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Synthesis of fluorescent silica-coated gibbsite platelets

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Abstract

We describe the fluorescent labeling of gibbsite particles. Gibbsite particles are first stabilized with polyvinyl pyrrolidone. Subsequently the particles are covered with a silica layer in which a fluorescent dye is incorporated. Both fluorescein and rhodamine dyes have been used. The fluorescent labeling is applicable to gibbsite particles of various sizes. Particles are transferred to dimethyl formamide by vacuum distillation after dialysis. These particles are used for confocal scanning laser microscopy and confocal fluorescence-recovery after photobleaching.

Keywords: Colloidal gibbsite; Fluorescence; Silica coating

1. Introduction

Minerals in soils are often plate-like particles. Gibbsite (γ-Al(OH)$_3$) is one of those minerals that can be found in soils and may have different shapes and sizes ranging from 100 nm to 100 μm [1–3]. Colloidal gibbsite can for instance be prepared by heating an acidified aluminum alkoxide solution at 85 °C for three days [4]. The resulting hexagonal platelets vary in size from 150–200 nm with a polydispersity of 25–30% and can be used as seeds for further growth. The polydispersity of the seeds is lowered to about 20% by centrifugation [5]. Because of its well-defined shape and the relatively low polydispersity, colloidal gibbsite is used as a model system to study phase behavior [6,7] and electrostatic properties of minerals present in soils [8,9]. To be able to study particles in real space by means of confocal scanning laser microscopy (CSLM) [10,11], fluorescently labeled gibbsite particles are necessary. Here we describe a way to synthesize such particles.

Gibbsite is a crystalline material. This makes it difficult to incorporate dye into its dense crystal structure. Silica may be used to facilitate a dye [12–15]. Gibbsite particles can be coated with silica by two methods [16,17]. Both have in common that an intermediate stabilization step is needed before silica growth. We choose polyvinyl pyrrolidone (PVP) as stabilizer because the method is quick and reliable [18]. PVP-coated particles can be transferred to ethanol without loss of stability. By dispersing the particles in a Stöber mixture of water, ethanol, and ammonia [19], addition of tetraethoxysilane (TEOS) grows a layer of silica on the surface of the PVP-coated gibbsite particles. Subsequently fluorescein isothiocyanate (FITC) or rhodamine B isothiocyanate (RITC) is covalently bound to the amine group of 3-(aminopropyl)triethoxysilane (APS) and incorporated inside silica [12,13]. The silica layer with incorporated dye is grown directly onto the PVP-coated particle surface.

As a solvent we choose dimethyl formamide (DMF). Since it closely matches the refractive index of silica, van der Waals interactions between particles can be assumed negligible. Another advantage is that the optical matching makes the particles more suitable for confocal microscopy. Therefore we show how the particles are transferred to DMF without aggregation, a common problem when changing the solvent. The method turns out to be applicable to gibbsite platelets of different sizes. Finally, applications of these particles are shown.

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2. Experimental methods

PVP (40.000, Sigma) was dissolved in demineralized water (typically 100 g/L) by stirring. To this solution a few milliliters of a gibbsite dispersion were added, containing 2 g of gibbsite, and stirred for 24 h to let PVP cover the particle surface. The resulting solution was centrifuged for 24 h at 500g to remove excess PVP, which remains in the supernatant. The sediment was easily redispersed in ethanol [18].

After this treatment, gibbsite platelets of 200 nm diameter (G200) were covered with a layer of fluorescent silica. RITC (for fluorescence, Fluka) was coupled to APS (purum >98%, Fluka) by mixing 0.1102 g (0.2056 mmol) of RITC with 0.5326 g APS and 1.9840 g anhydrous ethanol in a dried vial. This mixture was left stirring for two days under nitrogen atmosphere. A Stöber mixture was made of 1.2 g/L gibbsite in ethanol with an ammonia (25% p.a., Merck) concentration of 6.7% (v/v). While vigorously stirring, 0.05 ml of APS-RITC was added under the surface of the Stöber mixture, followed by 0.75 ml (3.87 mmol) TEOS (purum >98%, Fluka). After 6 h another portion of 0.35 ml (1.81 mmol) TEOS was added.

The reaction is complete after 16 h. In order to remove unreacted APS and dye, the mixture was transferred to polyvinylidene difluoride (PVDF) dialysis tubes (Spectra/Por, MWCO 250 kDa) to dialyze against ethanol. The dialysis medium was refreshed twice a day. The tubes were turned a few times to prevent sedimentation of the particles. After two days the dispersion was transferred to a round-bottomed flask. Carefully the dispersion was diluted with one fourth of its original volume using DMF (99%, Acros). Subsequently the ethanol was distilled off under reduced pressure. Small aggregates, formed during this procedure, were removed by short centrifugation of the dispersion, 15 min at 300g. The aggregates which sediment to the bottom were discarded. These particles were coded G200R.

This method is also applicable to grown sizes of PVP-coated gibbsite platelets (G600). The concentration of added TEOS and APS-dye is adjusted slightly taking into account the total surface area of the gibbsite platelets. The ammonia concentration remained the same at 6.7% (v/v). Small amounts of second nucleation can be removed by short centrifugation. The small silica particles remained in the supernatant while the large platelets were sedimented. Both fluorescein and rhodamine dyes are used. APS-RITC was synthesized as described above and a detailed procedure for the synthesis of APS-FITC can be found in Ref. [11]. The 600 nm RITC labeled gibbsite platelets were coded G600R.

Particles were characterized with a Tecnai 12 (FEI Company) transmission electron microscope (TEM) operated at 120 kV. Samples were prepared by drying 4 µl of diluted dispersion on the carbon-sputtered, polymer-coated side of an electron microscope grid. The thickness of the silica layer is measured by use of AnalySIS Pro soft imaging system.

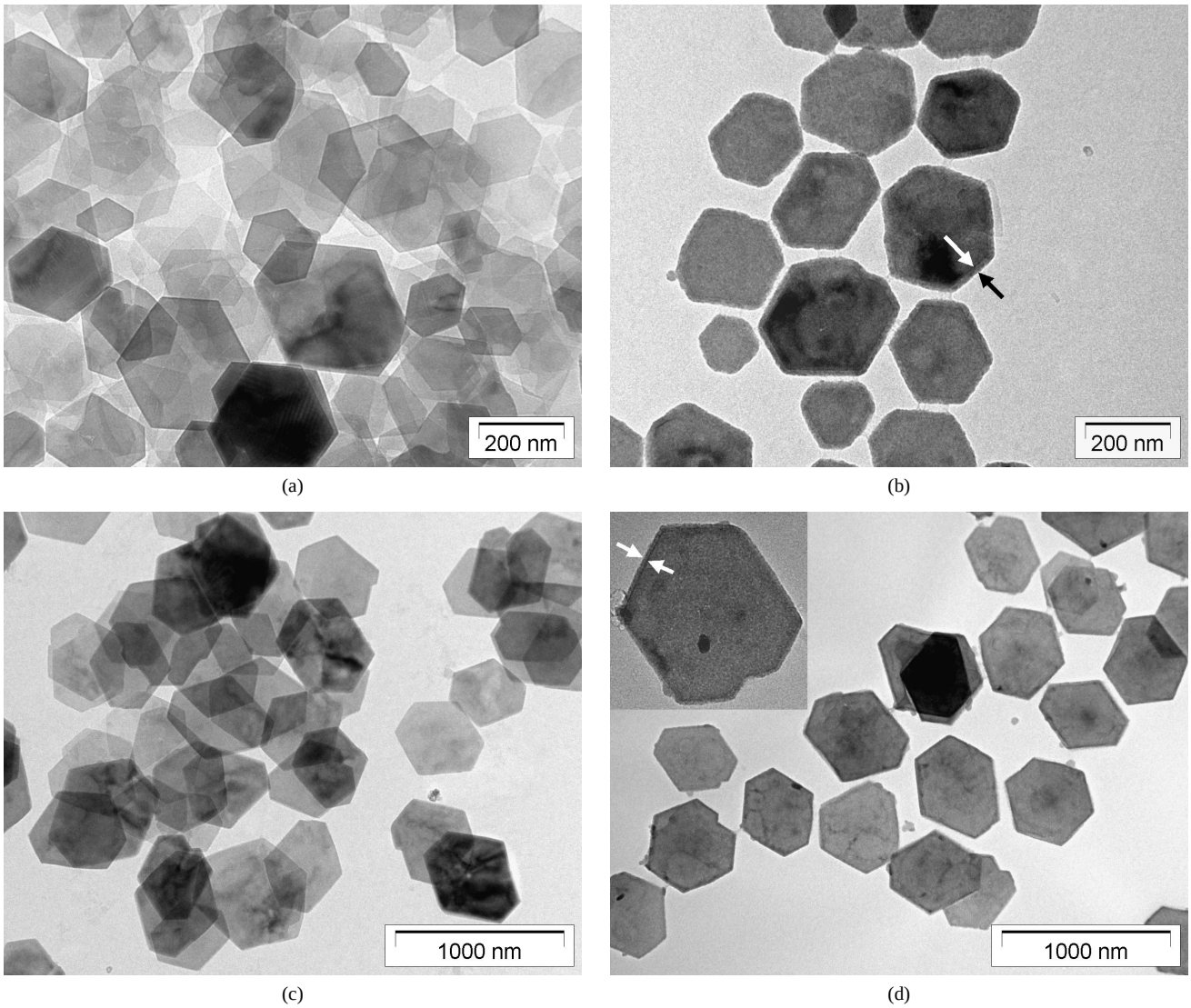
A Nikon Eclipse TE200-U inverted microscope equipped with a Nikon D-Eclipse C1 scanhead and a Plan APO 100× oil objective with a numerical aperture of 1.40 has been used for confocal imaging and confocal fluorescence-recovery after photobleaching (C-FRAP). Samples were prepared in a small vial from which the bottom was replaced by a microscope slip of 0.11 mm thickness.

3. Results and discussion

The PVP-coated gibbsite particles remained stable when transferred to the Stöber mixture. When the APS-dye and the TEOS were added, the reaction mixture became colored and after 30 min more turbid due to the formation of silica. At that moment the stirring rate was decreased for 4 h. By adding a new portion of TEOS, a second silica layer without dye is formed around the fluorescent shell. It can be shown that free, unbound dye molecules are not incorporated in this second silica layer [12,13]. The non-fluorescent layer is applied to exclude APS molecules from the particle surface. The latter would decrease the stabilizing negative surface charge of the silica layer by its basic amino groups, resulting in unstable particles [12,20]. The resulting mixture was stable but contained a few aggregates that were removed by short centrifugation as before.

The silica-coated fluorescent gibbsite platelets are first transferred to DMF by repeated centrifugation and redispersion. This resulted in irreversible aggregation of the particles, despite slow centrifugation. In order to prevent aggregation of the particles and destruction of the fluorescent dye, due to the high boiling point of DMF (153 °C), the platelets are transferred by use of distillation under reduced pressure to lower the boiling point of the solvents. Before that the dispersion is dialyzed against ethanol to remove free APS and dye. These molecules can react with the particle surface due to the slightly elevated temperature during distillation resulting in destabilized particles [16,20]. After two days of dialysis the ethanol is only slightly colored. There is no sign of instability of the particles currently dispersed in ethanol. This dispersion is mixed with DMF and the silica-coated gibbsite particles remain stable when DMF is slowly added. The refractive index of the suspension (1.4296 ± 0.00014) after distillation closely matched the refractive index of the pure DMF (1.4302 ± 0.00007).

In Fig. 1, TEM images of uncoated (G200, a) and coated (G200R, b) gibbsite platelets are shown. The 15.0 nm thick silica layer can be seen between the arrows. This silica layer consists of a fluorescent part directly onto the gibbsite surface and a non-fluorescent part on top of this fluorescent layer. Previous work on coating gibbsite with silica identified the resulting layer as silica by use of energy dispersive X-ray analysis (EDX) [18]. In the TEM image these layers cannot be seen separately. Figs. 1c and 1d show the large,



(a) (b) (c) (d)

Fig. 1. TEM images. (a) Uncoated gibbsite particles G200, $\langle D\rangle = 179 \pm 41$ nm. (b) Silica-coated fluorescent gibbsite G200R after transfer to DMF. The silica layer, 15.0 ± 1.6 nm thick, indicated between the arrows. (c) Uncoated gibbsite platelets G600, $\langle D\rangle = 574 \pm 47$ nm. (d) Silica-coated fluorescent gibbsite G600R after transfer to DMF, silica layer thickness 10.3 ± 1.5 nm. Inset: enlargement of a coated G600R platelet. The silica layer is indicated between the white arrows.

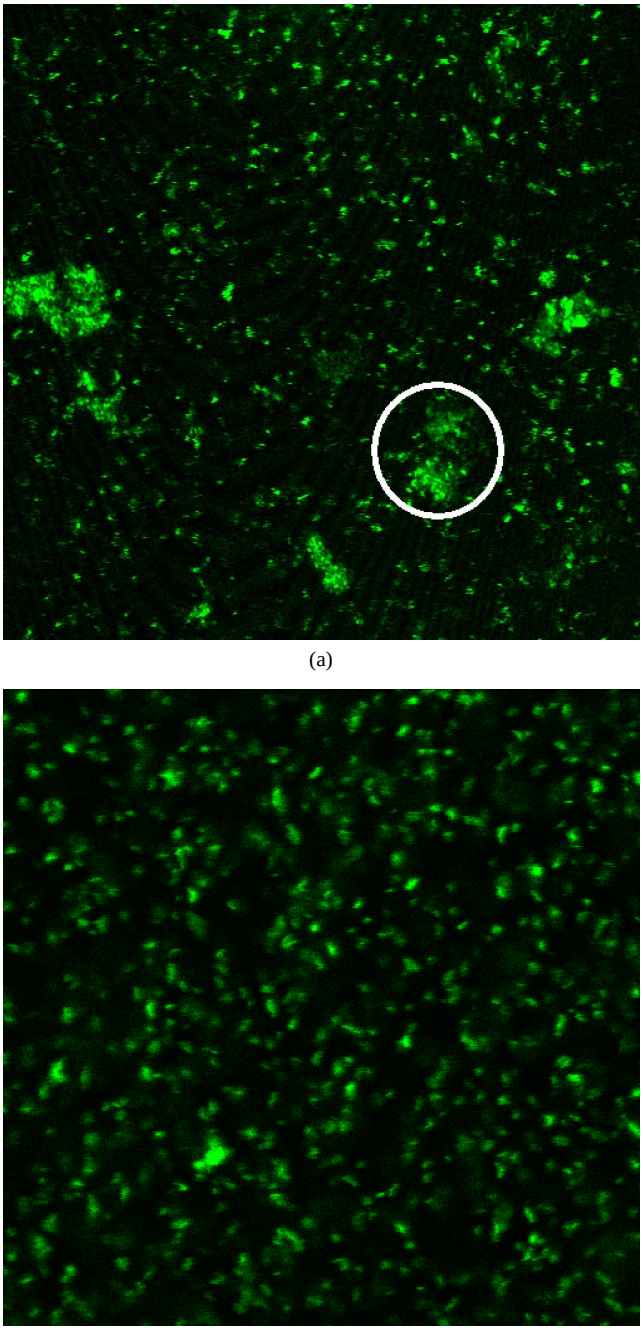
uncoated G600 gibbsite platelets and the coated G600R particles, respectively. Here, the silica layer is 10.3 nm thick. At first the coating of these grown gibbsite platelets accompanied a second nucleation of spherical silica particles. This is decreased by adjusting the amount of reactants to the surface area of the particles, keeping the ammonia concentration at 6.7% (v/v). The little amount of second nucleation can be removed by centrifugation as described before. The thickness of the silica layer can be varied by the amount of TEOS.

In Fig. 2, CSLM images of gibbsite platelets are shown. The size of the gibbsite platelets G200R is in the same order as the optical resolution of the used CSLM. Hence, the platelets can only be seen as small dots. The platelet-like shape can be observed when the G600R platelets are imaged. Large platelets have shown phase separation in which among others a columnar phase appeared [5]. These large fluorescent platelets open ways to observe different phases directly by use of CSLM.

Collective motion of the platelets may be observed, for instance, by C-FRAP. By bleaching a well-defined pattern, the recovery speed of the fluorescent intensity in that region is a measure for the collective motion of the platelets [21]. A rough estimate for the diffusion coefficient, using randomly oriented infinitely thin plates, yields [22]

$$D_0 = \frac{k_B T}{6\eta D} \approx 1.0 \times 10^{-12}\ \mathrm{m}^2/\mathrm{s}.$$

Here $k_B T$ is the thermal energy, η the solvent's viscosity and D the (average) diameter of a platelet. An example of C-FRAP is given in Fig. 3 where a square has been bleached in a sediment of rhodamine-labeled platelets. The decay of the (normalized) intensity profile provided us with a dif-



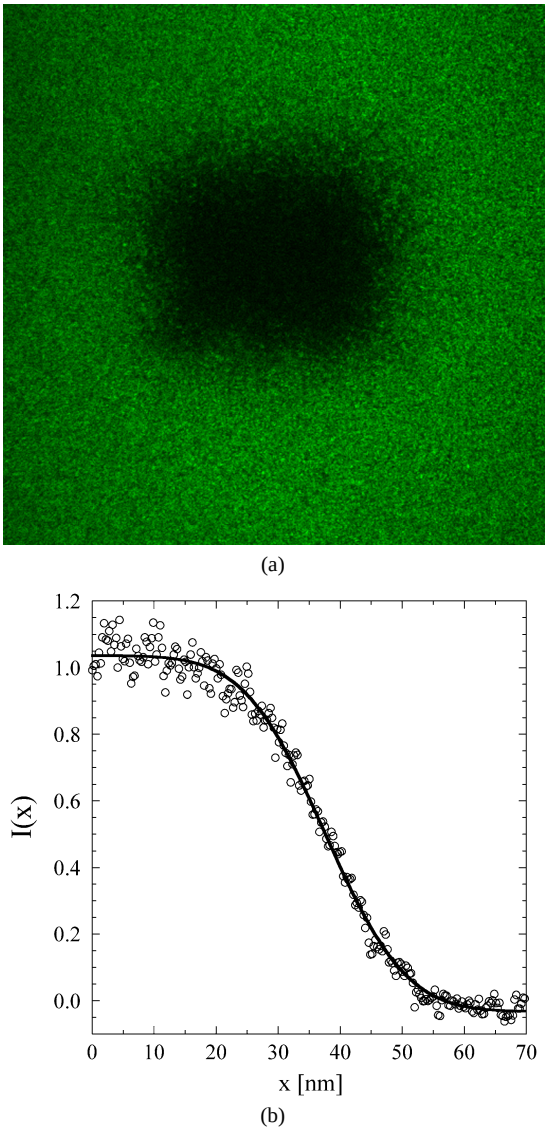
(a)

(b)

Fig. 2. CSLM images in a frame of 50 × 50 μm of (a) G200R, where some aggregated particles are present, like in the white rectangle, (b) G600R, individual particles are visible.

fusion coefficient of $D_0 = 0.5 \times 10^{-12}$ m^2/s. Since the density is much higher than for randomly oriented plates, this seems a reasonable value. Moreover, it relates well to data previously obtained by dynamic light scattering (DLS, $D_0 = 5 \times 10^{-12}$ m^2/s) [22] and from theory [23]. Note that C-FRAP allows us to access much longer time-scales than with, e.g., DLS.

When the G200R rhodamine-labeled gibbsite particles are being mixed with fluorescein-labeled spheres, using the



(a)

(b)

Fig. 3. (a) Bleached square (40 × 40 μm) in a sediment of rhodamine-labeled platelets ($\langle D \rangle = 574$ nm) in DMF. The total frame is 140 × 140 μm. (b) The normalized intensity profile along the edges of the square. The points are the data, the solid line the theoretical fit [21].

CSLM each species can be made visible individually. The position of the platelets during depletion-induced phase separation can now be visualized [24]. In Fig. 4, a CSLM image of such a phase-separating mixture is shown. The 700 nm (green) spheres can be seen separately. Microphases of (red) platelets are present in-between the spheres. A TEM image of a mixture is also shown to illustrate the size difference between the spheres and the platelets.

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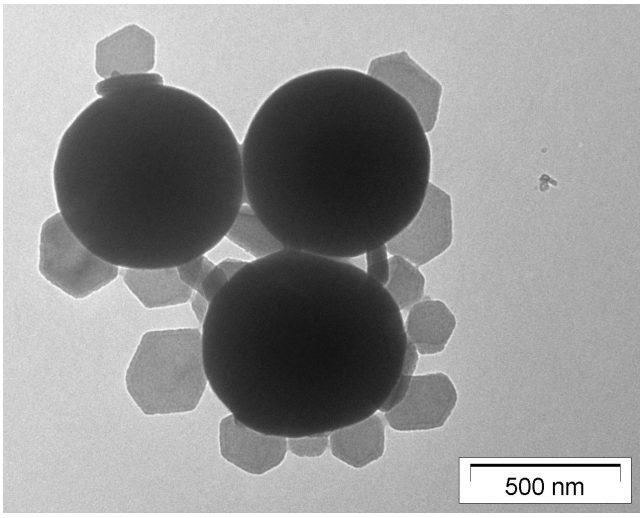
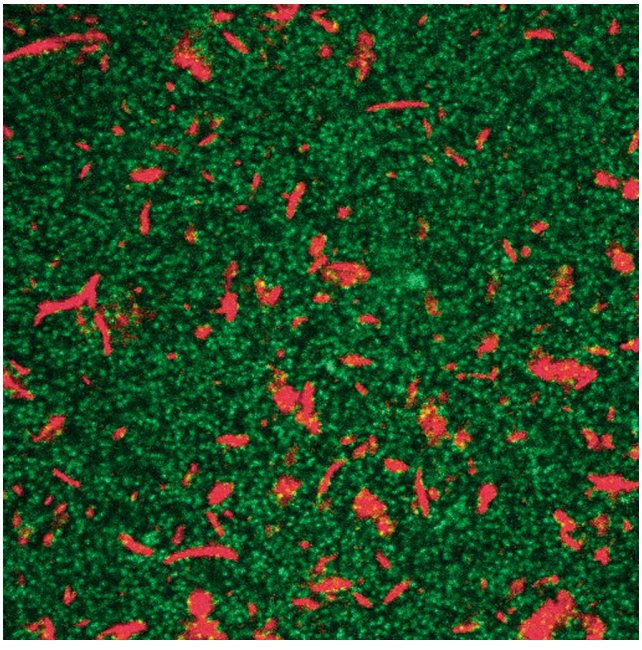


(a)



(b)

Fig. 4. (a) TEM image of a mixture of 700 nm FITC labeled spheres with RITC labeled gibbsite platelets G200R. (b) CSLM image of the same mixture, at a depth of 20 µm in the sample, frame 100 × 100 µm. FITC emission is imaged green and RITC emission is imaged red.

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