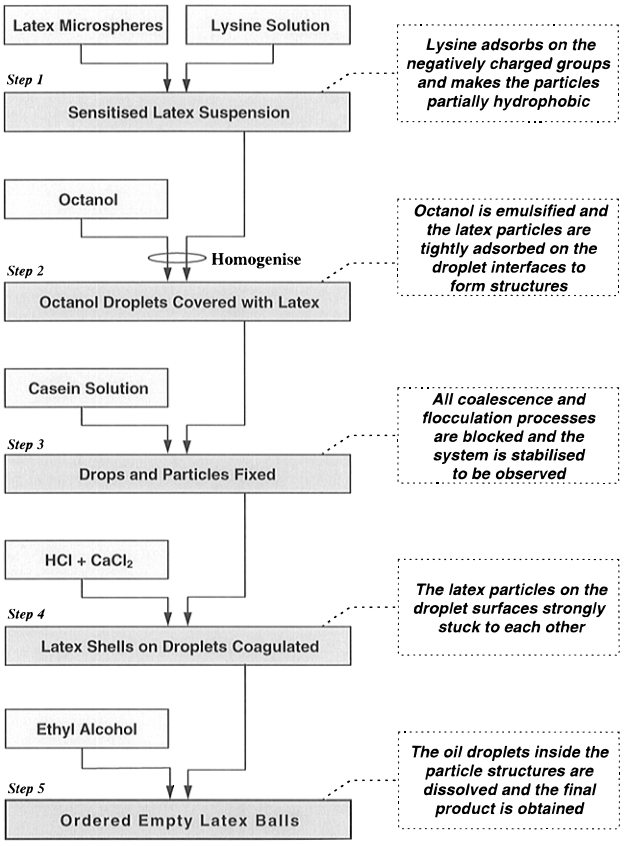

Figure 2. Concrete flowchart for the multistage, interaction-tailored assembly of ordered hollow supraparticles from the sulfate latex. The boxes on the right briefly describe the physical processes that take place at each step.

possibly because the rest periods provide some time for rearrangement of the latex microspheres adsorbed on the droplets.

The outcome of the homogenization step, if the process is carried out thoroughly enough, is octanol emulsion droplets covered with a shell of 2D ordered microspheres. A panel of such latex/droplet assemblies of different sizes is presented in Figure 3. The Ultra Turrax homogenizer produced emulsion droplets with a wide polydispersity, which results in supraparticles of diverse sizes. To demonstrate this, in all panels we present pictures of widely different size droplets or particles obtained in one or more experiments. The mean size of the droplets depended on the input energy (revolutions per minute) of the homogenizer. At 8000 rpm (min^{-1}) the droplet diameter was within the 10−100 μm range. At 14 000 min^{-1} the mean droplet size fell below 10 μm but the coverage with particles become poor due to the very high interfacial area. All of the data reported hereafter were obtained in the range 8000−10 000 min^{-1}. After the treatment with the homogenizer, it is very important to check by observation that the obtained droplets are fully covered by an ordered latex shell. A figure illustrating the appearance of the droplets in this check is included in the Supporting Information. This observation should be done quickly, as the droplets covered with microstructured latex shells are highly unstable. If the test tubes are left at rest, in 5−10 min the droplets will cream and coalesce, forming a layer of oil and latexes on top of the lysine solution.

To prevent breakdown, the next immediate step undertaken in the overall assembly scheme (Figure 2) was the stabilization of the latex/droplet assemblies. This was achieved by quick mixing of the emulsion with casein solution—Step 3 (Figure 2). The concentration of casein in the system was adjusted at 0.5−1 mg/mL. A few minutes of vortexing was required to prevent creaming before the protein adsorption is completed. After stabilization with casein, the protected particle/droplet assemblies can be stored for tens of minutes and easily examined by microscope. The casein adsorption and steric stabilization step is irreversible. It blocks altogether any further latex adsorption or flocculation. The success of the overall assembly is therefore determined by the quality of the latex structures before the addition of casein blocker.

We found that casein is able to gently bind the latex particles within the shells around the droplets. This possibly results from bridging of the adjacent particles by protein molecules adsorbed on their surfaces. The strength of this bridging is not sufficient to preserve the supraparticle structure during the dissolution of the carrier droplets. Therefore the addition of a stronger binding agent (coagulant) is necessary. We used a mixture of HCl and $CaCl_2$, both of whose components are known to be strong coagulants of the used latex beads.[14] The final concentration of the substances in the system after step 4 of the process (Figure 2) amounted to 10^{-2} M HCl and 5×10^{-3} M $CaCl_2$. In a separate experiment, when the above mixture was added to a suspension of latex microspheres, it induced their immediate coagulation and in 10 mins most of the microspheres were gathered in shapeless fractal-like aggregates (Figure 4).

It is important to note that in 2−3 min the coagulant mixture was not able to induce aggregation of the latex/droplet assemblies or of the free unadsorbed latex beads, obviously due to their protective coverage with casein. It however bound the compressed particles within the microstructured latex cores. The microstructured shells were thus prepared for the droplet extraction that was carried out by adding 50 vol % ethanol. We found empirically that the best yield is obtained when the ethanol is introduced as quickly as possible—this was achieved by injection. After the carrier octanol droplets are dissolved, the supraparticle assemblies remain suspended in the water/ethanol environment.

The supraparticles obtained in this way possess the structure of empty ordered shells from the coagulated latex. Microscopical pictures of suspended assemblies of different sizes are presented in Figure 5. It is seen that the particles within the shell preserve the 2D ordered pattern that was formed during the adsorption on the droplet surface. As the focal depth of the objective is only a few microns, the bigger supraparticles could be optically "cross-sectioned" by focusing on their top, bottom, or equatorial planes. The "cross-sectioning" technique allows us to prove that most of the obtained spherical shells are ordered all around and that no oil droplet is present inside. Such pictures are arranged in Figure 6.

Apparently not all of the latex shells around the droplets survive the droplet dissolution stage to form intact spherical supraparticles. Some of the adsorbed shells, particularly the incomplete ones, break into small 2D fragments or disassemble during the ethanol injection. When Figures 3 and 5 are compared, it is seen that the biggest assemblies are no longer present because most of them have broken during the removal of the droplets. The yield of the dissolution step appears to be about 30−50%; that, combined with the losses during the homogenization and from nonadsorbed particles, lowers the final yield below 10% (calculated as latex microspheres converted into intact supraparticles). The concentration of intact supraparticle spheres in the output suspension typically ranges around 2×10^5 cm^{-3}. The microstructured spheres in this suspension can be stored in a closed container for

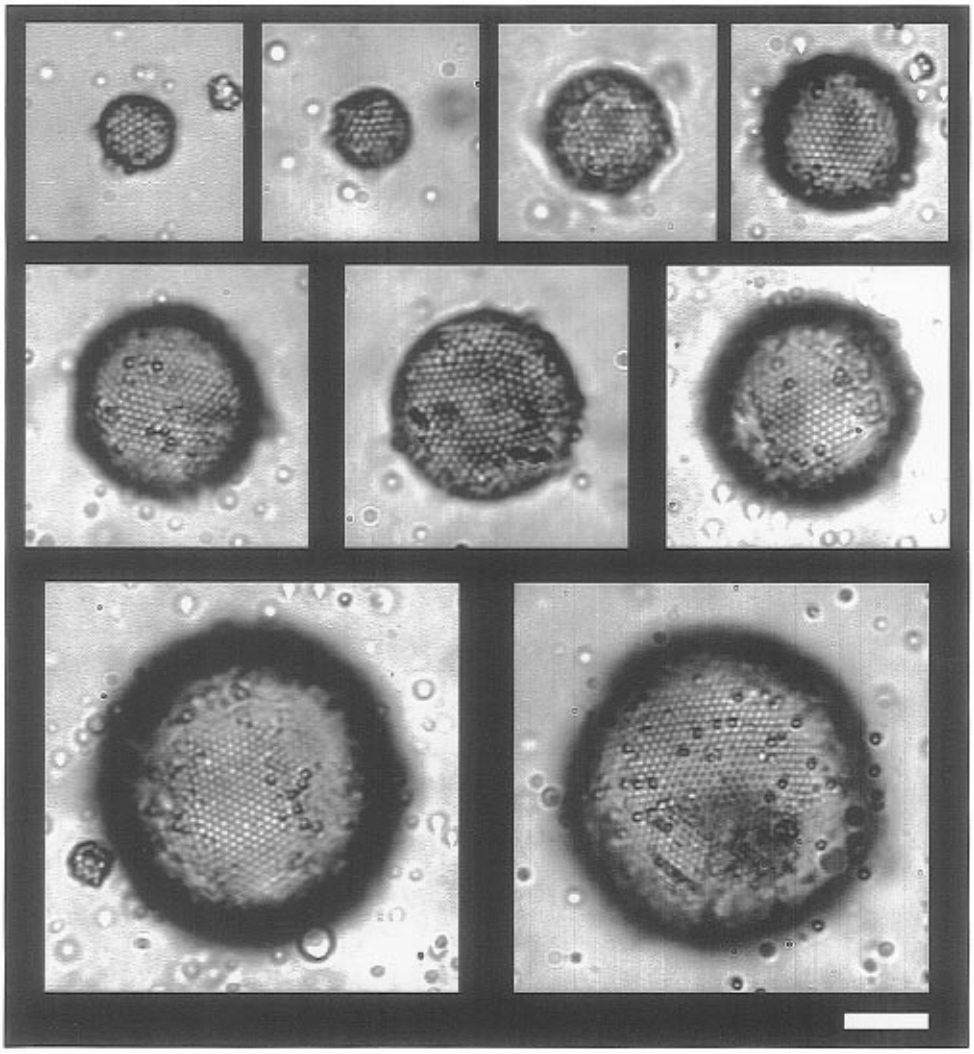

Figure 3. Digitized pictures of octanol droplets covered by ordered shells of the sulfate latex. These are obtained after step 2 of the flowchart in Figure 2. Particles of various sizes obtained in a single experimental run are presented. The white bar at the bottom is equal to 10 μm.

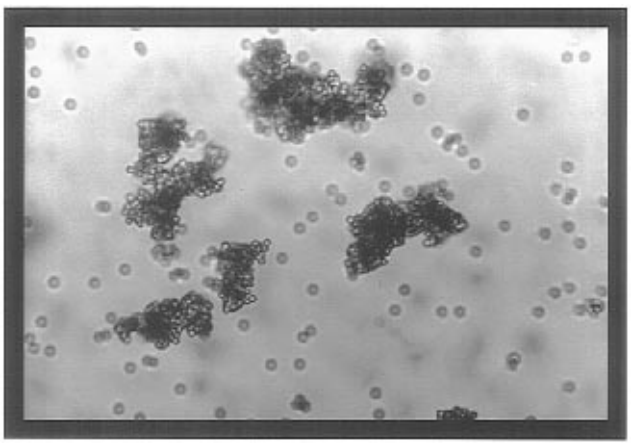

Figure 4. Picture of irregular coagulates obtained by adding a mixture of HCl + $CaCl_2$ to the original latex suspension.

periods longer than a week. In a few days they sediment on the bottom of the container but can be readily redispersed by shaking. Gentle centrifugation ($\leq$700 G) can be used to concentrate and purify the supraparticles, as these sediment before the smaller fragments and single beads. By centrifugation, one can also replace the water/ethanol/octanol environment with pure water.

Though the sulfate latex spherical shells appeared stable in a suspended state, they could not survive drying over a solid surface (with the exception of a few ones of very small size). Microscopic observation of the drying process of particles in a pure water environment was carried out. It turned out that the empty supraparticles break into disordered agglomerates on the surface at the moment when they are pressed down by the drying air/water interface.

Alternative Agents for Surface Modification. Below we describe some alternatives for the substances in Figure 2 that provide clues about the colloid interactions in the system.

Phenylalanine at a concentration of 0.014 M could be used to sensitize the microspheres. No sensitization of the latex suspension was observed at neutral pH. At pH = 3, below the p*I* of phenylalanine (p*I* = 5.48[16]), the microspheres easily adsorbed on the droplet surfaces. We have not routinely used this method for sensitization because it is a bit more complex than the lysine one and on some occasions aggregation of the microspheres was recorded.

Cationic Surfactant—HTAB. Hexadecyltrimethylammonium bromide (HTAB) is a cationic surfactant that readily adsorbs on the latex beads, adding positive charges to the surface. When it was used as a sensitizing agent at a concentration of 2.2×10^{-4} M, the obtained assemblies

(16) *CRC Handbook of Chemistry and Physics*, 72th ed.; CRC Press: Boca Raton, FL, 1992; Section 7.

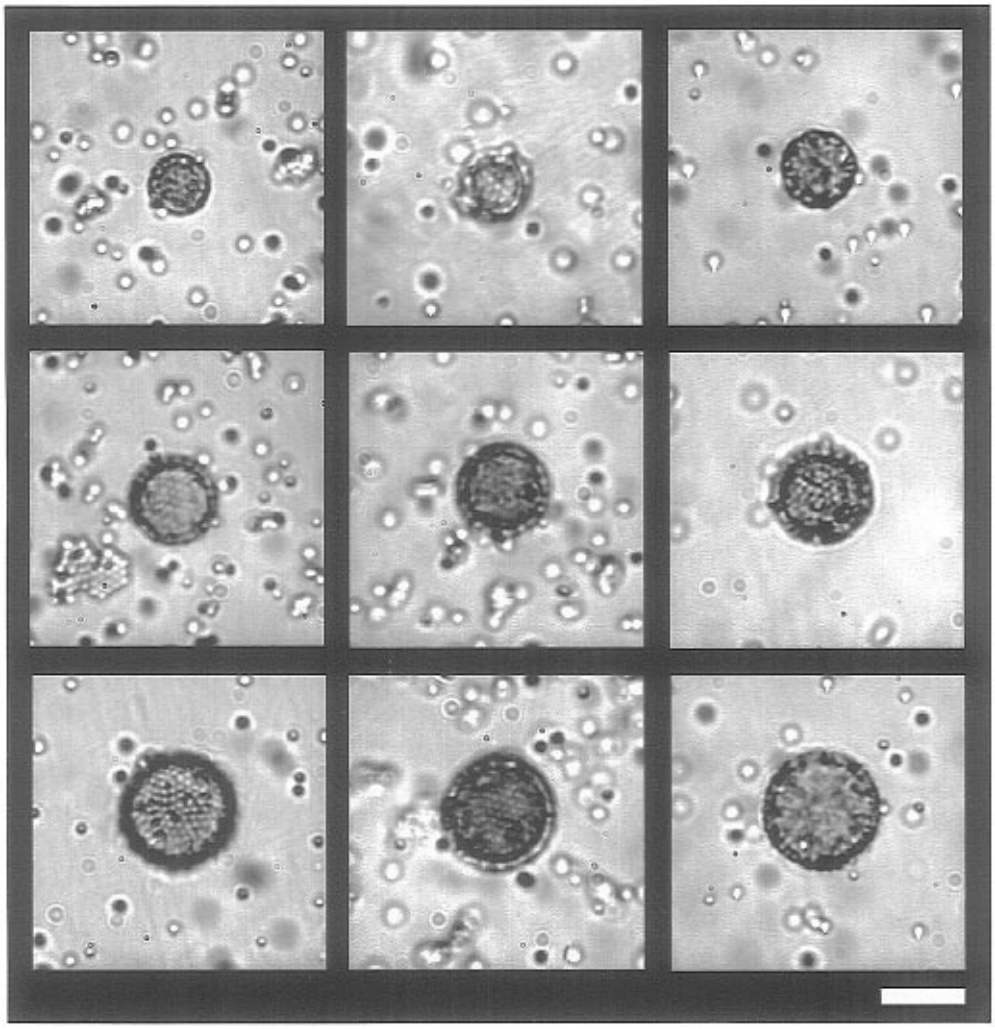

Figure 5. Shell-like spherical supraparticles from the sulfate latex obtained by the scheme in Figure 2. The assemblies are suspended in an aqueous environment. Bar = 10 μm.

appeared spherical, but without clearly visible ordering. At a concentration of 4.4×10^{-4} M the aggregates lost their spherical shape and ordering altogether (a figure is provided in the Supporting Information). At both concentrations the aggregates could be extracted from the droplets without applying the coagulating mixture (omitting step 4 in Figure 2).

Nonionic Blocker—Tween 20. One effective alternative for casein as a steric protection of the latex assemblies is the use of a nonionic surfactant. We have investigated the effect of adding Tween 20 (polyoxyethylene 20 sorbitan monolaurate) at a concentration of 4×10^{-4} M during step 3 of the assembly scheme. We found that Tween stabilizes the latex/droplet assemblies within a few seconds and keeps the particles dispersed throughout the following steps. The sole disadvantage of Tween 20 is that if the coagulating mixture is not added within a few minutes, the particle shells around the droplets slowly start to disassemble and desorb. The observed desorption of the latex is possibly a result from the high activity of Tween on the oil/water interface.

Solubilization of the Droplets by Surfactant Micelles. One promising alternative approach to the droplet dissolution in step 5 of the assembly process is the use of a micellar surfactant solution as an agent that removes the oil droplets from within the latex shells by solubilization. We carried out experiments using micellar solutions of Tween 20. The results are described in the Supporting Information.

Alternative Products of the Assembly Scheme. A few modifications of the scheme presented in Figure 2 that lead to other types of structures are presented below.

Suspensions from 2D Sheets. A qualitatively different result is obtained if the coagulant adding step (step 4 in Figure 2) is omitted. In this case the adsorbed ordered shell is not able to survive intact the droplet dissolution stage. The resulting structures are in the form of fragments that appear as a suspension of sheets of a 2D ordered latex (Figure 7). The fragmentation process seems to involve some degree of unfolding, as the sheets usually do not display significant curvature. Like the spherical particles, the suspensions of ordered sheets could be stored for a long time. These species do not get destroyed by drying and could be deposited and examined on a solid surface.

Cornlike Elongated Particles. In some of the experimental runs, a minute portion of ordered hollow assemblies of elongated "cornlike" shape was obtained. Pictures and a few more details are provided in the Supporting Information.

Latexes Fixed with a Protein Membrane. One major modification that was tried in the assembly flowchart in Figure 2 is the use of glutaric dialdehyde instead of the coagulation mixture in step 4. Glutaraldehyde cross-binds the casein molecules adsorbed on the surfaces of the particles and the droplets. The supraparticles obtained in this way were fixed by a protein membrane around and

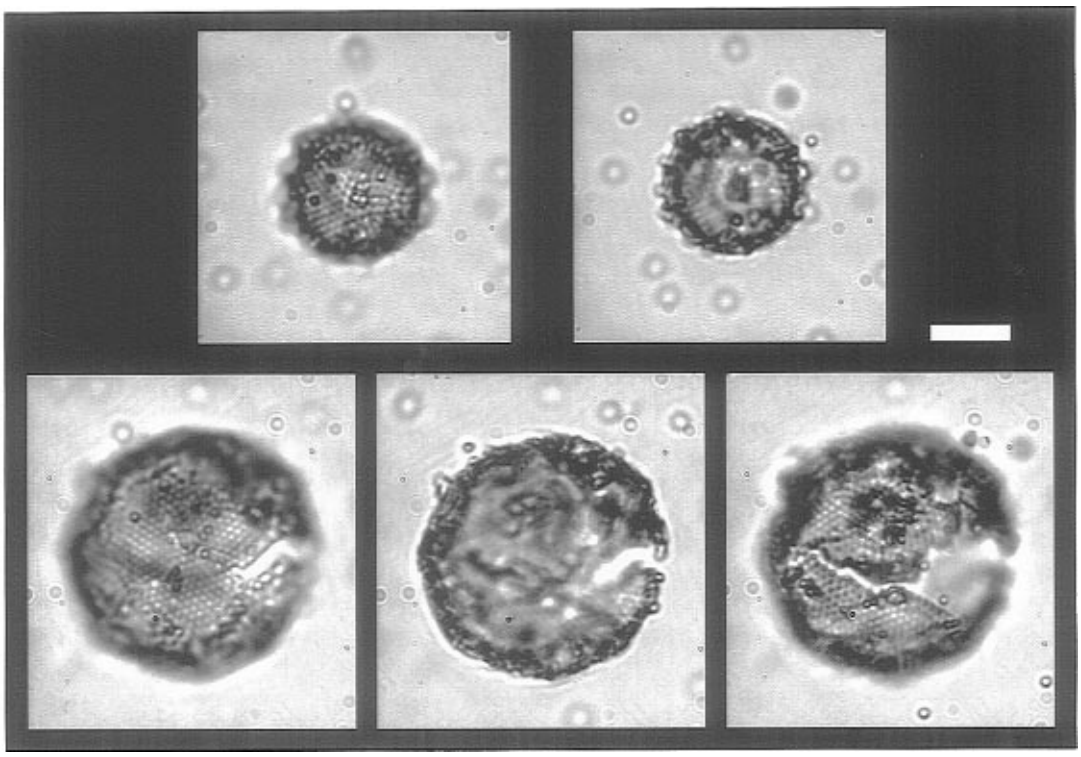

Figure 6. Optical "cross-sectioning" of two of the obtained supraparticles. By moving the focal plane of the objective up and down, one is able to observe the outer shell structure or the inside of the assemblies. The crack in the big particle allows us to demonstrate that the oil droplet inside it has been dissolved. Bar = 10 μm.

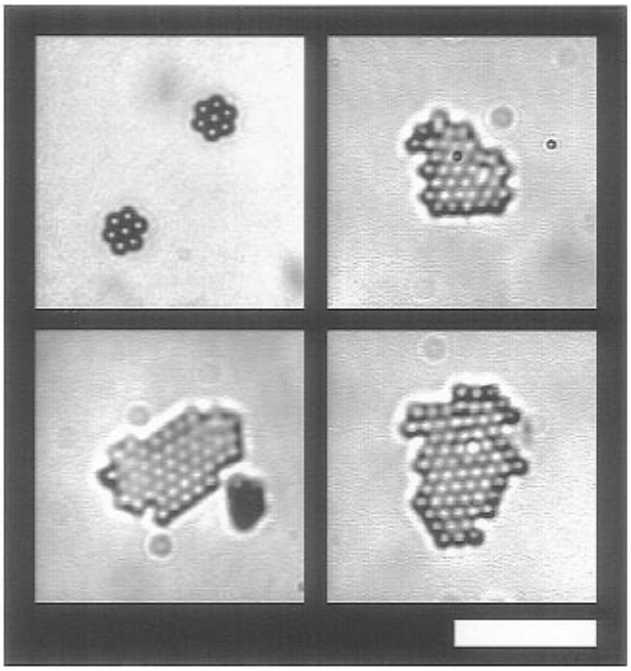

Figure 7. Small 2D sheets of aggregated latex obtained when step 4 of the assembly scheme in Figure 2 is omitted. Bar = 10 μm.

between the latex beads. Details and pictures are provided in the Supporting Information.

ζ-Potential of the Sulfate Latex Particles at the Different Stages of the Process. The multistage assembly scheme presented in Figure 2 involves and makes use of many types of colloid interactions. However, especially in the initial steps, electrostatics seems to be crucial. To clarify further this point, we carried out electrophoretic measurements on the ζ-potential of latex beads immersed in environments similar to the ones in the assembly process. The obtained data are presented in Table 1. The table also includes data for the measured ζ-potential of the octanol emulsion droplets.

3.2. Results on Amidine Latex Assemblies. To demonstrate that the interaction-tailored assembly scheme presented in Figures 1 and 2 could be extended to colloid species other than the sulfate latex, we performed experiments with latex microspheres of positive surface potential. According to the producer's specifications,[14] the only source of charge on the surface of the used polystyrene latex beads is the amidine groups ($C(NH_2)=NH_2^+$; more data are included in the Supporting Information).

We were able to find appropriate modifying agents that allowed the assembly of microstructured spheres from the amidine latex. The flowchart of the process is presented in Figure 8. Being highly charged, the amidine microspheres in their original state are too hydrophilic to adsorb on the surfaces of the oil droplets. For sensitization, we used a solution of the monosodium salt of glutamic acid at a concentration of 0.055 M. The concentrations of latex and octanol and the homogenization conditions were similar to the ones in the sulfate latex experiments (above). When the latexes were introduced into this solution and then were homogenized with octanol (steps 1 and 2 in Figure 8), they adsorbed on the droplet surfaces and after 8−10 mins started to penetrate inside the oil.

The homogenized system with the particles on or in the droplets was stabilized by adding Tween 20 (concentration = 4×10^{-4} M; step 3 in Figure 8). The amidine microspheres are insensitive to low pH and divalent ions.[14] Therefore we used glutaraldehyde as a coagulant. The amidine group easily reacts with one of the aldehyde groups of the glutaraldehyde molecule, leaving the group on the other end active.[12,14] We expect that with closely situated particles the glutaraldehyde is able to cross-link amidine groups across the gap between the particles. To prove this, we carried out a separate experiment and observed quick and irreversible coagulation (similar to the one shown in Figure 4) when any two amidine

Table 1. ζ Potentials of the Colloid Species Involved in the Assembly of the Sulfate (Negatively Charged) Latex[a]

system	ζ-potential, mV	pH	notes
sulfate latex	-82.0 ± 4.4	6.0	
sulfate latex + lysine	-62.8 ± 5.8	5.5	
sulfate latex + lysine + casein	-43.9 ± 3.2	5.6	
sulfate latex + lysine + casein + HCl + $CaCl_2$	$0 \sim -15$	2.1	difficult to measure because of high ionic strength and low pH
sulfate latex + phenylalanine	-89.1 ± 5.1	5.7	
sulfate latex + phenylalanine + HCl	-32.9 ± 4.1	3.0	
sulfate latex + HTAB	$+115.2 \pm 5.6$	5.6	almost immediate recharging
octanol emulsion droplets	-20.4 ± 9.9	6.1	samples survive less than 20 min
octanol emulsion droplets + lysine	-14.3 ± 7.1	5.8	samples survive less than 20 min

[a] 5×10^{-4} M NaCl is added to all the samples. The concentrations of the other substances are similar to those in the assembly process.

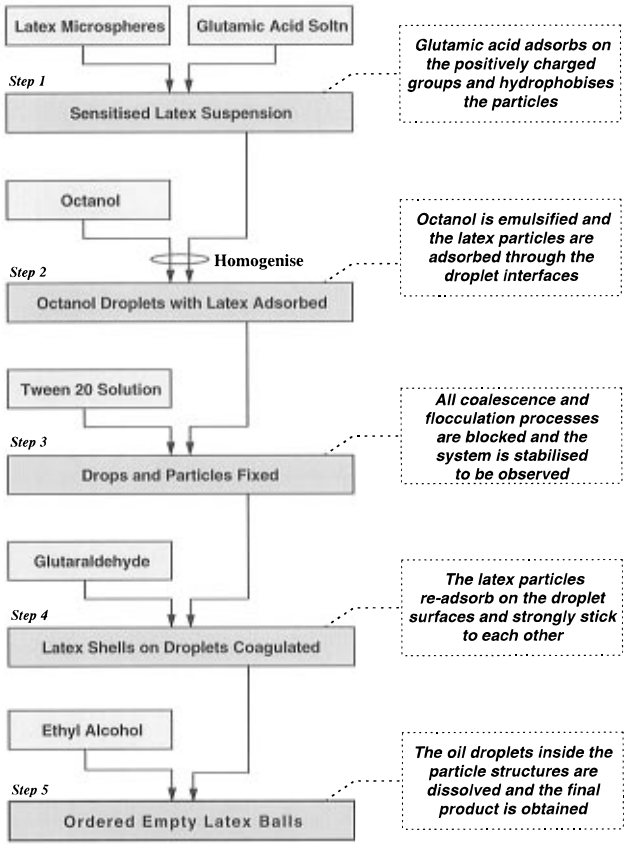

Figure 8. Flowchart for the assembly of hollow spherical supraparticles from the positively charged amidine latex.

microspheres treated with glutaraldehyde collide within the latex suspension.

After glutaraldehyde was added to the stabilized octanol droplets (step 4 in Figure 8) a readsorption of the latex particles from the droplets bulk back onto the interface was visible. When the final glutaraldehyde concentration was adjusted to 0.5 vol %, the obtained assemblies had a good ordering of the outer shell, but only the ones $\leq 15\,\mu$m in diameter survive the octanol dissolution step. At a glutaraldehyde concentration of 2 vol % even the biggest coagulated shells can be extracted from the carrier droplets, but the ordering of the latexes around the shell is very poor, probably due to the quick, strong coagulation. The best results are obtained by a two-step coagulation, first adding 0.2 vol % of glutaraldehyde and then storing for 5 min. This leads to the formation of not very strongly bound but microstructured shells. The supraparticles are then fixed strongly by increasing the glutaraldehyde concentration up to 2 vol %. Ethyl alcohol is finally injected to dissolve the octanol droplets and extract the supraparticles.

The final product of the amidine latex assembly scheme is again empty spherical aggregates with a microstructured shell (Figure 9). Inside the biggest shells some coagulated latex can be noticed. The smaller and medium-size supraparticles sporadically exhibit very symmetrical and ordered shells—see middle row of Figure 9.

We made electrophoretic measurements of the different colloid intermediates under conditions analogous to the ones in the process. The data, recalculated as ζ-potentials, are presented in Table 2.

4. Discussion

4.1. Modification of the Colloid Interactions throughout the Sulfate Latex Assembly Scheme. The assembly scheme presented in Figure 2 is based on interplay and modifications of different types of interactions. The sensitization and adsorption of the particles onto the droplet surfaces (steps 1 and 2) seems to be closely related to the electrostatic charge of the particles. This charge might prevent adsorption in two ways: (i) by electrostatic repulsion between the particles and the droplets, as both of these are negatively charged, and (ii) because of hydration forces[11]—the penetration of the charged surface groups of the latex into the oil phase is highly unfavorable, due to the incurred loss of the hydration shells around these groups. The high negative potential of the sulfate latex at the beginning of the process makes the adsorption process impossible. After sensitization with lysine, a decrease of the potential magnitude of about 20 mV is recorded (Table 1). The pK values of the lysine molecule (pK's = 2.18, 8.95, 10.53[16]) indicate that at neutral pH it possesses three ionized groups, the net charge being $\approx +1$. We expect that the positively charged lysine molecules decrease the surface potential of the microspheres by binding to the negative sulfate groups. The properties of polylysine as a high molecular weight polyelectrolyte that binds to the latex surface, decreases its potential, and causes flocculation are well-known.[17] The activity of monomolecular lysine at the used concentration appears to be lower, so it modifies the surface charge but without inducing flocculation. This is an important point, as we found that latex flocculation in the suspension or on the droplet surfaces leads to disruption of the structuring or even of the shape of the obtained aggregates (see e.g. the section about HTAB above). On the basis of the well-known formulae[7] and using the typical parameters from the literature,[7] we have estimated the homocoagulation threshold of the used latex to be ≈ -20 mV. The ζ-potential of the lysine-sensitized latex is above that point, and it cannot coagulate or spontaneously adsorb onto the droplet surfaces. The sensitized microspheres however are able to adsorb on the oil surfaces when propelled and pressed against the oil droplets by the

(17) (a) Gregory, J. *Trans. Faraday Soc.* **1969**, *66*, 2260. (b) Corry, W. D.; Seaman, G. V. F. *J. Colloid Interface Sci.* **1987**, *63*, 136. (c) Corry, W. D.; Seaman, G. V. F. *J. Colloid Interface Sci.* **1987**, *63*, 151. (d) Furusawa, K.; Sakai, H.; Watanabe, N.; Tomotsu, N. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 997.

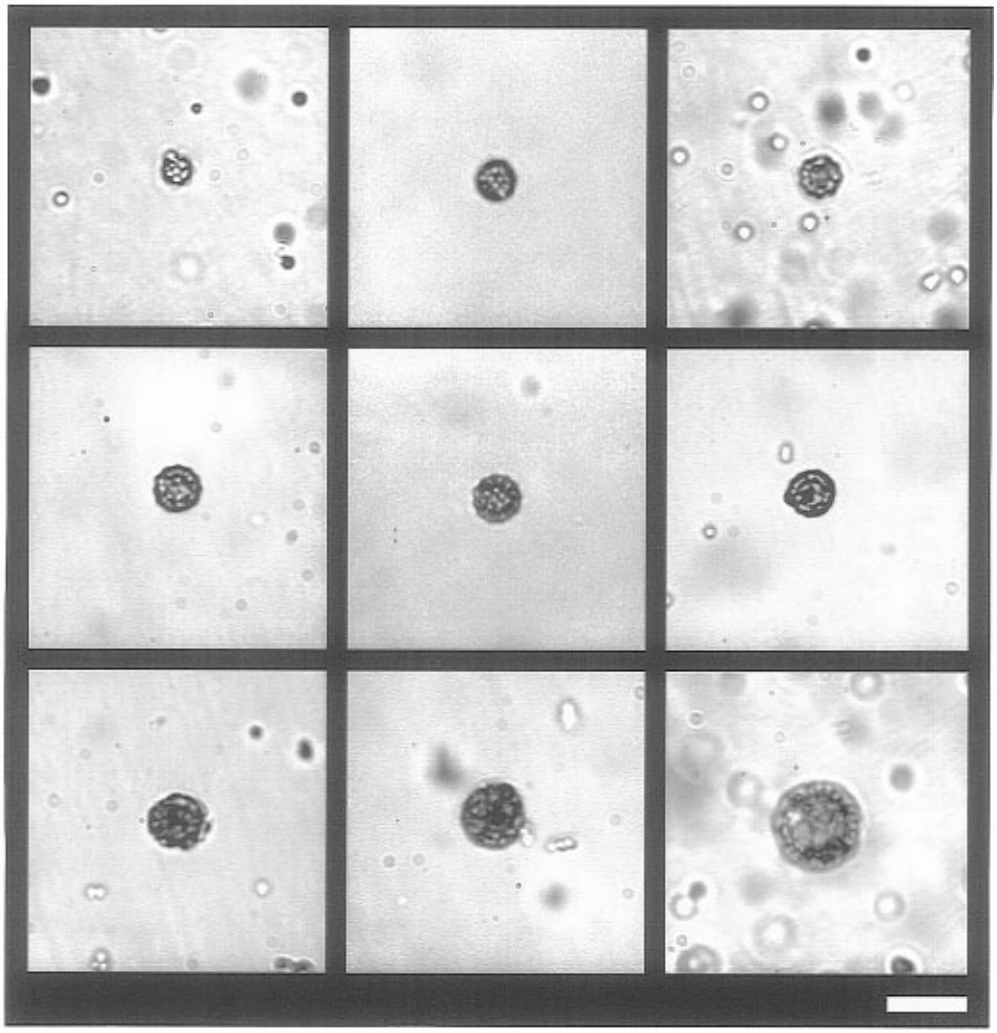

Figure 9. Digitized panel of hollow spherical assemblies of different sizes from the amidine latex. Bar = 10 μm.

Table 2. ζ Potentials of the Colloid Species Involved in the Assembly of the Positively Charged Amidine Latex[a]

system	ζ-potential, mV	pH	notes
amidine latex	117 ± 13.5	5.7	high potential, difficulties with measuring encountered
amidine latex + glutamic acid	≈0	6.7	falls to ~40 mV in the first 20 min and then to zero in the next 30 min
amidine latex + glutamic acid + Tween + glutaraldehyde	−15.7 ± 6.3	6.5	recharging has occurred
octanol emulsion droplets	−20.4 ± 9.9	6.1	samples survive less than 20 min
octanol emulsion droplets + glutamic acid	−13.9 ± 6.9	6.7	samples survive less than 20 min

[a] 5×10^{-4} M NaCl is added to all the samples. The concentrations of the other substances are similar to those in the assembly process.

homogenizer action. After adsorption, we observe that the beads remain mobile on the droplet surface and structure is induced by the restricted 2D space and the repulsion between the adsorbed microspheres. The lysine may also play a role by decreasing the hydration repulsion between the latex and the oil and by involving depletion interactions in the system, but at present we do not have direct evidence how these interactions are involved in the process.

A further decrease of the potential is recorded after casein is introduced to the system and adsorbed on the latex surfaces (Table 1). On the basis of literature data[10,18] this decrease of the ζ-potential can be attributed to the movement of the shear plane further into the solution rather than an actual decrease of the surface charge of the particles. The major role of casein however is not to modify the electrostatic interactions but to evoke steric repulsion by covering the latex and droplet surfaces with a voluminous protein adsorption layer.[7,11] This prevents any further processes of interdroplet coalescence or latex flocculation. The high binding ability of casein to different surfaces that blocks all adsorption or flocculation of other species is well-known[19] and used in applications like emulsion or suspension stabilization and biological assays.[20] At this stage of the process we observe that the microspheres again become unadsorbable on the droplet surfaces and the droplet/latex assemblies cannot coalesce or deposit on the test tube walls. The deduction that the

(18) Tadros, Th. F.; Vincent, B. In *Encyclopedia of Emulsion Technology*; Becher, P., Ed.; Marcel Dekker: New York, 1983; Chapter 3.

(19) Izmailova, V. N.; Rebinder, P. A. *Structure-formation in Protein Systems*; Nauka: Moscow, 1974 (in Russian).

(20) Pratt, R. P.; Roser, B. *Nunc Bull.* **1989**, *7*, 1.

major contribution of casein is the steric protection of the latex and droplet surfaces is further supported by the successful replacement of casein by Tween 20, a simple and well-known steric stabilizer of emulsions and suspensions.[21]

The ζ-potential of the latex falls down to negligible values after HCl and $CaCl_2$ are introduced. This leads to very strong sticking of the microspheres in contact (due to van der Waals and hydrophobic attraction) and blocking of any electrostatic repulsion that could lead to disassembly after the template droplets are removed. As suggested in the literature,[22] the Ca^{2+} ions may also be involved in electrostatic bridging of the closely situated particle surfaces. The high effectiveness of the coagulating mixture is demonstrated by Figure 4. The low pH and the calcium ions may lead to changes within the casein adsorption layer, though they obviously do not affect too much its steric protection barrier around the assembled supraparticles (this protection is preserved even in the water/alcohol environment). Thus, the main role of the coagulation step in the overall assembly scheme is to increase the structural stability within the latex shells. This is directly envisaged by comparing Figures 5 and 7, presenting the results of the assembly scheme with and without the coagulation step.

In Table 1 we also present the ζ-potential data when phenylalanine and HTAB are used as latex sensitizers. The contribution of the adsorbed phenylalanine to the charge of the latex turns out to be strongly dependent on pH. At neutral pH the negative potential of the latex increases slightly. At this pH there is possibly weak adsorption of phenylalanine on the beads by hydrophobic attraction between the latex surface and the hydrocarbon chain of the negatively charged molecules. Decreasing the pH leads to a radical decrease of the ζ-potential, as the phenylalanine molecules attain a positive charge. At pH = 3 the molecules may decrease the surface potential both by adsorption and by electrostatic binding to the sulfate groups of the latex. The ζ-potential data is in good correlation with experimental observations that show no adsorption at pH = 6 and strong adsorption at pH = 3 (combined with some degree of coalescence, as the potential is close to the homocoagulation point). The fact that the phenylalanine molecules act as an effective sensitizer, once they obtain a net positive charge, supports the hypothesis about the electrostatic nature of the latex/lysine interaction. The susceptibility of the polyelectrolytes like lysine and phenylalanine to the pH of the environment appears to be an effective way for indirect control of the surface properties of the sensitized latex beads.

The ζ-potential of the latex microspheres in the presence of HTAB quickly decreases to zero and in about 30 min becomes positive (Table 1). As the potential will fall below the homocoagulation threshold of −20 mV, a coagulation of the adsorbed particles is expected and is experimentally observed. Therefore, we presume that the addition of HTAB leads to the following quick and uncontrollable sequence of events as a result of recharging: particle adsorption, partial penetration inside the droplets, and strong irreversible coagulation. This data confirms again that the potential of the sensitized latex should be kept above the homocoagulation threshold. Though HTAB and other surfactants appear very effective in changing the surface properties, this very effectiveness makes the process difficult to control: even minute quantities of surfactant are strongly bound to the latex surfaces and lead to drastic changes in their electrostatic charge.

In general, by comparing Figure 2 and Table 1, the overall assembly scheme may be envisaged as a twofold decrease of the particle electrostatic potential, first to allow particle adsorption and ordering (step 1 in Figure 2) and then to remove any residual repulsion within the assembled structures (step 4). The microspheres within the assemblies are bound together by van der Waals and hydrophobic attraction and possibly electrostatic and protein bridging. The stability of the supraparticles against coagulation with each other is meanwhile assured by introducing strong steric protection around the assemblies (step 3).

4.2. Colloid Interactions throughout the Amidine Latex Assembly Scheme. The behavior of the amidine latex throughout the assembly schemes could again be related to the electrostatic interactions within the system (Table 2). The original latex sample is highly positively charged, which is in agreement with the producer's data. The octanol droplets display a slightly negative potential, which does not change significantly in the presence of glutamic acid (Table 2). The amidine latex and the droplets bear charges of opposite sign, when they are measured separately, yet no heterocoagulation of the droplets and the latex is observed. This can be explained in purely electrostatic terms, bearing in mind that the droplet and latex surface charges are largely different in magnitude. It has been established theoretically that the short-range forces between such surfaces are repulsive.[23] It is highly possible that hydration forces[11] are also involved, as, in order for the latex microspheres to adsorb (partially penetrate) inside the oil, a dehydration of the charged surface groups will be required. For highly charged surface groups, this process is energetically unfavorable.

The situation is changed abruptly after glutamic acid is added to the latex. The glutamic acid molecules at neutral pH should carry two negatively charged and one positively charged ionizable group (p*K*'s = 2.19, 4.25, 9.67[16]). Thus it may bind to the positive charges on the surface of the microspheres and neutralize them. In a period of about 1 h the potential of the microspheres goes down to values close to zero. The observed decrease is slow, and the data scattering of the results is rather big, some particles losing charge much more slowly than the others. This is consistent with the observation that some particles penetrate inside the oil, while others stay adsorbed on the interfacial boundary. Notably, unlike the sulfate latex microspheres, the amidine beads adsorb and penetrate inside the oil droplets before their homocoagulation threshold in the surrounding water phase is reached. This may occur due to the opposite charges of the droplets and the microspheres and is investigated in more detail in the second paper of this series. When Tween 20 and glutaraldehyde are added to the system in the next step, the latexes gain some negative potential. The particles should again become slightly hydrophilic, and we have observed them to readsorb from the droplet interior back on the interface. The origin of the thus gained negative potential is still not quite clear, as it may come from partial oxidization of the aldehyde groups of the glutaraldehyde molecules, as well as from OH adsorption on the Tween 20 polyoxyethylene chain[24] or ionic impurities present on either of the substances. It is notable that

(21) Benson, F. R. In *Nonionic Surfactants*; Schick, M. J., Ed.; Marcel Dekker: New York, 1967; p 247.
(22) Matsumura, H.; Furusawa, K. *Adv. Colloid Interface Sci.* **1989**, *30*, 71.
(23) Derjaguin, B. V.; Churaev, N. V.; Muller, V. M. *Surface Forces*; Consultants Bureau: New York, 1987; Section 6.8.
(24) Becher, P.; Schick, M. J. In *Nonionic Surfactants: Physical Chemistry*; Schick, M. J., Ed.; Marcel Dekker: New York, 1987; Chapter 8.

the use of glutaraldehyde for particle cross-linking is the only truly chemical method in the assembly schemes discussed here.

4.3. Possibilities for Further Development. The experimental results described above are of somewhat exploratory character, and they cannot provide all of the basic or technological knowledge for the supraparticle fabrication. The most important possibilities for further development are listed below.

Quantitative Characterization of the Colloid Interactions at the Different Steps of the Assembly Scheme. Our results of the ζ-potential of the particles and droplets are in agreement with the expected electrostatic interactions within the system. For a precise theoretical model of the processes within the system, one also has to obtain further parametric data on the electrostatic interactions and also account for the steric, hydrophobic, and hydration interactions. We intend to address this point in our future investigations.

Optimization of the Assembly Scheme. The assembly schemes presented in Figures 2 and 8 are based on our empirical selection of substances, concentrations, and experimental conditions. As the number of factors that affect the result is quite large, we do not expect that these conditions are optimal or even close to optimal. Both the yield and the quality of the supraparticles could be improved, should the colloid interactions at the different stages of the processes be quantified and optimization experiments with alternative modification agents be performed. In particular, by using alternative emulsification methods, one can decrease the polydispersity of the emulsion droplets and consequently of the obtained supraparticles.

Assembling Particles Other than Latex. This study makes use of latex microspheres as model colloid species. By choosing appropriate phases and modifying substances, it should be possible to apply the assembly method to other types of organic or inorganic particles.

Fabrication of New Types of Supraparticles. In the second paper of this series, we present data on ball-like and composite supraparticles. We believe that this is far from exhausting the number of different types of particles that could be assembled by the emulsion template method.

5. Concluding Remarks

Our study demonstrates the applicability of the emulsion assembly method to the fabrication of ordered colloid aggregates of defined size, shape, and morphology. The investigations are performed with both positively and negatively charged latex microspheres. To achieve the assembly and extraction of the supraparticles, we need to design, control, and modify in a number of steps the colloid interactions within the system ("interaction-tailored colloid assembly"). The modification is done by chosing appropriate substances and environment composition.

Exciting possibilities are opened by the principal ability of the emulsion template scheme to assemble colloid aggregates from multiple particles of divergent types. This issue is addressed in the second paper of this series. Another stimulating possibility for investigation is scaling the process down to nanometer dimensions. In this case the molecular-size colloid components could be gathered and assembled on microemulsion droplets or lipid vesicles.

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Supporting Information Available: Specification of the used latex suspensions; pictures of high-quality ordered shells on octanol droplets; HTAB-induced supraparticle formation and cornlike assemblies; description and pictures of fixing the supraparticles by crosslinking of the adsorbed casein layer; and use of micellar solution of Tween 20 for dissolution of the octanol droplets (7 pages). Ordering information is available on any current masthead page.

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