Letters

Imaging the Volume Transition in Thermosensitive Core−Shell Particles by Cryo-Transmission Electron Microscopy

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Received November 26, 2005. In Final Form: January 31, 2006

We report a study of colloidal thermosensitive core−shell particles by cryo-transmission electron microscopy (cryo-TEM). The particles consist of a solid core of poly(styrene), onto which a network of cross-linked poly(*N*-isopropylacrylamide) (PNIPAM) is affixed. In water, the shell of these particles swells when the temperature is low. Raising the temperature above 32 °C leads to a marked shrinking of the shell. In this letter, we present the first study of these core−shell particles by cryo-TEM in situ, that is, in aqueous solution. We demonstrate that the core−shell particles are well-defined and exhibit a narrow size distribution. In particular, the PNIPAM shell is compact and has a defined outer surface of a slightly irregular shape. The micrographs show that there are density fluctuations within the network. Cryo-TEM of the system above and below the transition temperature furnishes information about the thermosensitive particles that had not been available through other methods employed in previous investigations.

Introduction

Gels composed of cross-linked poly(*N*-isopropylacrylamide) (PNIPAM) chains can undergo a phase transition as a function of temperature in which the network shrinks in a continuous or discontinuous fashion.[1] The volume transition in macroscopic networks has been studied extensively by Tanaka and others.[1,2] A comprehensive review on this work was given by Shibayama.[3] If cross-linked PNIPAM chains are affixed to the surface of polymer latex spheres, then thermosensitive colloidal particles

are obtained. Such systems were prepared some time ago by Makino et al.,[4] by Okubo et al.,[5] and by others.[6−17] Figure 1 displays the volume transition of these particles in a schematic

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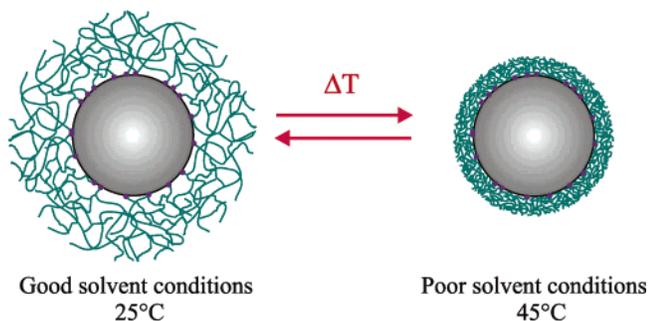(11) Dingenouts, N.; Seelenmeyer, S.; Deike, I.; Rosenfeldt, S.; Ballauff, M.; Lindner, P.; Narayanan, T. *Phys. Chem. Chem. Phys.* **2001**, *3*, 1169.

Figure 1. Schematic representation of the volume transition in core−shell micronetworks. The polymer chains are affixed to the surface of the core, which thus provides one boundary of the network. The solvent, water, is taken up by the network at low temperature but is expelled when the shell undergoes a volume transition at 32 °C. The shrinking is fully reversible upon cooling the suspension to room temperature again.

fashion: Immersed in water, the PNIPAM shell of the particles swells when the temperature is low. However, raising the temperature in the system beyond 32 °C leads to a volume transition in which the network in the shell shrinks by expelling water. The marked reduction of the radius of the particles during this process can be easily monitored by dynamic light scattering (DLS).[6,9,10,12] This method leads to a precise determination of the hydrodynamic radius R_H of the particles that is identical to the outer shear plane of the PNIPAM shell. Given the narrow size distribution of the core particles, R_H yields the thickness of the thermosensitive shell.

Whereas DLS gives only the outer radius of the particles, the internal structure of the network can be studied by small-angle X-ray scattering (SAXS)[12] and by small-angle neutron scattering (SANS).[11] These investigations have demonstrated that the method of preparation[9] leads to a rather compact shell. This was inferred from SAXS and SANS data obtained from the same system.[12] However, no direct analysis could be given because conventional transmission electron microscopy allows studies only in the dry state.

Recently, these thermosensitive core−shell particles have attracted renewed interest as model colloids, in particular, for a comprehensive study of the flow behavior of concentrated suspensions. Thus, Senff et al. presented investigations of the rheology of PNIPAM microgels and of the core−shell particles under consideration here.[18] The basic idea of these studies can be derived directly from Figure 1: the effective volume fraction, ϕ_{eff}, determining the hydrodynamic volume of the particles in solution can easily be adjusted by the temperature. Hence, dense suspensions can be achieved out of a rather dilute state by lowering the temperature. Using thermosensitive core−shell particles, Senff et al.[18] could thus present a comprehensive investigation of the flow behavior of dense suspensions up to effective volume fractions close to random close packing of the spheres. These data in turn have recently been used to check the predictions of mode-coupling theory for the strong shear thinning observed in concentrated colloidal suspensions.[19] All data available so far

point to the fact that thermosensitive core−shell particles present a model colloid that is highly suitable for investigations of the dynamics of concentrated dispersions. (See also refs 20 and 21.)

Here we present the first study of thermosensitive core−shell particles and their volume transition (cf. Figure 1) by cryogenic transmission electron microscopy (cryo-TEM[22−25]). This method is well established for the study of complex fluids[22−25] and biological systems.[26,27] The volume transition is directly made visible at different temperatures, including temperatures below and above room temperature, and can be compared to data obtained by small-angle scattering and dynamic light scattering.[9,11,12]

Experimental Section

Materials. *N*-isopropylacrylamide (NIPAM; Aldrich), *N,N′*-methylenebisacrylamide (BIS; Fluka), sodium dodecyl sulfate (SDS; Fluka), and potassium persulfate (KPS; Fluka) were used as received. Styrene (BASF) was washed with KOH and distilled prior to use. Water was purified using reverse osmosis (MilliRO; Millipore) and ion exchange (MilliQ; Millipore). The core−shell-type PS−PNIPAM particles were synthesized, purified, and characterized as described recently.[9]

Methods. Dilute samples (0.2 wt %) were kept at a given temperature (23 or 45 °C) and vitrified rapidly by the method described in refs 22−25. Specimens were prepared by applying a small (ca. 3 mL) drop on a perforated film supported on a copper TEM copper grid held by tweezers of the controlled environment vitrification system (CEVS). The chamber was kept at 45 °C and was saturated with water vapor to prevent evaporation from the specimens. After blotting the drop into a thin film, the specimen was plunged into liquid ethane at its freezing point. The vitrified specimens were loaded into an Oxford CT-3500 (now Gatan, Pleasanton, CA) cryo-holder and were examined in an FEI (The Netherlands) T12 G^2 dedicated cryogenic-temperature transmission electron microscope. Images were recorded at low electron-dose conditions (to minimize electron-beam radiation-damage) with a Gatan UltraScan 1000 bottom-mounted, high-resolution, cooled CCD camera using the Gatan Digital Micrograph software package.

For the data at room temperature, a few microliters of diluted emulsion were placed on a bare copper TEM grid (Plano, 600 mesh), and the excess liquid was removed with filter paper. This sample was cryo-fixed by rapidly immersing into liquid ethane cooled to −170 to −180 °C in a cryo-box (Carl Zeiss NTS GmbH). The specimen was inserted into a cryo-transfer holder (CT3500, Gatan, Munich, Germany) and transferred to a Zeiss EM922 EFTEM (Zeiss NTS GmbH, Oberkochen, Germany). Examinations were carried out at temperatures around 90 K. The TEM was operated at an acceleration voltage of 200 kV. Zero-loss filtered images were taken under reduced dose conditions (500−2000 e/nm2). All images were recorded digitally by a bottom-mounted CCD camera system (UltraScan 1000, Gatan) and processed with a digital imaging processing system (Digital Micrograph 3.9 for GMS 1.4, Gatan).

Dynamic light scattering (DLS) was done using a Peters ALV 4000 light-scattering goniometer. The solution was diluted to 2.5 × 10^{-3} wt %, and the salt concentrations were set to 10^{-4} and 5 × 10^{-2}

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mol·L^{-1}. The scattering angle mostly was 90°. For several temperatures (see filled points in Figure 1), the scattering angle was varied between 45 and 150° in steps of 15°.

Results and Discussion

The synthesis of the core−shell particles proceeds through a well-defined two-stage process: at first, a core latex consisting of 95% styrene and 5% NIPAM is synthesized by conventional emulsion polymerization.[9] This latex is practically monodisperse and defines the radius of the cores precisely. In a second step that takes place at elevated temperature (80 °C), these core particles are used as seeds for the core−shell particles. NIPAM and BIS are thus polymerized onto these seed particles to form the thermosensitive shell that consists of cross-linked PNIPAM chains. The advantages of this synthesis are that the core and the core−shell particles can be analyzed separately and there exists a well-defined surface between the core particles and the shell. A possible problem, however, might be incomplete binding between the network of the shell and the surface of the core particles. Comprehensive analysis of these particles by SANS and by SAXS has revealed that the particles have a well-defined core−shell structure, as expected from this synthesis.[11,12] In particular, the analysis of the SANS data performed at elevated temperatures (40 °C) demonstrated unambiguously that the shell is rather compact, that is, the outer radius of the particles is well-defined.[11] Moreover, the SAXS data taken at higher scattering angles showed that the network of the shell exhibits the density fluctuations expected for a swollen network.[12] One problem with this analysis was the lack of agreement between the hydrodynamic radius of the particles and the profiles derived from the analysis of SANS and SAXS done at room temperature. A comparison between rheological data taken at much higher concentration, however, revealed that the hydrodynamic radius is a well-defined quantity that governs the flow behavior up to rather high volume fractions.

It should be noted that the core particles have a residual surface charge originating from the first emulsion polymerization.[9] These charges lead to active repulsion at all temperatures. Thus, suspensions of the core−shell particles remain stable even at temperatures above the volume transition. If, however, this electrostatic repulsion is screened by a 50 mM salt solution, then the particles attract each other above the volume transition and coagulate.[28] This reversible coagulation is the subject of a forthcoming investigation. Here, the particles are studied in pure water to avoid possible problems related to aggregation at elevated temperatures.

Figure 2 displays the dependence of the hydrodynamic radius, R_H, as a function of temperature, calculated from DLS data. These data show clearly the marked shrinking of the particles upon temperature rise and corroborate previous results. Also, the shrinking and reswelling upon lowering the temperature are fully reversible because the residual charges on the surface of the core particles prevent flocculation at elevated temperature.

Figure 3a and b displays the micrographs obtained by cryo-TEM at two different temperatures. We first consider the micrographs related to room temperature (Figure 3a). The micrograph shows that the core−shell particles are indeed narrowly distributed. Moreover, the thermosensitive shell is clearly visible in these pictures. Note that no contrasting agent was used to enhance the contrast of any part of the particles. The shell covers the cores in all cases seen here but does not assume an entirely spherical shape. This is accompanied by lighter and denser parts of the network. The density fluctuations within the

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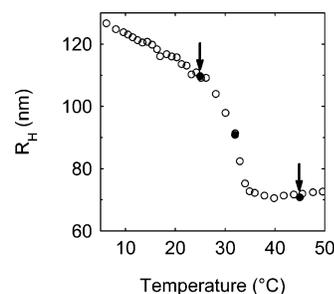
Figure 2. Hydrodynamic radius of the particles, as measured by dynamic light scattering (90° scattering angle) as a function of temperature. Solid circles mark the temperatures where measurements have been done at angles ranging from 45 to 150° in steps of 15°. Arrows indicate the temperature where the cryo-TEM measurements have been made.
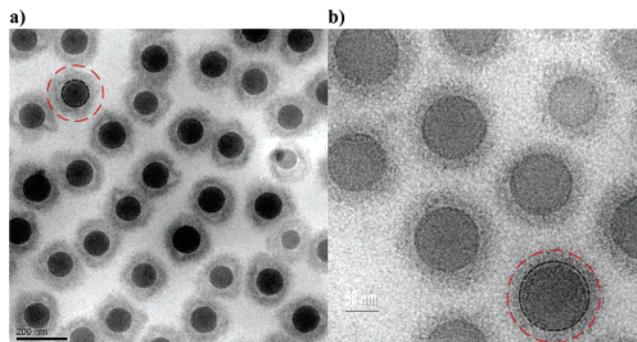
Figure 3. Cryo-TEM micrographs of a 0.2 wt % aqueous suspension of the PS/PNIPAM core−shell particles. The sample was maintained at (a) 23 and (b) 45 °C before vitrification. The core consists of polystyrene and the corona of cross-linked PNIPAM with BIS. The circle around the core marks the core radius determined by dynamic light scattering in solution. The circle around the entire particle gives the hydrodynamic radius R_H of the core−shell particles, again determined by dynamic light scattering taken from Figure 2.

network can be assigned to the additional contribution seen in the SAXS measurements of similar core−shell particles.[12] As argued in refs 9 and 12, the scattering intensity contains a term related to spatial inhomogeneities of the network found for macroscopic networks and predicted by theory. (See the review by Shibayama.[3]) Hence, Figure 3a provides a direct image of an important conclusion drawn from previous SAXS measurements.[12] Moreover, the present micrographs suggest that these fluctuations lead to a slightly irregular shape that may be also embodied in the contribution to the scattering intensity measured at higher scattering angles.

Figure 3a also suggests that the thermosensitive shell is in some cases not fully attached to the cores. We examined ca. 600 particles from various micrographs and found that this is a general phenomenon. It seems that the shell in some case is not fully "stitched" onto the cores. This sheds new light on the second step in the synthesis of the core−shell particles as follows: The analysis of the core particles by SAXS showed that the 5% NIPAM leads to a thin shell of PNIPAM on the cores.[9] In the second step, chain transfer of the growing PNIPAM network to this thin shell leads to a connection between the core and shell. This process is carried out at high temperatures, at which the growing shell collapses onto the core particles.[9] Chain transfer does not lead to complete attachment of the shell to the cores, as seen in Figure 3a, and the strong swelling of the shell at room temperature may lead to a partial detachment of the shells. However, there is close coverage of the cores by the respective shells for most particles seen by cryo-TEM.

The hydrodynamic diameter is shown by a dashed circle in Figure 3a for one core−shell particle. The hydrodynamic radius slightly overestimates the overall size of the particles as seen by cryo-TEM. The average diameter as derived from the analysis of ca. 600 particles amounts to 103 nm whereas DLS gives 109 nm for 23 °C (Figure 2). This difference can be traced back to a few dangling chains of the network, not visible by cryo-TEM or scattering, that stick out and enlarge R_H. These dangling ends of the network are directly visible for some particles in Figure 3a and b. Moreover, R_H measures an average that is also influenced by the irregularity of the shape of the particles. In general, however, R_H seems to provide a good measure of the average radius of the particles.

Figure 3b is an example of the micrographs resulting from the system quenched from 45 °C. Here we chose a higher magnification to display the details of the particles more clearly. Naturally, this experiment is more difficult because vitrification must be much faster then the relaxation time characterizing the shrinking kinetics of the particles. However, Figure 3b in comparison to 3a clearly shows that the particles have shrunken considerably. Moreover, the shell has been compacted by this shrinking process and now provides a tight envelope of the cores. This is to be expected given the fact that the shell has been attached to the core at even higher temperatures.[9] Moreover, the compactness of the shell had already been deduced from SANS measurements.[11] The micrographs, however, demonstrate that the surface of the shells is quite fuzzy. The fuzziness of the outer surface together with the irregular shape found at room temperature may also be responsible for the difference between the hydrodynamic radius R_H and the profiles found in scattering experiments.[12,29,30]

Comparing the cryo-TEM data to data taken from DLS (Figure 2), we find that the overall radius of the particles obvious from these micrographs is in good agreement with the hydrodynamic radius measured at 45 °C (dashed circle). This indicates that the process of quenching the high-temperature structure of the particles is faster than the uptake of water with decreasing temperature. This finding is quite important inasmuch as it shows that the method of preparation does not disturb the structure of the thermosensitive particles.

Conclusions

We presented the first study of thermosensitive latex particles by cryogenic transmission electron microscopy at different temperatures. The micrographs thus obtained show the particles in situ; no staining is necessary to enhance the contrast of core and shell. Cryo-TEM reveals that the shells are rather tightly bound to the core particles and exhibit fluctuations of their density and shape. Moreover, the binding between the thermosensitive shells and the cores is not complete for all particles studied here. This has been traced back to the two-step process used for the synthesis of the particles. Raising the temperature leads to the marked shrinking already measured by dynamic light scattering. Hence, the analysis of the thermosensitive core−shell particles by cryo-TEM demonstrates that the schematic picture shown in Figure 1 is in general correct. However, cryo-TEM elucidates a new feature that is not obvious from previous studies: the thermal fluctuations of the network are followed by a slightly irregular shape. The overall radii of the particles deduced from these micrographs compare favorably with the hydrodynamic radii deduced from dynamic light scattering. All data demonstrate that the thermosensitive core−shell particles present well-defined model colloids.

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