

Letters

Monodisperse Core–Shell Poly(methyl methacrylate) Latex Colloids

Roel P. A. Dullens,^{*,†} Maria Claesson,[†] Didi Derks,[‡] Alfons van Blaaderen,[‡] and
Willem K. Kegel[†]

*Van't Hoff Laboratory for Physical and Colloid Chemistry, Debye Institute, Utrecht University,
Padualaan 8, 3584 CH Utrecht, The Netherlands, and Soft Condensed Matter, Debye Institute,
Utrecht University, Princetonplein 5, 3584 CC, Utrecht, The Netherlands*

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Monodisperse cross-linked composite PMMA latex particles have been developed. The chemical cross-linking of the PMMA facilitates the preparation of particles consisting of a fluorescent core and a large nonfluorescent shell. These core–shell spheres can be dispersed in a density and refractive index matching mixture. This results in an ideal colloidal hard sphere model system that can be used to study many fundamental problems such as freezing, melting, and the glass transition using quantitative confocal scanning laser microscopy. Furthermore, precise control over the size and the properties of the core and the shell(s) facilitates other applications of this model system.

Introduction

Over the past few decades, confocal scanning laser microscopy (CSLM) has been established as a powerful technique in a variety of research fields. In the early 1980s confocal microscopy was introduced in medical and life sciences.^{1–4} More recently, the technique was applied in material research⁵ and colloid science^{6–13} as well. Colloids are particles in the size range of 1–1000 nm and exhibit

similar phase behavior as atomic or molecular materials.^{14,15} Whereas atomic systems are difficult to study in real space and time because of the small sizes and the fast time scales, colloidal systems are, by contrast, very suitable to be studied directly. Using CSLM, the dynamics and structure of colloidal dispersions can be investigated in real space and fundamental issues such as freezing, melting, and the glass transition can be looked at in great detail.^{6–13}

One of the simplest interparticle interactions is the hard sphere potential. Two commonly used colloidal hard sphere model systems are silica spheres in refractive index matched solutions with high ionic strength¹³ and sterically stabilized poly(methyl methacrylate) (PMMA) particles in apolar solvents.^{10,14,16,17} The silica particles developed

* Corresponding author. E-mail: r.p.a.dullens@chem.uu.nl.

[†] Van't Hoff Laboratory for Physical and Colloid Chemistry, Debye Institute, Utrecht University.

[‡] Soft Condensed Matter, Debye Institute, Utrecht University.

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for CSLM consist of a fluorescent core and a nonfluorescent shell. As a result, the (time-dependent) particle coordinates can be obtained with high accuracy in three dimensions (3D) using tracking algorithms similar to those described in ref 18. It is, however, hard to match the high mass density of the silica particles. Thus, effects of gravity in this system are far from negligible. PMMA colloids, on the other hand, have a low mass density so that it is possible to match both the refractive index and the mass density. This allows experiments which are hardly affected by gravity.¹⁰ However, the PMMA particles used so far are completely fluorescent,¹⁷ which seriously complicates the accurate location of the particles in 3D.

In this study we developed monodisperse cross-linked composite PMMA latex particles, e.g., with a fluorescent core and a nonfluorescent shell. These particles facilitate quantitative confocal scanning laser microscopy studies in 3D with negligible effects of gravity.

Experimental Section

Synthesis. By modifying a well-known dispersion polymerization method to synthesize latex particles,^{17,19,20} we obtained fluorescent cross-linked particles in a single step.²¹ (Ethylene glycol) dimethacrylate (EGDM, Merck, 90%) was used as cross-linking agent.¹⁹ These core particles were covalently labeled with the fluorescent monomers NBD-MAEM (4-methylaminoethyl methacrylate-7-nitrobenzo-2-oxa-1,3-diazol) and RAS (RITC-aminostyrene, which were prepared as described in ref 17. It was observed that the NBD-MAEM dye bleached rapidly, whereas the RAS dye was rather stable against photobleaching. We are currently also incorporating other dyes.²² Prior to particle synthesis, the NBD-MAEM was dissolved in acetone. The RAS was dissolved in acetone (Merck, p.a.) and the monomers and then filtered (1 μ m diameter Fluoropore filter). The monomers, methyl methacrylate (MM, BDH, >99%) and methacrylic acid (MA, Fluka, 98%) were distilled under nitrogen atmosphere (MA at reduced pressure as well) prior to use. The particles were sterically stabilized by the graft copolymer poly(12-hydroxystearic acid). For the synthesis we used a 50% solution in a 2:1 (w/w) ethyl acetate/butyl acetate mixture (obtained from ICI); the preparation of PHS is described in, for example, ref 19. This copolymer will be referred to as PHS. Subsequently, the cores were grown larger with PMMA that was cross-linked during growth to obtain core-shell particles.

To synthesize the particles, a standard reflux setup expanded with a dropping funnel with a water jacket was evacuated and brought under a nitrogen atmosphere. The dropping funnel was removed while an overpressure of nitrogen was present. The initiator azo-bis-isobutyronitrile (ADIB, Janssen Chimica, recrystallized from acetone before use) was poured into the reaction flask. To prepare NBD-MAEM labeled particles, the MM and MA were mixed in a separate beaker. A small amount of the MM/MA was added to the reaction flask to dissolve the ADIB while magnetically stirring. To the remaining MM/MA (in the beaker) the PHS solution, hexane (Baker, >95%), Exxsol D 100 (Exxon Chemical Europe Inc., a high boiling hydrocarbon mixture, see Supporting Information), and the NBD-MAEM/acetone mixture were added. After mixing, this was poured into the reaction flask. For the synthesis of RAS labeled particles, the RAS/acetone/MM/MA mixture was poured in a separate beaker. A small amount was added to the reaction flask to dissolve the

Table 1. Details of the Single-Step Fluorescent Cross-Linked Latex (amounts in g)^a

	PNX17	PRX1	PRX3
Reaction Flask			
ADIB	0.102	0.106	0.101
MM	10		
MA	0.202		
NBD-MAEM/acetone mixture ^b	0.72		
RAS/acetone/MM/MA mixture ^c		8.87	8.54
PHS	1.125	0.97	1.05
hexane	8.38	6.13	6.06
Exxsol D 100	4.38	3.11	2.95
octyl mercaptan ^a	0.063	0.055	0.055
Dropping Funnel			
hexane	3.98	3.99	4.0
Exxsol D 100	2.01	2.0	2.0
EGDM ^a	0.138	0.142	0.14
dropping time (min)	23	18	28
R_{SLS} (nm)	175	197	215
σ_{SLS} (%)	12	10	9

^a For the determination of the amount of octyl mercaptan and EGDM, the desired mass was converted in volumina via the densities: $\rho_{\text{octylmercaptan}} = 0.84$ g/mL and $\rho_{\text{EGDM}} = 1.05$ g/mL. ^b The concentration of the NBD-MAEM in acetone: 11.5 mg/mL. ^c The weight ratio in the RAS mixture: RAS/acetone/MM/MA = 0.20/3.77/94.15/1.88.

Table 2. Details of Seeded Growth Steps (amounts in g)^a

	17P#1	17P#2	1P#1	1P#2	3P#1	3P#2
Reaction Flask						
ADIB	0.102	0.10	0.11	0.106	0.102	0.404
cores	5.1	4.54	5.1	4.66	5.14	18.6
Exxsol D 100		0.48		0.37		1.51
octyl mercaptan ^a	0.065	0.065	0.061	0.061	0.063	0.252
Dropping Funnel						
MM	10.0	10.0	10.03	9.98	10.05	40
MA	0.20	0.211	0.207	0.21	0.204	0.84
PHS	2.75	2.74	2.75	2.78	2.79	11.1
hexane	7.02	7.08	7.01	7.09	7.4	23
Exxsol D 100	3.58	3.48	3.48	3.49	3.5	19.05
EGDM ^a	0.103	0.095	0.104	0.108	0.1	0.4
R_{SLS} (nm)	315	555	375	660	405	635
σ_{SLS} (%)	7	6	7	6	7	6

^a The coding of the sample numbers is explained in the text. For the determination of the amount of octylmercaptan and EGDM the desired mass was converted in volumina via the densities: $\rho_{\text{octylmercaptan}} = 0.84$ g/mL and $\rho_{\text{EGDM}} = 1.05$ g/mL.

ADIB while magnetically stirring. To the remaining RAS/acetone/MM/MA mixture (beaker) the PHS solution, hexane, and Exxsol D 100 were added. After mixing, this was poured into the reaction flask as well. Finally the chain-transfer agent octyl mercaptan (Fluka) was added directly in the reaction flask. The dropping funnel was replaced on the flask and was filled with a mixture of hexane, Exxsol D 100, and EGDM that was mixed in a second beaker. The contents of the round-bottom flask were subsequently heated to 80 °C using an oil bath. A few minutes after the reaction had started, the reaction mixture became turbid, indicating nucleation. The addition of the mixture in the dropping funnel was started at the moment that nucleation was observed. After complete addition, the reaction mixture was refluxed for 1 h. Next, the oil bath was removed and the contents of the flask were cooled to room temperature. The seeded growth steps were done as described in ref 17, except that EGDM was added to the feeding mixture. The amounts of chemicals and the dropping times are summarized in Table 1 (core-synthesis) and in Table 2 (seeded growth steps). All the chemicals were weighed unless stated otherwise. The samples are coded as follows: 17P#1 corresponds to the seeded growth of the PNX17 cores and 17#2 corresponds to the seeded growth of the 17P#1 particles, thus the second growth step of PNX17 cores.

Characterization. To determine the size and polydispersity of the particles, angle-resolved static light scattering (SLS) measurements on highly diluted dispersions were obtained using

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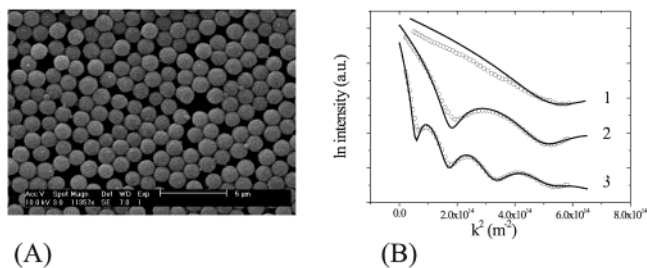


Figure 1. (A) SEM image of RAS labeled core-shell PMMA particles (3P#2). (B) The SLS curves (circles) and the theoretical fits (lines) of NBD-MAEM labeled core-shell particles: (1) the cores (PNX17), (2) the particles after first seeded growth step (17P#1), and (3) the final particles (17P#2).

a homemade light scattering apparatus. The wavelength λ_0 of the incident light was 632.8 nm. Prior to the measurements the dispersions were filtered through Mitex filters. The used solvent was hexane, and the background was not subtracted from the data. A calculated form-factor,²³ fitted to the experimental scattering data, provided the radius (R_{SLS}) and the polydispersity (σ_{SLS}). Polydispersities inferred from SLS are in general an upper limit.¹⁷

Scanning electron microscopy (SEM) was performed on a Philips XLFEG30 microscope to obtain information about the shape and the surface roughness of the particles. The samples were prepared by dipping formfar-coated grids into dilute dispersions and allow the solvent to evaporate. Subsequently, the grids were coated with a 5 nm thick layer of platinum/palladium to minimize particle damaging such as melting and shrinking due to the electron beam. For CSLM, the particles were dried on a microscope slide. To suppress the scattering during imaging, immersion oil was added to the dried particles. The particles were imaged using a Leica DM IRB confocal scanning laser microscope in combination with an argon laser ($\lambda_0 = 488$ nm), a Leica TCS NT scanhead, and an oil-immersion lens: Leica 100 \times , NA 1.4 was used in fluorescence mode. UV-vis absorption spectra were measured using a Cary 1E spectrophotometer.

Results and Discussion

Figure 1A shows a SEM micrograph of RAS-labeled cross-linked core-shell particles (3P#2), and Figure 1B shows the SLS curves of NBD-MAEM labeled particles (17P#2) during the subsequent stages of the preparation. The radii and polydispersities are summarized in Table 1 and Table 2. The SEM image and the SLS data show that the particles are monodisperse and spherical.

To avoid migration of dyed material throughout the particle,¹⁷ cross-linking of the PMMA was essential. The cross-linking suppresses but does not completely prevent the migration of non-cross-linked polymers.^{24,25} It was observed that especially the NBD-MAEM migrated, whereas the mobility of RAS was negligible. However, non-cross-linked PMMA dissolves in good solvents, e.g., tetrahydrofuran (THF), whereas cross-linked PMMA does not. Therefore, we succeeded in removing the migrating non-cross-linked fluorescent polymers by centrifugation after dissolving them. As a result, particles consisting of immobile cross-linked PMMA chains only were obtained. Absorption spectra of the supernatants obtained during the centrifugation confirmed the removal of the non-cross-linked fluorescent PMMA, as shown in Figure 2. The peak in the spectra corresponds to the excitation wavelength of the fluorescent dye NBD-MAEM. It is observed that

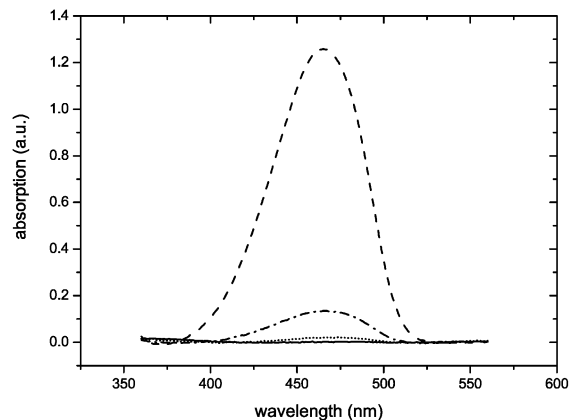


Figure 2. Absorption spectra of the supernatants obtained during the transfer of the NBD-labeled particles from hexane to tetrahydrofuran: first (dash-dash), second (dash-dot-dash), third (dot-dot), and fourth supernatant (solid).

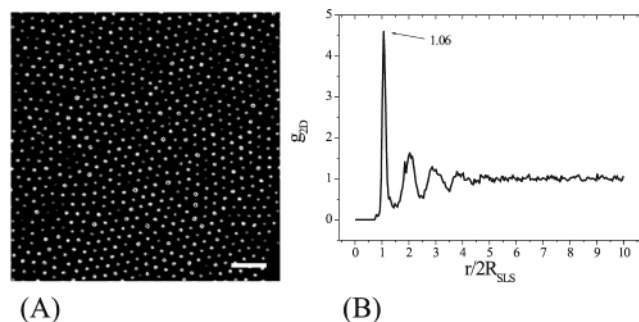


Figure 3. (A) CSLM image of RAS core-shell particles dried on a microscope slide (1P#2). The bar corresponds to 5 μ m. (B) The corresponding 2D radial distribution function g_{2D} as a function of the distance $r/2R_{SLS}$.

the absorption strongly decreases when refreshing the solvent. When the particles are sedimented four times, the absorption is virtually zero, indicating that all the non-cross-linked polymer chains are removed from the particle. Higher cross-link densities might avoid the migration completely. However, aggregation was observed for cross-link densities typically larger than 1.5% (w/w).

After readsorption of the stabilizer, which was removed by the good solvent, the particles could be redispersed in apolar solvents (see Supporting Information). In some solvents, PMMA particles are highly charged and the charge distribution can be anisotropic.⁶ However, in a mixture of tetralin, *cis*-decalin, and carbon tetrachloride, which matches the mass density as well as the refractive index, PMMA particles behave as hard spheres.^{10,17} Thus, fluorescently labeled cross-linked core-shell particles in this mixture are ideal colloidal model hard spheres for quantitative confocal microscopy. In Figure 3 a CSLM micrograph of RAS-labeled particles and the corresponding 2D radial distribution function, g_{2D} , as a function of the distance $r/2R_{SLS}$ are shown. Before imaging, the particles were dried on a glass slide; during the drying process the particles are driven together due to the capillary forces. The position of the first peak of the g_{2D} is in good agreement with the diameter obtained by static light scattering (SLS), confirming that the particles are in contact. The fluorescent cores are clearly separated by the nonfluorescent shells, as observed by CSLM (Figure 3A), showing that the particles are of core-shell morphology.

The properties of the core and each seeded growth layer, i.e., size (core), thickness (growth layer), cross-link density, and fluorescent dye can be tuned independently. This

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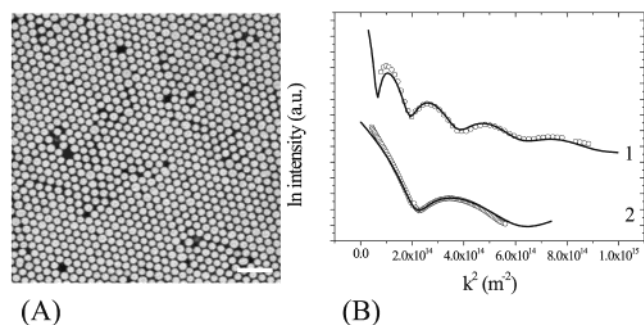


Figure 4. (A) CSLM image of swollen NBD-MAEM labeled cross-linked particles in THF. The bar corresponds to 5 μm . (B) The SLS curves (circles) of cross-linked PMMA particles in respectively (1) hexane ($R_{\text{SLS}} = 282$ nm) and (2) in tetrahydrofuran ($R_{\text{SLS}} = 550$ nm). The solid lines represent the theoretical fits.

facilitates further applications of this model system. First, the cross-linked particles can be dispersed in solvents in which the particles swell, whereas non-cross-linked particles dissolve therein. For example, in THF, the cross-linked PMMA particles can swell up to a factor of 7.4 in volume,²¹ as observed in Figure 4, which shows a CSLM image of cross-linked PMMA particles in THF and the SLS curves in hexane and THF. Moreover, since different solvents may cause different particle interactions,²⁶ the strength and range of the interactions can be tuned. For instance, the particles have a soft potential in THF.²¹ Third, particles with different morphologies can be prepared. For example, we prepared a PMMA particle consisting of a non-cross-linked core that was labeled with NBD-MAEM, a thin shell of nonlabeled, cross-linked PMMA, and finally a thick shell of non-cross-linked, nonlabeled PMMA. Moreover, the control over the particle morphology enables systematical variation of the glass temperature, T_g , throughout the particle, since the T_g is different for cross-linked and non-cross-linked PMMA.^{24,27}

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All these different particles may be used in fundamental research as well as in coating industries.

In conclusion, we have developed monodisperse spherical core-shell PMMA colloids. Cross-linking of the PMMA allows preparation of particles with a fluorescent core and a large nonfluorescent shell. These particles can be dispersed in a density and refractive index matching mixture, in which the colloids interact like hard spheres. This is an ideal hard sphere model system to be studied with quantitative confocal scanning laser microscopy, since particle positions can be resolved in three dimensions with high accuracy. Furthermore, cross-linked particles can be dispersed in solvents in which the particles swell, enabling control of interparticle interactions. Also, the size and the properties of the core and the shell as well as the number of shells can be tuned. Our current approach opens up the possibility of preparing hollow cross-linked particles²⁸ and PMMA particles doped with quantum dots.²⁹ We are currently working on the development of these systems.

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Supporting Information Available: Description of the procedure to readsorb the PHS on the particles after the removal of the migrating polymers and information about Exxsol D 100. This material is available free of charge via the Internet at <http://www.acs.org>.

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