Unraveling Complexity: A Strategy for the Characterization of Anisotropic Core Multishell Nanoparticles

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In this work, a widely applicable routine to characterize the core, surface, stability, and optical properties of CdSe/CdS/ZnS core–shell–shell nanorods after multiple growth steps is established. First, size, shape, and shell thickness of the nanorods are characterized by transmission electron microscopy (TEM), analytical ultracentrifugation (AUC), and small angle X-ray/neutron scattering (SAXS/SANS). In the next step, Fourier-transform infrared (FT-IR) spectroscopy, thermogravimetric analysis (TGA), and SANS measurements are applied to determine the surface species of nanorods. Then, the colloidal stability of the nanorods is investigated by UV–vis spectroscopy and dynamic light scattering (DLS) after different washing cycles. Finally, photoluminescence quantum yield (PLQY) of the nanorods during washing and sample storage is determined. With this highly complementary routine for particle characterization, the core, surface, stability, and optical properties of nanorods after multiple growth steps are resolved. The results demonstrate the importance of the developed toolbox to characterize such highly complex, anisotropic nanorods for a technical environment. This is of major importance for the handling of colloidal quantum materials and their quality control in industrial applications.

1. Introduction

Colloidal nanoparticles (NPs) have been widely applied in various fields like photocatalysis, bioimaging, energy conversion, and displays. [1–5] Various methods have been proposed to fabricate a variety of nanostructures with tailored size and shape, e.g., semiconductor, silver, and gold NPs. [6–14]

Due to their unique optoelectronic product properties, quantum confined colloidal semiconductor core–(multi)shell nanorods are one important class of nanostructures. However, unsaturated surface atoms with dangling bonds often lead to trap states within the band gap, which usually have a negative effect on the optical properties of the NPs. To overcome this limitation, shell(s) are grown around the NPs to obtain desired end-use properties, e.g., high and stable photoluminescence quantum yield (PLQY). [15–22]
Moreover, thin films made of anisotropic particles, e.g., rods or wires, are beneficial for end-use applications like optoelectronics due to the higher contact area when compared with spherical particles.[1,2,23–26] Usually, colloidal core–(multi)shell nanorods are synthesized via multiple, consecutive growth steps. Consequently, in this process a comparatively complex reaction chemistry is needed to control the anisotropic growth. This is based on the varying affinity of distinct growth directing molecules to different materials or facets.[27,28] Therefore, both the core and the surface of NPs need to be characterized. For spherical particles, various methods have been applied to characterize their core and surface. Regarding the NP core, dynamic light scattering (DLS)[29] and small angle X-ray scattering (SAXS)[30] were used to measure the particle size distribution (PSD) in liquid state based on the scattering of the particles. Moreover, analytical ultracentrifugation (AUC) is an appealing alternative technique for measuring the PSD of well-dispersed colloidal NPs with Ångström resolution.[31,32] Ultraviolet–visible (UV–vis) spectroscopy is also promising as it is a fast and easy method to obtain the PSD of semiconductor NPs in liquid state based on their size-dependent absorbance.[33] In addition, scanning electron microscopy (SEM) and/or transmission electron microscopy (TEM) are very popular to obtain the PSD of NPs after drying on a suitable substrate or support by visualization.[34] Regarding the surface of NPs, it is certainly of major importance for processability and end-product quality due to the high surface-to-volume ratio of all kinds of NPs but in particular in case of quantum materials.[35] For example, it was found that the purity of trioctylphosphine oxide (TOPO), which is a common surface ligand of such quantum materials, has an influence on the molecular composition at the surface and hence the stability of PLQY of the NPs.[36,37] There are some investigations using Fourier-transform infrared (FT-IR) spectroscopy and X-ray photoelectron spectroscopy (XPS) to identify different types of the surface molecules.[38–42] However, in most of these studies quantitative results rely on assumptions or constrains that may fail for anisotropic particles. Nuclear magnetic resonance spectroscopy (NMR) was applied to access both qualitative and quantitative information of the surface of NPs.[43–47] However, small angle neutron scattering (SANS) and NMR measurements need to be performed in deuterated solvents, which are different from the original dispersing liquids and often require a solvent exchange, e.g., by introducing an additional washing step. Noteworthy, in our previous work, a widely applicable analytical strategy with a combination of complementary methods for surface characterization was proposed.[48] Knowing the advantages of nanorods and the importance of the core and surface characterization, in this work our previously developed analytical strategy for surface characterization was adopted and further developed to characterize core, surface, stability, and optical properties of highly complex anisotropic nanorods after multiple growth steps. As shown in Scheme 1A, CdSe/CdS/ZnS core–shell–shell nanorods (referred to as green rods in the following) were synthesized by three consecutive growth steps (quasi-spherical CdSe seeds, rod-like CdS as first shell, rod-like ZnS as second shell) and can be used as model system for anisotropic quantum materials. During these three growth steps, different molecules, namely trioctylphosphine (TOP), TOPO, octadecylphosphonic acid (ODPA), hexylphosphonic acid (HPA), and oleic acid, were used to control the size and shape of the particles. As shown in Scheme 1B, the challenge was to establish a widely applicable characterization routine to not only access the size and shape of the green rods, but also the surface molecular species which can be manifold due to the complex synthesis chemistry. First, the size and shape of the green rods were obtained from TEM, AUC, and SAXS measurements. Second, FT-IR and thermogravimetric analysis (TGA) were combined to determine the surface species of the green rods. The results were in good agreement with the assumptions made by the authors. The scheme shows the proposed widely applicable routine to characterize the nanorods after multiple growth steps.

Scheme 1. A) Scheme of the synthesis of CdSe/CdS/ZnS core–shell–shell nanorods (green rods). B) The proposed widely applicable routine to characterize the nanorods after multiple growth steps.
agreement with SANS measurements. Then, the colloidal stability of the green rods during washing was investigated by UV–vis spectroscopy and DLS. Finally, the PLQY was investigated during washing and sample storage. By putting our findings together, both core and surface, stability, and PLQY of core–shell–shell nanorods after multiple growth steps were resolved. We believe that this concept is an important step toward knowledge-based formulation of highly complex anisotropic nanorods and quality control on technical scale.

2. Results and Discussion

2.1. Size and Shape of the Green Rods

As mentioned in the introduction, various fast analytical techniques can be applied to measure the size of spherical NPs. Yet, size and shape determination for nanorods from one technique is not easy and straightforward. As one recent example, scanning mobility particle sizing (SMPS) in combination with AUC was able to determine the size and shape of ZnO nanorods. However, this methodology was not applicable in the current case due to the lab restrictions for heavy metal materials. AUC is able to deal with anisotropic structures with better statistics as it can be used for multidimensional particle characterization. However, the density of the nanorods investigated in this study is not known due to their highly complex core–shell–shell structure, which impedes the application of AUC alone. Hence, TEM was applied to measure size and shape. However, as high spatial resolution analysis method, the number of particles being analyzed and thus the statistical information from TEM is orders of magnitude lower than AUC and other scattering methods. On the other side, the dry samples required for TEM (prepared by drop-casting) studies might be different to that in liquid phase. Moreover, even when applying programmed evaluation procedures using software like ImageJ, the total number of analyzed particles is still limited. Thus, it is important to complement the measurement of the size and shape distribution in the liquid phase by other techniques with better statistics, e.g., AUC and SAXS. In the given case, the mean length and diameter of the green rods from TEM measurements was used as input for the AUC analysis to reduce the number of unknown parameters during evaluation.

TEM investigation over a large area reveal very homogeneous size and shape of the green rods (see Figure S1, Supporting Information S1 for more details). Figure 1A shows a representative scanning transmission electron microscopy (STEM) Z-contrast image of the green rods. The shape and distribution is quantified manually by measuring 100 rods from STEM images along their length and diameter axes (subdivided into end 1, middle, end 2, to reflect the varying thickness along the rod axis) were counted as shown in Figure 1B (inset). The length and diameter distributions are plotted in Figure 1C,D, respectively. Both, the length and diameter showed a narrow distribution with average values of 25.6 ± 4.7 and 4.8 ± 0.5 nm, respectively. Interestingly, few tetrapods were also found as indicated by the white dashed circles in Figure 1A. The formation of the tetrapod structure is rather likely attributed to the missing of shape-controlling molecules on certain crystal facets.

The AUC results of the green rods after different washing cycles can be found in Figure 2. The main peak at about 150 S (Svedberg, unit for sedimentation coefficient) is ascribed to the green rods. After different washing cycles, the main peak stayed nearly the same, which means that the particle size of the main fraction remains almost constant. Moreover, the unknown peak around 100 S disappeared after washing and

![Figure 1](image-url)
is therefore attributed to an unknown residual from synthesis. In addition, the peak at around 275 S can be attributed to the larger tetrapods. The observed peak of the tetrapods is in line with the TEM results. Noteworthy, no additional peaks at larger sedimentation coefficients occurred during the washing cycles. Hence, it is concluded that the green rods are highly stable in toluene even after four washing cycles.

It needs to be mentioned that the AUC measurements complement the TEM results with superior statistics and resolution. With the known mean length and diameter of the green rods from TEM image analysis, the average hydrodynamic dimensions of the green rods were determined using the average sedimentation and diffusion coefficient. As shown in Table 1, the average hydrodynamic length and diameter of the green rods were derived as 27.8 and 7.0 nm, respectively. Based on volume conservation, the measured sedimentation and diffusion coefficients as well as the translational friction coefficient of the nanorods were derived using the expression of Hansen. Then, the effective density of the green rods (including solid core, two shells, organic stabilizer shell, and dispersing liquid shell) was fitted to be 2.1 g cm$^{-3}$ and the organic stabilizer shell thickness determined to be 1.1 nm. Details about these calculations are provided in Supporting Information S2.

The size and shape distribution of the green rods can also be deduced by simultaneously fitting a multilayer model to SAXS and SANS data. As shown in Scheme 1A, the nanorods were approximated by a spherical CdSe core with one ellipsoidal CdS, one cylindrical ZnS, and one cylindrical organic stabilizer shell. Details about the calculations are provided in Supporting Information S3. The scattering lengths of the inorganic core of the green rods (CdSe/CdS/ZnS) and the dispersion medium (toluene/d8-toluene) were calculated using their bulk densities and data from the NIST Center for Neutron Research database (https://www.ncnr.nist.gov/resources/activation/). The semi-axes of the CdS ellipsoids were fixed to be 10 and 0.1 nm larger than the central CdSe sphere. These parameters were estimated from the TEM measurements of the red rods (see more details in Supporting Information S4). Free parameters of the fit were the radius of the CdSe core, the length and diameter of the ZnS cylinder, as well as the layer thickness and scattering length of the organic stabilizer shell. The polydispersity of the particles was taken into account by a log-normal distribution of the core radius.

The data and fitting results for the green rods are plotted in Figure 3. As depicted in Table 1, the fitting results for the core of the nanorods are in good agreement with the TEM results. According to our previous work, the thickness of the organic layer was obtained from SANS. The thickness turned out to be 1.4 nm, which relates well to the difference in diameter between TEM and AUC. Thus, a combination of TEM, AUC, and SAXS/SANS measurements enables the determination of size and shape of the anisotropic particles, even after multiple growth steps with high accuracy.

**Table 1.** TEM, AUC, and SAXS/SANS results of CdSe/CdS/ZnS nanorods (green rods).

|          | Length [nm] | Diameter [nm] | Aspect ratio [–] | Density [g cm$^{-3}$] | Organic stabilizer shell thickness [nm] |
|----------|-------------|---------------|------------------|------------------------|----------------------------------------|
| TEM      | 25.6 ± 4.7  | 4.8 ± 0.5     | 5.3 ± 1.1        | –                      | –                                      |
| AUC      | 27.8$^{a}$  | 7.0$^{h}$     | 4.0$^{h}$        | 2.1                    | 1.1                                    |
| SAXS/SANS| 22.9 ± 6.1  | 4.7 ± 1.3     | 4.9 ± 1.8        | –                      | 1.4                                    |

$^{a}$Dimensions including the organic stabilizer shell and using TEM data for the unsolvated particles as input to the fitting routine.
Notably and as an independent test for the validity of our comprehensive characterization approach, the mass conservation must be fulfilled for the individual phases of the nanorod. The exact composition of the nanorod is unknown, but an approximation can be given. Using 4.4 g cm\(^{-3}\) as a reasonable estimate for the density of the unsolvated nanorod excluding its organic shell and a density of toluene for the organic and solvent layer, we can reproduce the density of 2.1 g cm\(^{-3}\) derived by AUC when using the SAXS/SANS shell thickness as input (see Supporting Information S2 for further details). In contrast, the calculated density would be overestimated for the organic stabilizer shell thickness measured by AUC, which is smaller by 0.3 nm in comparison to the SAXS/SANS data (see last column in Table 1). Previously, we have shown that organic shell thicknesses below 1 nm on small NPs can be determined by AUC.\[^{[53]}\] The observed deviations are to be expected as uncertainties exist for the determination of the shell thickness due to irregularities of the nanorod shape in general or the unknown end-cap geometry in particular. Especially the latter can have considerable influence on the derived shell thickness.\[^{[49,50]}\]

2.2. Characterization of Surface of Nanorods

Until now, size and shape of green rods were discussed. However, as already mentioned, the surface species on the green rods also need to be determined as surface plays a key role for all nanomaterials, with regard to both product performance and processing. In the current case, TOP/TOPO, ODPA, HPA, and oleic acid were used during the synthesis of the green rods. In contrast to typical protocols, similar to other works reported in literature,\[^{[2,34,35]}\] no fatty amines were applied during ZnS shell growth. All of the former are possible candidates that can attach to the surface of the green rods. Herein and as described in the following, FT-IR spectroscopy was applied to determine the functional groups on the surface of the nanorods, while TGA was employed to determine the amount and the type of surface molecules.

Noteworthy, during the synthesis TOP was also used. However, TOP is very easy to be oxidized to TOPO. As reported by Jasieniak and Mulvaney, TOP in liquid phase was exposed to an ambient environment for 14 h, resulting in a very strong peak of TOPO (intensity higher than TOP) in \(^{31}\)P-NMR results.\[^{[56]}\] In current case, due to the available infrastructure, it was unavoidable that the nanorods were exposed to oxygen during washing and sample storage. Moreover, during the sample preparation, i.e., multiple flocculation-redispersion cycles during washing, the drying of pellets for FT-IR and TGA measurements, TOP was exposed to oxygen for quite a long time (in total it took several days to weeks). Therefore, in the current case, it is very unlikely that TOP is, after sample preparation and analysis, still not oxidized at the surface of the nanorods. Hence, only TOPO will be discussed in the following.

As mentioned in the experimental part, the nanorods of this work were synthesized on a pilot scale setup for industrial production and their size, shape, and optical properties are highly reproducible. Furthermore, the aim of this work is to establish a blueprint for quality control of advanced quantum materials in terms of size, shape, stability, main components on the surface, and optical properties in industrially relevant environments. Therefore, the impurities in TOP/TOPO and/or new ligands generated by the high temperature reaction were neglected and only the organic stabilizers used in the synthesis were considered for the surface characterization of nanorods.

2.2.1. Functional Groups on the Surface of Green Rods

The FT-IR spectra of the green rods as well as of TOPO, HPA, ODPA, and zinc oleate as reference are shown in Figure 4. When going from higher wavenumbers to lower wavenumbers, the peak at 1720 cm\(^{-1}\), which is very pronounced in case of zinc oleate, is attributed to the carboxyl group (black curve). This characteristic peak is clearly not observed in the spectrum of the green rods (green curve), which leads to the conclusion that no noticeable amount of carboxyl groups is bound to the surface of the NPs. Therefore, the absence of the C=O band around 1720 cm\(^{-1}\) already indicates that there are no surface species with a carboxyl group, e.g., oleic acid, on the surface of the green rods. Moreover, as indicated by the vertical black dashed line in Figure 4, the rocking vibration of the C–H peak of the ODPA molecule (gray curve) shows a clearly different position (1472 cm\(^{-1}\)) when compared with the other samples (1465 cm\(^{-1}\)). This implies that also ODPA molecules are not bound to the surface of the green rods because this is a relatively strong peak. In addition, no signal was detected when adding 5 mg of ODPA in 800 µL deuterated toluene (see Figure S6 in the Supporting Information S5 for P-NMR results). This indicates a low solubility of ODPA molecules in toluene. Poorly soluble ligands on the surface of NPs are expected to collapse and thus may induce agglomeration, which is, however, not the case for the highly stable green rods.

![Figure 4. FT-IR results of TOPO (blue line), HPA (magenta line), ODPA (gray line), zinc oleate (black line), and green rods after one washing cycle (green line).](image-url)
rods (stability is discussed in Section 2.3). Therefore, we conclude that also ODPA is not on the surface of the green rods, at least not in a detectable amount. Thus, the surface of green rods mainly contains TOPO and/or HPA molecules, which are not distinguishable from FT-IR results alone. Noteworthy, impurities in TOP/TOPO and/or new ligands generated by the high temperature reaction are not considered.

2.2.2. Amount of Molecules on the Surface of Green Rods

Based on the aforementioned FT-IR results, the absence of oleic acid and ODPA was confirmed for the green rods. To further distinguish different surface species based on their decomposition temperatures, TGA was applied. The results of the green rods after one, two, and four washing cycles are shown in Figure 5A. Negligible differences of weight loss at 600 °C were found after different washing cycles. This strong binding of the molecules on the surface is again in agreement with the high colloidal stability. Moreover, as can be seen from the differential thermogravimetric (DTG) results in Figure 5B, no weight loss in the temperature range from 200 to 300 °C (indicated by the gray arrow in Figure 5A) was observed. As this temperature range is typically ascribed to phosphine species,[57] it can be concluded that no phosphine species (HPA, as ODPA was excluded in the previous section) is present on the surface of the green rods. On the contrary, a significant weight loss around 500 °C was recorded, which is ascribed to TOPO molecules as reported in literature.[57] Therefore, we conclude that TOPO is the main and strongly bound ligand at the surface of the green rods that nearly does not desorb during washing. Again it needs to be mentioned here that impurities in TOP/TOPO and/or new ligands generated by the high temperature reaction are not considered.

A quantitative in situ analysis of the stabilizer shell in the colloidal state can be performed by simultaneous fitting of SAXS and SANS data (see more details on the fitting procedure in Supporting Information S3).[52] In this case, the model parameters of the inorganic core were fixed and only the scattering length and thickness of the organic shell were fitted. This was carried out for samples in different mixtures of toluene and deuterated toluene to achieve a wide range of scattering length densities (SLDs) of the dispersing liquid. For each washing step (one, two and four times), the SAXS and SANS data were fitted simultaneously. By applying linear fitting to the SLD of the organic shell versus the SLD of the dispersing liquid, the content of dispersing liquid in the shell is the slope of the linear fitting. This is shown in Figure 6 for all washing steps.

From these calculations, the volume fraction of organic stabilizers was determined to be $0.56 \pm 0.03$, $0.80 \pm 0.03$, and $0.84 \pm 0.03$ after washing the green rods one, two, and four times, respectively. After the individual washing steps, the thickness of the organic shell is 1.44, 1.44, and 1.35 nm. From this data, we conclude that during the washing procedure, solvent is expelled from the shell. At the same time, in agreement with literature,[30] the stabilizer molecules rearrange in a denser structure. This densification blocks the solvent molecules from re-entering the stabilizer shell during and after redispersion.

When the SLD of the organic molecules on the surface is known, the volume fraction of stabilizer can also be calculated with the consideration of a mixed SLD. The SLD of TOPO can be calculated by considering the volume fraction of TOPO and the SLD of the dispersing liquid. This is shown in Figure 6 for all washing steps. The best fit is obtained for $0.56 \pm 0.03$, $0.80 \pm 0.03$, and $0.84 \pm 0.03$ after washing the green rods one, two, and four times, respectively. After the individual washing steps, the thickness of the organic shell is 1.44, 1.44, and 1.35 nm. From this data, we conclude that during the washing procedure, solvent is expelled from the shell. At the same time, in agreement with literature[30] the stabilizer molecules rearrange in a denser structure. This densification blocks the solvent molecules from re-entering the stabilizer shell during and after redispersion.

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be calculated as SLD(TOPO) = −0.27 × 10^{10} \text{cm}^{-2} with its density of 0.88 \text{g cm}^{-3}. Based on this, the volume fraction of organic molecules can be calculated as 0.69 ± 0.03, 0.72 ± 0.03, and 0.82 ± 0.03 for samples after one, two, and four washing cycles, respectively. Comparing this to the results of the aforementioned contrast variation measurements, the TOPO shell is less dense than bulk TOPO. After four washing cycles, the volume fractions are nearly the same, which means that TOPO on the surface of nanorods is now almost as densely packed as in the bulk. From these findings, the surface coverage of TOPO molecules on the surface of the green rods can be calculated. After one, two and four washing cycles, the density was determined to be 1.6, 2.3, and 2.2 molecules per nm², respectively. Noteworthy, impurities in TOP/TOPO and/or new ligands generated by the high temperature reaction are not considered. Thus, SAXS/SANS not only further proves the previous conclusions from FT-IR and TGA results, but also gives clearly more insights on the nanorods in colloidal state.

2.2.3. Functional Groups on the Surface of Red Rods

It is surprising that mainly TOPO molecules are present at the surface of the green rods as a total of four molecular species were applied during multiple growth steps. Here it needs to be noted again that impurities in TOP/TOPO and/or new ligands generated by the high temperature reaction are not considered. Therefore, the surface of the intermediate product, the so-called red rods (CdSe/CdS without ZnS shell) was investigated to gain further insight into the surface of the green rods. As shown in Figure 7, the characteristic features in the FT-IR spectra of the green and red rods are quite similar. An absence of the C=O band around 1720 cm⁻¹, indicating the absence of oleic acid, and the same C–H peak around 1465 cm⁻¹, indicating the absence of ODPA (highlighted by black dashed line), were observed. Thus, also for the red rods as intermediate product, no detectable amount of oleic acid and ODPA was found at the surface. However, the peak of P=O around 1100 cm⁻¹ as indicated by the gray dash-dotted line clearly differs between the red rods and green rods. As the reason for this difference cannot be resolved by FT-IR alone, TGA measurements were further conducted.

2.2.4. Amount of Molecules on the Surface of Red Rods

Based on the aforementioned FT-IR results, the absence of oleic acid and ODPA was confirmed for both the finally obtained green rods and the intermediate red rods. As shown in the thermograms of Figure 8A, a weight loss at 500 °C was observed for the red rods, which is again attributed to TOPO molecules. Moreover, the weight loss in this region remains unchanged (16 wt%) after one and two washing cycles, which also indicates the strong binding of TOPO to the surface of the red rods. In contrast, a small but non-negligible additional weight loss at 248 °C was found, which is becoming clear from the DTG data of the red rods after one and two washing cycles in Figure 8B. From the enlargement of the peak at 248 °C (from 175 to 300 °C), shown in the inset of Figure 8B, it can be seen that the weight loss at 248 °C became much smaller after two washing cycles. As mentioned, in literature this peak is attributed to phosphine species (in current case HPA).\[57\] Interestingly, the weight loss between 200 and 300 °C decreases from 0.8 wt% after one washing step.
to 0.3 wt% after the second washing step. This indicates, again in line with the literature,[57] that HPA molecules can be removed by washing and hence, HPA has a weak affinity to the particle surface. It could be possible that the HPA molecules desorb from the surface during the last shellning with ZnS, do not bind strongly to the ZnS surface and thus stay to a large extend in solution. Therefore, based on the FT-IR and TGA results, at least within the detection limits of both techniques, it can be concluded that mainly TOPO molecules and a small amount of weakly bound HPA are present on the surface of the red rods. Again, it needs to be mentioned that the impurities in TOP/TOPO and/or new ligands generated by the high temperature reaction are not considered.

Noteworthy, it is usually unfavorable that L-type (in current case TOP/TOPO) ligands replace the X-type (in current case ODPA, HPA, oleic acid) ligands on the surface of semiconductor NPs. Surface ligands can be divided into L-, X-, and Z-types depending on the electronic structure of particle-ligand binding motifs (see more details in ref. [58]). However, as reported in the literature, this can be rationalized as a ligand-promoted Z-type displacement process.[58–60] Moreover, during the synthesis the molar concentration of TOP/TOPO used was much higher than the X-type ligands, e.g., $>20$ times than ODPA, HPA, and $>5$ times than oleic acid. Therefore, it is reasonable that the surface of nanorods was mainly covered by TOPO.

### 2.3. Colloidal Stability against Agglomeration

In the previous sections, the size, shape, and surface of the green rods were determined by TEM, AUC, SAXS/SANS, FT-IR, and TGA measurements. However, it is also necessary to check the colloidal stability of the samples after multiple growth steps. Therefore, washing experiments were applied to study the stability of the green rods. Due to the small size of the primary particles, the scattering in the visible range is negligible. Hence, extinction spectra can be applied to determine the colloidal stability of a sample based on the scattering of aggregates at larger wavelengths in the visible range. As shown in Figure 9, the extinction spectra of the green rods before and after one to four washing cycles were recorded. The peak around 515 nm (see ten times enlargement in the wavelength range from 480 to 550 nm) is attributed to the first exciton peak of the CdSe core, while the peak around 440 nm is ascribed to the CdS shell.[1] The PSD of CdSe core can be derived from extinction measurements as previously published in literature with the assumption that the CdS and ZnS shells do not change the optical response of the CdSe core (see Figure S7 in Supporting Information S6 for PSDs).[13,61] In case of the green rods without washing, the mean volume equivalent particle diameter turned out to be 2.66 nm, which is in agreement with literature.[1] It is clearly seen that the peak position did not change after four washing cycles. Moreover, no scattering in the range of 550 to 600 nm (no optical response of the green rods) is observed. This means that no remarkable agglomeration of the primary particles occurred even after four washing cycles.[62] Therefore, based on extinction measurements, it can be concluded that the green rods remained highly stable. Neither agglomeration nor any other sort of ageing occurred during ongoing washing. These findings are in agreement with the aforementioned AUC results that already pointed toward a high colloidal stability.

To cross-validate the UV–vis analysis, DLS was applied to check the PSD of the diffusion coefficient equivalent sphere with regard to the correlation function. Although the commercial DLS applied for this study cannot evaluate shape anisotropy, it is a good technique to qualitatively detect changes in the scattering, e.g., due to an agglomeration-induced size change of a sample.[26] As shown in Figure 10, the green rods after one to four washing cycles were measured by DLS. As can be seen from the aforementioned AUC data, the unwashed sample contains some residuals. Hence, the DLS result is not shown here. The peak of the green rods was found at about 26 nm and the PSD almost did not change during washing. This means that the dispersity of the samples remained unchanged. This is again in agreement with the results of AUC and UV–vis measurements. As an independent validation of the DLS data, we calculated the hydrodynamic diameter of

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**Figure 9.** Extinction spectra of the green rods before and after one to four washing cycles.

**Figure 10.** DLS results of the green rods after one to four washing cycles.
the green rods using the minimum and maximum diameter (3.5 and 6.0 nm) and length (15.0 and 33.0 nm) measured by TEM (see Figure 1). Using a shell thickness of 1.4 nm and the frictional properties derived from Hansen’s expression,[51] we estimate a lower hydrodynamic diameter of about 11 nm and an upper size limit of about 21 nm. While the peak position is well represented by DLS, it tends to overestimate the width of the distribution due to insufficient resolution for polydisperse systems as already shown previously for spherical particles.[63] In addition, larger species are overrepresented in the scattering intensity-weighted distribution when comparing the results to the number weighted-distribution obtained by TEM analysis. Nevertheless, as relatively cheaper UV–vis spectroscopy and DLS are more common in laboratory usage than AUC, they can alternatively be applied to analyze colloidal stability, always depending on their availability that certainly changes from facility to facility.

2.4. Optical Properties of the Green Rods

2.4.1. Dilution Effects on the PL Intensity

Until now, the size, shape, and surface as well as the stability of the green rods after several washing cycles were determined. With regard to optoelectronic applications like LEDs, it is necessary to check the optical properties of the green rods during washing and sample storage. As the particle–particle interactions affect the optical properties and hence reduce the PLQY, the normalized PL intensity of the samples during dilution needs to be investigated.[64] As shown in Figure 11A and Table 2, the PL peak position (λ_{exc} at 380 nm) shifted during dilution and stayed constant at 522 nm (indicated by a gray vertical line) when exceeding a dilution factor of 25. The blue shift of the PL peak position is caused by the concentration-quenching effect as reported in literature.[65,66] In brief, high concentrations lead to a lower PLQY and a red shift of the PL peak position. In order to determine the proper dilution range, the normalized PL intensity at different concentrations was determined as shown in Figure 11B. Although a higher amount of particles should lead to higher PL intensities, the PL intensity of the green rods at the original particle concentration (3 wt%) is extremely low. This is due to the self-quenching effect (also known as concentration-quenching effect) at high particle concentration. As indicated by the gray arrow in Figure 11B, the normalized PL intensity leveled off when more than 1000 times dilution was conducted. This defines the minimum dilution for determining the PLQY of the green rods (see more details in Supporting Information S7). Noteworthy, the slight deviation of normalized PL intensity is attributed to the low signal-to-noise ratio when more than 1000 times dilution was conducted.

2.4.2. PLQY of Green Rods after Washing and During Storage

After determining the appropriate dilution conditions, the PLQY of the green rods at different excitation wavelengths after washing and storage over time was investigated. Especially for industrial applications, the optical properties of the green rods during processing (washing) and storage need to be carefully controlled. As illustrated in Figure 12, the black and gray bars are the PLQY of the green rods at λ_{exc} of 380 and 400 nm, respectively. It can be seen that the PLQY value remains almost constant during the different washing cycles. This is in line with the aforementioned high colloidal stability.

To check the reproducibility and storage stability, the sample was stored for two months at ambient environment and the same experiments were repeated. The results are shown in Figure 12 by blue bars with tilted stripes and olive bars with horizontal stripes, respectively. It can be seen that the PLQY before (black bars) and after storage (blue bars with tilted stripes) is

| Volumetric dilution factor [-] | Peak position [nm] |
|--------------------------------|-------------------|
| 0                             | 537               |
| 5                             | 528               |
| 10                            | 526               |
| 25                            | 522               |
| 100                           | 522               |

Figure 11. A) PL intensity of the green rods with different dilution factors measured for an excitation wavelength of 380 and 1 nm slit. B) Normalized PL intensity of the green rods at the excitation wavelength of 380- and 1 nm slit.

Table 2. Influence of dilution on the PL peak position.
almost the same. Moreover, in comparison to the results at \(\lambda_{\text{exc}}\) of 380 and 400 nm, the PLQY at \(\lambda_{\text{exc}}\) of 450 nm (green bars) is lower. This is attributed to the insufficient excitation of all green rods at \(\lambda_{\text{exc}}\) of 450 nm. The excitation wavelength at 450 nm (lower energy than 400 nm) was chosen due to the fact that it is more relevant for end applications, e.g., in the field of displays. Based on this, it is concluded that the samples can be washed and stored without losing their optical performance.

### 3. Conclusions

In this work, a widely applicable routine with a unique combination of different analytical methods for the characterization of nanorods after multiple growth steps with respect to the particle core, surface, stability, and optical properties was established. First, size, shape, and shell thickness of the CdSe/CdS/ZnS core–shell–shell nanorods (green rods) were resolved by TEM, AUC, and SAXS/SANS measurements. In the next step, FT-IR spectroscopy, TGA, and SANS were employed to shed light on the main components on the surface of the green rods. Noteworthy, impurities in TOP/TOPO and/or new ligands generated by the high temperature reaction were not considered. In particular, SANS provided unique insights into the densification of the organic ligand shell during washing in the colloidal state. Then, the colloidal stability of the green rods was studied during washing by DLS and UV–vis spectroscopy. Finally, the PLQY of the green rods was investigated after washing and storage over time. Our hierarchical characterization strategy with a combination of different analytical methods allowed to gain in-depth understanding of highly complex, anisotropic, multishell NPs. This strategy is not only regarded as blueprint for the multidimensional characterization of anisotropic colloidal nanorods with regard to quality control after synthesis but also is an important step toward a more effective formulation of nanomaterials on technical scale.

### 4. Experimental Section

**Materials:** All chemicals were used as received. Methanol (anhydrous, 99.8% Sigma-Aldrich), trietylphosphine oxide (99%, Sigma-Aldrich), quinine hemisulfate monohydrate (99%, Alfa Aesar), 4-(dicyanomethylene)-2-methyl-6-(4-dimethylaminostyryl)-4H-pyran (DCM, 98%, Sigma-Aldrich), and coumarin153 (99%, Sigma-Aldrich). CdSe/CdS/ZnS core–shell–shell nanorods (QGR 225L, refer to “green rods”) with a concentration of 3 wt% in toluene, CdSe/CdS core–shell nanorods (QRR 16/TU/028, refer to “red rods”) with a concentration of 3 wt% in toluene (CdSe/CdS without ZnS shell), Cd-phosphonate (15/TU/044), and zinc oleate (15/TU/026) were provided by Merck KGaA. To investigate the long-term stability of the nanorods, washing experiments and subsequent monitoring of the samples over time were applied. The PLQY was used as a highly sensitive criterion for the stability of the nanorods against agglomeration.

**Synthesis of Green and Red Nanorods:** The synthesis was carried out in a pilot setup by industrial partners from Merck KGaA in Darmstadt (Dr. Greve and Dr. Härter, both included as coauthors of this work) according to a patent owned by Merck KGaA. The patent provides a novel class of seeded nanorods with well-controlled content of Zn-chalcogenides in the rods’ architecture.\(^{[67]}\) Both red rods and green rods were synthesized in a pilot equipment by Merck KGaA. Briefly, first a batch of spherical CdSe seeds was synthesized and then used to grow anisotropic seeded nanorods (CdSe/CdS nanorods) in a second step. Finally, the zinc precursor was added to synthesize the CdSe/CdS/ZnS nanorods. During these three consecutive growth steps, different molecules, namely trietylphosphine (TOP), triethylphosphine oxide (TOPO), octadecyolphosphonic acid (ODPA), hexylphosphonic acid (HPA), and oleic acid, were used to control the size and shape of the particles.

**Washing Experiments of Green and Red Nanorods:** For each washing step, a volume ratio of suspension (toluene as continuous phase) to MeOH (poor dispersing liquid) of 1:0.59 was adjusted. This induced flocculation of the particles and allowed a solid-liquid separation by centrifugation of the sample for 5 min with a speed of 4000 RPM (corresponding to 1789 times gravity) at 25 °C. After centrifugation, the supernatant was discarded and the pellet containing the nanorods was redispersed in toluene with the help of ultrasonication. In total, the green rods and the red rods were washed four times and two times, respectively. To check the long-term stability, the PLQY measurements were repeated after two months of storage.

**Measurement Techniques–Transmission Electron Microscopy:** The samples for TEM studies were drop casted on lacy carbon grids and dried in ambient environment for the measurement. Contamination was the major challenge to achieve high resolution and analytical (e.g., mapping with energy dispersive X-ray spectroscopy, EDXS) studies. Different incident electron beam energies (200, 80, and 30 kV) were attempted but not helpful. 10 s of plasma cleaning helped to reduce the contamination, but the rods became unstable under the electron beam illumination. TEM images were recorded with a double Cs-corrected ThermoFischer (former FEI) Titan Themis transmission electron microscope operated at an acceleration voltage of 200 kV. The length and diameter of the nanorods in STEM Z-contrast images were measured with the free software ImageJ.

**Analytical Ultracentrifugation:** A preparative ultracentrifuge, type Optima L-90K from Beckman Coulter (USA), equipped with a multilength detector was used to perform the AUC sedimentation velocity (SV) experiments.\(^{[32]}\) Titanium centerpieces from Nanolytics (Germany) with a path length of 12 mm were applied. SV data was acquired at 6000 rpm and 20 °C in toluene. The solvent density and viscosity were 0.8669 g cm\(^{-3}\) and 0.587 m Pas, respectively. Intensity data were recorded and converted to extinction data using the air regions in the measurement cells above the samples’ menisci. The extinction

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**Figure 12.** PLQY of the green rods at excitation wavelengths of 380 nm (black bars) and 400 nm (gray bars) with 1 nm slit. The blue bars with tilted stripes and olive bars with horizontal stripes are the results of repeated measurements after two months at excitation wavelengths of 380 and 450 nm, respectively.
Photoluminescence Spectroscopy: PL spectra were measured by a Horiba Fluorolog-3 spectrofluorometer (Horiba Jobin Yvon GmbH, Germany) using 380, 400, and 450 nm as excitation wavelengths and 1 nm slit. In order to minimize particle-particle interference as well as to exclude self-quenching during the PL measurement, all samples were diluted by a factor of >2000 until the absorbance at the excitation wavelength was lower than 0.05. All the diluted samples for PLQY determination had a total volume of about 20 mL. Therefrom a portion (~2.5 mL) of each sample was taken for the PL measurement. After the PL measurement, this portion was discarded. The rest of the suspension was stored at room temperature but strictly in the dark for two months. To analyse reproducibility, three different standards were used as reference, e.g., quinine hemisulfate monohydrate (99%, Alfa Aesar), 4-(dicyanomethylene)-2-methyl-6-(4-dimethylaminostyryl)-4H-pyran (DMC, 98%, Sigma-Aldrich) and coumarin153 (99%, Sigma-Aldrich).

Dynamic Light Scattering: A Zetasizer Nano ZS from Malvern Instruments was used for the DLS measurements. All samples were dispersed in a glass cuvette at a solid concentration around 0.001 wt%. The scattered light of the particles was detected and the signal was converted to size using the Stokes-Einstein equation. Noteworthy, in this study, DLS was only applied to detect changes in the scattering behavior of a sample, for instance due to agglomeration. With the commercial device available, it was not possible to evaluate shape anisotropy.

Fourier-Transform Infrared Spectroscopy: The FT-IR spectra were recorded by an FT-3100 FT-IR spectrometer (Varian GmbH, Germany), under nitrogen atmosphere. The FT-IR samples were prepared as follows. For each FT-IR measurement, first, the particles were isolated by flocculation with MeOH and subsequent centrifugation (see Supporting Information for the green rods in toluene, using 380, 400, and 450 nm as excitation wavelengths and 1 nm slit). The obtained pellets were dried under vacuum prior 1 mg of the powder was mixed with 300 mg KBr in a mortar (analytical standard, Sigma-Aldrich). The mixture was compressed into a disk by a laboratory press at a pressure of 195.16 bar (model 1ST, Maassen GmbH).

Thermogravimetric Analysis: All TGA data were recorded by a TGA Q50 spectrometer (TA Instruments, USA) with a nitrogen loading rate of 40 L min⁻¹ as balance gas and 60 L min⁻¹ as sample gas. The heating rate was 10 K min⁻¹ and the temperature prior starting the measurement was maintained at 413 K for 1 h to eliminate water residuals.

Small Angle Scattering: SAXS measurements were performed with the VAXSTER instrument at the Institute for Crystallography and Structural Physics (FAU). This instrument was a modified Ganesha instrument from SASSLAB using a liquid gallium jet X-ray source from Excillum and a Pilatus 300 K detector. SANS measurements were performed at the SANS-1 instrument at the MLZ in Garching. Neutron measurements were performed for green rods that were washed one, two, and four times. The samples were prepared in toluene and completely deuterated toluene. The two dispersions were mixed to create different neutron scattering length contrasts of the dispersion medium while maintaining the initial particle concentration. SANS measurements were performed with deuteration percentages of 0, 12, 25, 37, 50, 62, 75, 87, and 100 vol%. The data were fitted with the software SASfit (https://sourceforge.net/projects/sasfit/).

Determination of Photoluminescence Quantum Yield: To determine the PLQY, the integrated PL intensity of a reference (quinine hemisulfate monohydrate) and the integrated PL intensity of the green rods were plotted versus the absorbance at 380 nm as shown in Figure S8 (see Supporting Information S7). The quantum yield was calculated as follows:[66]

\[
QY_x = \frac{QY_{stds, t}}{\varphi_{std} \left( \frac{n_x}{n_{std}} \right)^2}
\]

where \(QY_x\) stands for the quantum yield of the analyte, \(n_x\) and \(n_{std}\) are the refractive indices of the dispersing liquid for the analyte under investigation (for the green rods in toluene, \(n_x = 1.30\)) and the reference material (in 0.1 M H₂SO₄, \(n_{std} = 1.33\)), respectively, \(\varphi\) denotes the slope of the linear fitting for the analyte and \(\varphi_{std}\) is the slope of the linear fitting for the reference material. Quinine hemisulfate monohydrate was used as standard with a PLQY of 50.8% at 380 nm.[66] Noteworthy, sufficient dilution (typically absorbance at excitation wavelength below 0.1) was needed in this approach to avoid particle-particle interactions and self-quenching.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest
The authors declare no conflict of interest.

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