1. Introduction

Sedimentation velocity (SV) analytical ultracentrifugation (AUC) experiments of high quality require precisely defined sector-shaped cells to allow for perfect radial migration of sedimenting species. In order to obtain information on the species under investigation using AUC, the sedimentation boundaries are monitored using an optical setup for analysis of extinction, emission, or interference data. These measurement setups provide the sound basis for a direct boundary model (DBM) analysis of the acquired sedimentation data based on Lamm’s equation, which is formulated in cylindrical coordinates and valid for sedimenting and diffusing species in a sector-shaped centrifugal cell.

State-of-the-art data analysis of SV-AUC experiments based on DBM determines the sedimentation coefficient distribution with the highest possible precision. For the analysis of diffusing species, DBM provides the possibility to derive the diffusion-corrected sedimentation coefficient distribution alongside the diffusion coefficient of the individual species. This 2D analysis provides valuable additional information on the system, such as the determination of individual molar masses of protein oligomers in biological systems, for example, the analysis of building blocks of amyloid fibrils. Recently, the simultaneous measurement of size and density has been demonstrated for core-shell Au and Si nanoparticles (NP) as well as polydisperse ZnO and CuInS2 NPs. Furthermore, analytical centrifugation (AC) can be applied to NPs for the determination of size and shape anisotropy as well as size and density.

AC is widely applied to study the long-term stability of suspensions without exploring the full potential of AC for particle characterization. Recently, AC has been extended further for thorough particle analytics. In particular, DBM has been made accessible for the evaluation of AC data for the determination of the apparent sedimentation coefficient distribution. This significant step forward allows the evaluation of whole sedimentation profiles acquired during an AC experiment.

The objective of the present work is to push AC analysis to a new level by enlarging the measurement capabilities of AC using a new sample assembly, an increased maximum rotor speed, and by taking advantage of the strong expertise of the AUC community. To this end, a new sample assembly was designed, where a sector-shaped centerpiece is inserted into a...
metal housing for robust and well-defined measurement conditions. Moreover, the customized LUMiSizer(R) can now be operated at 32.5% higher rotational speeds (up to 5300 rpm), which, in this way, reduces measurement times by a factor of 1.7 compared to the standard setup. The optimized design is more compact, which significantly minimizes temperature gradients and layering effects.\(^\text{[20]}\)

In the first part of this manuscript, the measurement window of AC was evaluated based on Peclet (Pe) numbers. Therefore, sedimentation data were simulated with an established Brownian dynamics (BD) algorithm\(^\text{[21–25]}\) for SiO\(_2\) NPs and Au NPs. For each particle system, the rotor speed was varied between 300 and 5300 rpm in order to cover the entire range of AC. Our results show that it is possible to determine both sedimentation and diffusion properties at intermediate Pe numbers. At higher Pe numbers, merely sedimentation properties can be retrieved from data analysis. With this, we provide a way for estimating relevant parameters such as particle size, rotor speed, or experimental time for centrifugation experiments via AC in a 3D parameter space. For thorough sedimentation analysis, the NPs must be spectroscopically observable. This requires certain optical properties, which can be related to particle mass concentration using Lambert–Beer’s law. We derived limiting particle mass concentrations for spherical particles applying Mie’s theory.

AC already serves as a powerful tool for particle characterization\(^\text{[15–18,20,21]}\), and can now be applied to a considerably wider measurement range, enabling, for example, the analysis of low density polymers or core–shell particle systems with sizes below 20 nm, which have not been accessible for AC before. Experimental evaluation and validation of the new setup was carried out in this work with SiO\(_2\) and PS particles of different sizes. A comprehensive evaluation was conducted by comparing measured sedimentation properties with the results of AUC. Furthermore, the detection range was evaluated based on the limiting extinction signal, which can directly be converted to limiting particle mass concentration and the respective refractive indices. Next, diffusion-corrected DBM analysis was made accessible for AC data, which is demonstrated by analyzing synthetic data. This enables the retrieval of the effective particle densities alongside the diffusion-corrected sedimentation coefficient distribution for cases where diffusion sufficiently superimposes sedimentation in acquired sedimentation profiles. With the sector-shaped centerpieces and diffusion-corrected DBM analysis at hand, the simultaneous analysis of sedimentation and diffusional parameters enabled for the first time the comprehensive 2D analysis of Au NPs via AC, where the core–shell properties of the NPs were measured and validated by scanning transmission electron microscopy (STEM) analysis and literature data.

### 2. Theoretical Background

#### 2.1. Fundamentals of Sedimentation

The fundamentals of centrifugation can be found in literature.\(^\text{[1]}\) In short, based on a mass conservation approach in a sector-shaped centrifuge cell, Lamm’s equation describes the evolution of concentration, \(c\), based on sedimentation and diffusion coefficients, \(s\) and \(D\), respectively, according to\(^\text{[26]}\)

\[
\frac{\partial c}{\partial t} = \frac{1}{r} \frac{\partial}{\partial r} \left[ r \cdot D \frac{\partial c}{\partial r} - s \cdot \omega^2 \cdot r^2 \cdot c \right]
\]

the distance to the axis of rotation is denoted as \(r\) and the angular velocity is \(\omega\). The sedimentation coefficient is given by the particle’s velocity normalized to the acceleration \(\omega^2 r\)

\[
s = \frac{u}{\omega^2 r}
\]

For a classical sedimentation experiment, the concentration is uniform in the centrifugal cell at the beginning. When a centrifugal field is applied, particles having a higher density than the solvent sediment from the meniscus at position, \(r_m\), to a radial position, \(r\), within a certain time, \(t\), according to Equation (3)\(^\text{[27]}\)

\[
s = \frac{1}{\omega^2 t} \ln \left( \frac{r}{r_m} \right)
\]

A simple method to determine the sedimentation coefficient is to observe the midpoint of the sedimentation boundary as a function of time. Equation (4) links the sedimentation coefficient and the sedimentation equivalent diameter \(x\) based on Stokes’ law

\[
x = \sqrt{\frac{18\eta_s s}{(\rho_p - \rho_f)}}
\]

the liquid dynamic viscosity is given as \(\eta\) and the densities of the particles and fluid are \(\rho_p\) and \(\rho_f\), respectively. For small particles, diffusion phenomena can be observed during an SV experiment. The Stokes–Einstein relation provides the diffusion coefficient as a function of the frictional coefficient \(f\)\(^\text{[28]}\)

\[
D = \frac{RT}{N_A f}
\]

where, \(N_A\) denotes Avogadro’s constant, \(R\) is the universal gas constant, and \(T\) is the absolute temperature. The sedimentation coefficient and the diffusion coefficient can be put in relation using the Svedberg equation\(^\text{[29]}\)

\[
D = \frac{sRT}{M(1 - 6\bar{v} \rho_f)}
\]

\(M\) and \(\bar{v}\) are the molar mass and the partial specific volume of the particles, respectively.

### 3. Experimental Section

#### 3.1. Materials

##### 3.1.1. Commercial SiO\(_2\) NPs

Spherical SiO\(_2\) NP dispersions of various sizes (nominal diameters of 200, 300, and 500 nm) were obtained from Micromod
The Au NPs were prepared by seed-mediated growth in cetyltrimethylammonium bromide (CTAB) micelles. For the synthesis, the protocol of Ye et al. was modified by using $10^{-3}$ M CTAB solution instead of 0.1 M CTAB in the growth step to obtain spherical Au NPs instead of rods.[31] The Au seed particles were prepared by adding 0.25 mL of 0.01 M sodium tetrachloroaurate(III)-hydrate solution and 0.25 mL of 0.01 M trisodium citrate solution to 10 mL of ultrapure water. A total of 0.6 mL of freshly prepared 0.01 M sodium borohydride solution was added to this mixture under magnetic stirring with 1000 rpm that resulted in a color change from yellow to red-brown due to the formation of spherical elemental Au particles <5 nm. After 2 min, the stirring was stopped and the suspension of the seed particles was stored at 25 °C for 2 h prior to use. For seed-mediated growth of spherical Au NPs, 0.5 mL of 0.01 M sodium tetrachloroaurate(III)-hydrate solution, 0.08 mL of 0.1 M ascorbic acid solution, and 0.2 mL of 1 M hydrochloric acid were added to 10 mL of $10^{-3}$ M CTAB solution. The ascorbic acid reduces the Au(III)-salt to Au(I) that is detectable by a change of the growth solution from yellow to colorless. The further reduction of the Au(I) salt to elemental Au by the weak reducing agent ascorbic acid is only possible on the surface of seed particles.[32] The growth of the Au NP was started by adding the seed suspension to the growth solution under gently shaking for 2 min. To obtain Au NPs of different size, the amount of seed suspension added to the growth solution was varied between 0.1 and 1.0 mL. Higher amount of seeds results in smaller Au NPs. The suspension was stored at 25 °C for 12 h to complete the growth of the Au NPs.

3.2. Methods

3.2.1. AC with Sector-Shaped Cells

For AC measurements with sector-shaped cells, a customized LUMiSizer(R) of LUM GmbH (Berlin, Germany) was used. The path length of the new cells was 2 mm. Data acquisition with this instrument is possible at two different wavelengths simultaneously (470 and 870 nm). The temperature was set according to the parameters mentioned in Section 4. The rotor can be spun at up to 5300 rpm with this instrument. Unless otherwise stated, the rotor speed was varied such that all NP systems sedimented within 2 h.

3.2.2. AC with Cells of Rectangular Cross Section

AC experiments of PS dispersions were carried out according to ISO 13318-2 using the standard setup of the LUMiSizer(R) LS611 from LUM GmbH. A wavelength of 470 nm was used and the temperature was set according to the parameters mentioned in Section 4. A comparison with the performance of sector-shaped cells, polycarbonate rectangular cells with path lengths of 2.2 mm were used in this study. The rotor speed was varied such that all NP systems sedimented within 2 h. More details can be found in Section 4.

3.2.3. Analytical Ultracentrifugation

A modified preparative ultracentrifuge, type Optima L-90 K, from Beckman Coulter was used for the SV-AUC experiments. The experiments were performed as mentioned in Section 4. Further details about the applied multiwavelength optics and data acquisition can be found in the literature.[33] The temperature was set to 20 °C and titanium centerpieces with path lengths of 12 mm were used for all experiments. SV AUC data were analyzed with the c(s)-model of SEDFIT to determine sedimentation coefficient distributions.[8,34]

3.2.4. BD Simulations

Sedimentation data as retrieved from AC experiments were simulated via a well-established BD algorithm. Details of the algorithm have been described elsewhere.[23–25] Sedimentation data were simulated for Au and SiO$_2$ NPs of various mean particle sizes as described in Section 4. The meniscus was set to 11 cm and the bottom of the cell was set to 13 cm from the rotor axis, representing the common parameters for AC experiments. The cell geometry was assumed to be sector-shaped. The scan interval was varied between 1 and 1500 s, which is a typical range for AC experiments, based on the theoretical sedimentation time of the NPs that depends on the rotor speed. More details can be found in Section 4.

4. Results and Discussion

4.1. Evaluation of Measurement Range of AC Based on the Pe Number

The most common operation mode for AC represents SV experiments, where the evolution of the concentration profile is monitored over space and time. In contrast, low rotors speeds allow for sedimentation equilibrium (SE) studies, where mass transport by sedimentation and diffusion is balanced throughout the cell.[35] While SE studies are in particular of interest for the study of molar masses and thermodynamic...
SV experiments allow for a 2D analysis of sedimentation and diffusional properties. For instance, this can be utilized to study the core–shell structure of NPs.[32–14] In the first part of this manuscript, we aim to evaluate the measurement range of AC for SV studies based on a dimensionless parameter, the Pe number. It provides the possibility to combine the material parameters, such as particle size, shape and density with the operational conditions temperature, rotor speed, and cell dimensions of the centrifuge. The Pe number is defined as the ratio of the advective, that is here the sedimentation flux, and the diffusional flux of the particles, as can be seen in Equation (7). After rearrangement, the Pe number is calculated according to

\[
\text{Pe} = \frac{\text{sedimentation flux}}{\text{diffusion flux}} = \frac{\pi^{3/2} x^{3} \rho_{p} N_{A} \left(1 - \frac{\rho_{p}}{\rho_{s}}\right) \omega^{2}}{2RT r_{sb} \Delta r}
\]  

\((7)\)

herein, \(\Delta r\) is the width of a representative sedimentation boundary and \(r_{sb}\) is the distance to the rotor axis. The width of a representative sedimentation boundary is introduced as it defines the concentration gradient of the diffusion flux and was determined at a fixed radial position \(r_{sb}\). The retrieval of this parameter is included in Figure S1, Supporting Information. For all calculations of Pe numbers, \(\Delta r\) and \(r_{sb}\) were treated as a constant length. With this concept, the Pe number provides an estimate for the interplay of sedimentation and diffusion for a broad parameter range, such as particle sizes, particle density, and rotor speeds of AC experiments. The detailed derivation of the Pe number is provided in the Supporting Information.

For demonstrative purposes, we calculated Pe numbers for SiO\(_2\) NPs dispersed in water as a function of the particle size and the rotor speed of AC experiments as shown in Figure 1. In the Supporting Information, we provide the Pe numbers for Au and PS NPs (see Figure S2, Supporting Information). Four different regions can be defined based on the Pe number as shown in Figure 2. At very low Pe numbers, sedimentation is balanced by diffusion due to very low rotor speeds and small particle sizes. At intermediate Pe numbers, sedimentation effects are observed, which are superimposed by the diffusion of the particles. Within this region, a full 2D analysis is possible. At higher Pe numbers, sedimentation effects dominate the sedimentation boundaries, and therefore merely the sedimentation coefficient distribution can be retrieved from these datasets. Finally, at even higher Pe numbers, particle velocity is too fast for accurate data analysis.

In order to evaluate the exact Pe limits for the four regions, we conducted BD simulations for SiO\(_2\) particles with mean diameters of 25 and 300 nm as well as Au NPs with a mean diameter of 20 nm. We tested different Pe numbers by simulating several experiments at rotor speeds between 300 and 5300 rpm. This is equivalent to Pe numbers ranging from 0.01 and 4000, thus covering the whole operation window of the AC. For each simulation, we analyzed the sedimentation and diffusion coefficients of the particles and compared them with the calculated values. If the ratio of the retrieved and the calculated values equal unity, the parameter could be successfully retrieved.

Figure 2 clearly shows that the sedimentation and diffusion coefficients are not reproduced at low Pe numbers (the ratio differs from unity). However, with increasing Pe number, the accuracy of the values increases. At a Pe number of 0.7, both the sedimentation and the diffusion coefficients can be successfully reproduced. This point marks the limit to region II. Within region II, both parameters can be analyzed with great accuracy. However, if the Pe number exceeds a value of 30, it is no longer possible to extract the diffusion coefficients accurately. Conclusively, we define the limits of the second region to be Pe numbers between 0.7 and 30. At higher Pe numbers, only the sedimentation properties can be accurately retrieved.

**Figure 1.** Pe numbers for SiO\(_2\) NPs dispersed in water for various rotor speeds in AC experiments.

**Figure 2.** Accuracy of sedimentation and diffusion coefficients for SiO\(_2\) and Au NPs for various Pe numbers of the centrifugation simulations. Values are retrieved from respective data analysis from simulated sedimentation data. The plot indicates four different regimes for the Pe number.
Beyond Pe numbers of 4000, it was impossible to retrieve sedimentation coefficients. Sedimentation is too fast and no individual sedimentation boundaries can be resolved. Thus, no data analysis was possible. It has to be noted that the maximum Pe number at which sedimentation properties can be accurately measured is closely related to the speed of the instrument’s detection system. Throughout AC experiments, acquisition of extinction data is sufficiently fast, especially compared to the scanning extinction systems in the AUC, because of the STEP-Technology, where the entire centrifugal cell is illuminated at once. Therefore, measurement intervals of <1 s are possible with a radial discretization of 30 µm. To test the limits of the AC detection systems, scan intervals of 1 s were simulated for Pe number exceeding 2000.

In summary, BD simulations permitted the identification of four different regions. For Pe numbers between 0.7 and 30, it is possible to analyze sedimentation and diffusion effects of the particles. For Pe numbers between 30 and 4000, only sedimentation effects can be analyzed. For even higher Pe numbers sedimentation is too fast and the detection of sedimentation boundaries is no longer possible. Notably, this is a theoretical limit.

As diffusion can be neglected for high Pe numbers (Pe > 30), pure sedimentation must be considered in the region. Notably, at higher Pe numbers (Pe > 30), the solvent viscosity must be taken into account, when defining the limiting experimental parameters, which can also be seen from Equation (4). Furthermore, for considerations based on Pe numbers (0.7 < Pe < 30), the solvent viscosity is negligible because it cancels out in the derivation. This is due to the fact that both, sedimentation and diffusion depend on the solvent viscosity and the Pe number is defined by the ratio of both. Here, we provide an evaluation of limiting Pe numbers and present calculations for the retrieval of sedimentation and diffusion coefficients at intermediate and moderate Pe numbers.

Based on the limiting Pe numbers, the measurement range of AC can be accurately determined using Equations (2)–(4) and (7). For the limiting Pe numbers of 4000 and 0.7, several combinations of particle size, density, and rotor speed were calculated for NPs dispersed in water based on Equation (7), which provided the parameter planes illustrated in Figure 3. Moreover, Figure 3 gives a schematic illustration of the measurement range of AC based on the limiting planes and the technical limitations due to the minimal and maximal possible rotor speeds. In particular, it is possible to study NPs of different densities and nominal sizes below 100 nm at certain rotor speeds.

These theoretical considerations allow for a thorough design of AC experiments. For this, the limiting planes of Figure 3 are provided as contour plots in the Supporting Information (Figure S3, Supporting Information) in order to provide a more detailed view. Furthermore, a schematic illustration for the design of AC experiments based on the Pe number is provided also in Figure S4, Supporting Information. For example, the sedimentation properties of a spherical NP with a mean size of 50 nm and a density of 10 g cm⁻³ can be accurately measured at 1200 rpm using AC based on Figure 3.

One further crucial parameter when designing AC experiments is the time of the centrifugation experiment. Considering Equations (2)–(4) for water-based systems, the time for sedimentation of spherical NPs within a centrifugation experiment is predetermined for a fixed Pe number. Based on these considerations, we estimated the sedimentation time of the spherical NPs for the parameter range provided in Figure 3. The results are presented in the left and right panels of Figure S5, Supporting Information.

Apart from the consideration based on Pe numbers, an estimation of the measurement range is further possible based on limiting sedimentation times for different particle sizes and densities considering Equations (2)–(4). This is especially relevant for high Pe numbers (Pe > 30), as diffusion is negligible in this region and therefore pure sedimentation effects dominate. Notably, the Pe number is independent of the solvent viscosity as it cancels out in the definition. However, in the sedimentation-dominated region, it must be taken into account as sedimentation effects are significantly influenced by viscosity effects. Conclusively, we performed calculations and estimated a parameter range for spherical NPs dispersed in water. Moreover, we repeated the calculations for NPs dispersed in ethanol and toluene. Our results are provided in the three panels of Figure S6, Supporting Information. Moreover, we included the consideration of limiting sedimentation times in our schematics in Figure S4, Supporting Information.

Finally, in order to analyze NPs via AC successfully, the sedimentation boundaries must be spectroscopically detectable. Therefore, the NPs must give sufficient optical signal. In this context, Ullmann et al. recently investigated the performance of AC with respect to the analysis of broad particle size distributions (PSDs) from sedimentation data acquired by a turbidity detector. It was shown how number-based PSDs can be accurately determined from intensity-based distributions.[18] For the purpose of a proper design of AC experiments, we assessed the detection range of our AC, which corresponds to an extinction signal of the samples between 0.4 and 1.2.[2] For spherical particles, this region can be directly converted to a particle mass concentration by Mie’s theory and Lambert–Beer’s law.[19] We estimated limiting detectable particle mass.
concentrations based on the minimal extinction signal of our AC and Mie’s theory as well as Lambert–Beer’s law. Our calculations were performed for model particle systems, namely spherical SiO$_2$ and Au NPs as shown in Figure 4A, B as well as Fe$_3$O$_4$ and PS particles as shown in the Supporting Information (Figures S7 and S8, Supporting Information). For each material system, a wavelength-dependent refractive index was taken into account. Notably, the detection wavelengths of the AC are 470 and 865 nm.

### 4.2. Development of Sector-Shaped Cells for AC

In order to conduct sedimentation experiments in sector-shaped cells, the geometry of double-sector AUC cells was adapted by LUM GmbH and transferred to AC. With the use of a metal cell housing that includes a centerpiece between two optical windows, centerpieces of variable customized geometries can be used. The optical accessibility of the centerpiece enables the detection of the sedimentation boundaries throughout SV-AC experiments. The design concept including all elements (cell housing, window holders, seals, windows, and centerpiece) is presented in Figure 5A and a single fully assembled cell inserted into an AC is shown in Figure 5B.

For the technical realization of sedimentation experiments via AC, the setup of the rotor was redesigned. The new design provides proper housing of the new cells as shown in Figure 5B and a rotor cover which reduces the volume of the measurement chamber for an improved temperature control. Moreover, the rotor can now be run at up to 5300 rpm, which significantly enlarges the measurement range towards smaller or less dense NPs, as can be assessed from Figure 3. In the following, the performance of the redesigned setup will be thoroughly investigated.

### 4.3. Reduction of Layering Effects in Sedimenting Particle Suspension

As the sedimentation of NPs is very sensitive towards absolute temperature and particularly temperature gradients within the centrifugal cell, AUC experiments are run under vacuum in order to minimize heat development through friction created by the spinning rotor. The advantage of such a vacuum system gets apparent when considering AC experiments, which are performed under ambient conditions. When a temperature gradient forms during an AC experiment, ordering of the sedimenting NPs in defined layer-like structures has been occasionally observed in cases of very small particles and/or minor density contrast for longer sedimentation times. It can be practically overcome by using a sedimentation distance of only 10 mm, shorter sedimentation times and by running the experiments below 10 °C. A quantitative description of these effects and the underlying mechanism was provided by Sloutskin and co-workers. The layering is sensitive towards small temperature gradients, which are in the order of 0.1–50 mK for NPs over the centrifugation cell. For micron-sized particles, Mueth and Esipov suggested that stratification effects are created by a convective instability due to coupling between a horizontal thermal gradient and a vertical gradient in the concentration of the suspended particles. The temperature gradient was measured to be 1 ± 2 mK in the absence of a heat source and 10 ± 2 mK with a heat source.
The theoretical approach was qualitatively supported by experiments for sedimentation of PS particles dispersed in water (low-density contrast) and NPs with a broad PSD.[43] The sector-shaped cell together with the revised rotor design allow us to address this well-known issue, in particular with respect to expanded AC applications. Due to high thermal conductivity, the metal cell housing does not give rise to temperature gradients evoking thermal sedimentation instabilities during experiments. Moreover, the new rotor design reduces friction and additionally creates a thermally insulated compartment for the sample assembly with the sector-shaped centerpiece. In consequence, stable sedimentation profiles could be observed throughout the entire measurement even for low-density particles, heterogeneous samples and higher temperatures. This is demonstrated in Figure S9, Supporting Information.

Sedimentation data analysis by DBM provides the possibility to detect small sedimentation irregularities when analyzing the systematic noise profiles of the fitted data.[7,20] In addition, thermal instability effects lead to a broadening of the retrieved sedimentation coefficient distributions.[43] Here, we demonstrate this effect by measuring PS with a mean size of 1000 nm in our AC using the standard setup with rectangular cells at various temperatures ranging from 15 to 45 °C. The acquired sedimentation data were analyzed for each temperature individually and the sedimentation coefficient distributions corrected to solvent properties of water at 20 °C are shown in Figure 6A.

Evidently, the sedimentation coefficient distribution was successfully retrieved from the respective data analysis for a set sedimentation temperature of 15 °C. However, increasing the temperature up to 35 °C led to the formation of thermal instabilities. In the case of the polydisperse PS particles under investigation, the phenomenon was dominating at 35 °C and individual sedimentation boundaries could no longer be detected. Stair-case-like profiles, which are typical for layering effects as appearing in the literature studies,[22,43] were not visible in our data. Exemplary sedimentation profiles are shown for a temperature of 35 °C in the left panel of Figure S9, Supporting Information. The profiles for 25 and 45 °C were similarly dominated by an instability of sedimentation boundaries (data not shown).

Data analysis via DBM revealed a significant broadening of the sedimentation coefficient distributions, which are further overlaid by artifacts, as displayed in Figure 6A. The instabilities in the sedimentation boundaries for temperatures above 15 °C can be explained by variations in the cooling and heating system of the centrifuge, while experiments at lower temperatures were not subjected to such data-influencing fluctuations.[22]

Following these observations, our new setup was tested by measuring the same PS NPs at 2000 rpm. The acquired sedimentation profiles at a temperature 35 °C are shown in the Supporting Information (right panel of Figure S9, Supporting Information). No layering effects could be observed for all temperatures. Figure 6B shows the retrieved sedimentation coefficient distributions for these experimental data corrected to solvent properties of water at 20 °C. As expected, the sedimentation coefficient distributions could be accurately determined at all temperatures.

In summary, significantly minimized temperature gradients within the rotor chamber and in the sector-shaped cells practically prevent thermal instabilities within the sedimentation boundaries. These developments enlarge the measurement range of AC in terms of particle analysis for low-density particles, offer the possibility to investigate particle systems exhibiting a high polydispersity, and allow for higher experimental temperature flexibility.

4.4. Application of Sector-Shaped Cells to Sedimentation Analysis of SiO₂ Particles

After successful evaluation of the thermal performance of the improved setup, the new sector-shaped cell compartment was further tested and validated using spherical SiO₂ NPs. AUC has been established as a standard method for particle characterization and has been shown to accurately measure the size and shape of various complex NPs[45] and serves herein as a well-established benchmark technique for NP

Figure 6. A) Retrieved sedimentation coefficient distributions from data analysis of PS particles with a mean size of 1000 nm that were measured in standard rectangular cells applying AC. All sedimentation coefficients were corrected to solvent properties of water at 20 °C. Temperature-induced sedimentation instabilities were observed above 15 °C, resulting in significant broadening of the sedimentation coefficient distributions. B) Retrieved sedimentation coefficient distributions from data analysis of the same PS particles that were measured in sector-shaped cells applying AC. All sedimentation coefficients were corrected to 20 °C in water. Due to the absence of temperature-induced instabilities, the same distributions could be retrieved at all temperatures.
characterization.\textsuperscript{[33,46–48]} Thus, SiO\textsubscript{2} NPs were analyzed by AC and AUC, and the sedimentation properties were extracted from both techniques. This serves as a validation tool for our new setup.

We measured the sedimentation properties of SiO\textsubscript{2} particles with nominal diameters of 200, 300, and 500 nm. The rotor speeds were 1300, 1000, and 500 rpm corresponding to Pe numbers of 92, 311, and 258, respectively. These Pe numbers are located within regions II and III, where sedimentation properties should be analyzed accurately (see Figure 2).

Exemplary sedimentation profiles obtained for 200 nm SiO\textsubscript{2} particles using AC are depicted in Figure 7A. Evidently, the sedimentation boundaries could be retrieved with high data quality and excellent signal-to-noise ratio. The calculated sedimentation profiles match the measured data very well. Slight contributions of systematic noise are observable close to the meniscus due to the high refractive index increment. Besides that, the residuals between model and measured data are randomly distributed. Notably, DBM is capable of extracting time- as well as radial-independent noise. In order to properly process the acquired sedimentation data, noise decomposition has been applied to all datasets presented in this manuscript.\textsuperscript{[20]}

The results from sedimentation analysis of these SiO\textsubscript{2} particles with nominal diameters of 200 and 300 nm, respectively, are presented in Figure 7B. For independent validation of the experimental studies in AC, the same particles were analyzed using AUC. The results of both techniques are compared in Figure 7B. Clearly, the obtained distributions from AC and AUC match very well for both analysis, hence validating the excellent performance of the sector-shaped cells.

In order to complete the evaluation of the performance of our sector-shaped cells for AC experiments, sedimentation coefficients of the spherical SiO\textsubscript{2} NPs measured by AC and AUC were converted to particle sizes based on Equation (4). The PSDs obtained from AC and AUC were found to agree well as can be seen in Figure S10, Supporting Information.

Overall, deviations found between AC and AUC are in line with the measurement accuracy of AUC retrieved from a multi-laboratory study.\textsuperscript{[49]} Notably, the AUC experiments were performed at higher rotor speeds (1500 rpm in this case) due to technical limitations. This in turn underlines the advantages of AC, which can be run at lower rotor speeds in order to access a wide spectrum of nano- and microparticles.\textsuperscript{[38]}

In order to further evaluate the detection range of the new setup, we measured the sedimentation properties of 200 nm SiO\textsubscript{2} NPs at various particle concentrations from 1 to 25 mg mL\textsuperscript{−1}, which correspond to different extinction signals at the two different detection wavelengths, covering nearly the whole detection range of AC. The sedimentation properties could be measured with great accuracy throughout the whole detection window of the AC, as can be seen in Figure S11. Supporting Information, further ensuring proper validation of the new setup. Slight changes can be explained by concentration non-ideality effects, which can safely be neglected in the present work.\textsuperscript{[28]}

4.5. Evaluation of Measurement Range for Bimodal PSD

In a next step, we tested the sector-shaped cells with respect to the analysis of bimodal systems by mixing two SiO\textsubscript{2} particle suspensions. SiO\textsubscript{2} particles were mixed with nominal diameters of 200 and 500 nm, respectively. When bimodal suspensions sediment, depending on the sedimentation coefficients, two distinguishable sedimentation boundaries form (data not shown). Data analysis revealed the existence of a bimodal distribution as can be seen in Figure 8A. Deconvolution of the individual peaks was possible and obtained data are in agreement with the results from the analysis of the individual monomodal dispersions as schematically demonstrated in Figure 8A. Moreover, the fractions of the individual species could be determined by integrating over the separated peaks as is further indicated in Figure 8A.
In order to test the performance of AC with respect to the lower detection limit of individual size fractions, we measured sedimentation coefficient distributions of six different bimodal particle dispersions. This investigation allowed drawing conclusions with respect to studying coarse and fine fractions of particle dispersions as well as samples with a broad size heterogeneity. SiO$_2$ particles with nominal sizes of 200 and 500 nm were chosen and subject to sedimentation within a single measurement at either 550 or 1300 rpm for 2 h. The signal contributions for both particle fractions were varied between 1:1 and 1:10 in order to reach the lower optical detection limit of the AC. For each suspension, sedimentation data were analyzed via DBM to retrieve the sedimentation coefficient distributions. Moreover, the experimentally found signal contributions were calculated from the sedimentation coefficient distributions. One representative sedimentation coefficient distribution is depicted in Figure 8A. Two representative results are depicted in the Supporting Information (see left panel of Figure S12, Supporting Information).

Apart from clearly identifying the main particle species with nominal diameters of 200 and 500 nm, we observed a lower detection limit of a signal contribution of 10% for the faster sedimenting species, which can be attributed to the broadness of the distribution, as can be seen in the left panel of Figure S12, Supporting Information. We confirmed the width of the sedimentation coefficient distribution for this particle dispersion by auxiliary AC measurements for the pure fraction with a nominal diameter of 500 nm, as can be seen in the right panel of Figure S12, Supporting Information. The detection limit can further change depending on the polydispersity of the respective fraction.

Below this threshold, it was no longer possible to identify individually sedimenting species in the respective sedimentation coefficient distribution as can be seen in the Supporting Information (Figure S12, Supporting Information) for the mixing ratio of 1:10. Minor shifts of the sedimentation coefficient distributions are attributed to concentration non-ideality. A schematic illustration of the measurement limit is also provided in the Supporting Information (see Figure S13, Supporting Information).

Notably, the signal contributions can be directly converted to respective particle mass concentrations for known extinction coefficients. In the detection limit, the absolute concentrations of the 200 and 500 nm SiO$_2$ NP fraction were 0.25 and 0.13 mg mL$^{-1}$ in the bimodal suspension, respectively (see Figure 8B). The critical particle mass concentration could be determined as a function of the particle size by applying Mie’s theory for the limiting extinction signal of 0.1. For SiO$_2$ NPs, our hereby reported values are within the theoretical predictions, as can be seen in Figure 8B. In conclusion, we have identified the detection limits of AC in terms of particle mass fraction for the larger and polydisperse fraction at the detection wavelength of our LUMiSizer(R).

4.6. Size and Effective Density Determination of Core–Shell Au NPs by AC

Recently, DBM has been transferred to AC for the analysis of sedimentation data while neglecting the effects of diffusion. However, diffusion-corrected DBM is of particular importance for the characterization of slowly sedimenting analytes, such as macromolecules or sub-20 nm NPs. The basic principle of diffusion-corrected DBM is the description of mass transport due to sedimentation and diffusion via the finite element solution of Lamm’s equation. Moreover, the diffusion coefficient can be expressed as a function of the partial specific volume and the sedimentation coefficient as well as the frictional ratio. In the case of spherical particles, the frictional ratio equals unity.

Diffusion-corrected DBM is a well-known and well-established tool for the determination of molecular weights, shape anisotropies, or particle densities and is available through various freely available software such as SEDFIT, UltraScan, and SEDANAL.

In this manuscript, we aimed to transfer diffusion-corrected DBM to AC and in particular apply it to the 2D analysis of NP systems. Details of the theoretical background of DBM can be found in literature and can be found in the Supporting Information. In order to test the application of DBM to data retrieved by AC in sector-shaped cells, sedimentation data of spherical Au NP were simulated first via BD with an effective size of 25 nm and an effective density of 8 g cm$^{-3}$ (mimicking a CTAB shell thickness of 3.3 nm) in water. The rotor speed was set to 1000 rpm that corresponds to a Pe number of 0.75. This
system serves as an ideal model system for 2D analysis via AC as it closely resembles the experimental parameters and conditions of our AC experiment, which will be presented later.

Next, analysis was carried out using diffusion-corrected DBM for the AC data by means of the well-established software SEDFIT.[8,50] Theoretical sedimentation profiles were fitted to the simulated data while minimizing the root-mean-square deviation, an estimate of the fit quality. The meniscus position and partial specific volume (inverse of the effective density) were treated as floating parameters during analysis. The diffusion-corrected sedimentation coefficient distribution alongside the best-fit partial specific volume was retrieved from the analysis.

In the case of AUC experiments, it was well-demonstrated that the results from diffusion-corrected DBM and BD simulations match perfectly.[28] Here, we observe equivalent results for the case of AC, as can be seen in Figure 9A. The diffusion-corrected sedimentation coefficient distribution perfectly reproduces the assigned sedimentation coefficient of the simulated model particles of 2413 S (see Figure 9B).

In summary, the simulations have shown that a combination of diffusion and sedimentation properties leads to the reproduction of the correct effective density of the simulated Au model particles. Conclusively, it should be possible to determine all necessary parameters from AC data, which is a prerequisite for successful experimental investigation of core–shell properties of Au NPs.

From an experimental point of view, it has been shown that core–shell properties of NPs can be determined from a 2D AUC SV experiment as long as diffusion sufficiently superimposes sedimentation.[12,14] For the characterization of a core–shell system, the effective particle density, \( \rho_{P,\text{eff}} \), is a key parameter during 2D analysis. Knowing the effective density and the hydrodynamic diameter of the particles from the diffusion coefficient, one can determine the shell thickness, \( h \), by the following equation:[12,13,58]

\[
h = x_{\text{eff}} \left( 1 - \frac{\rho_{P,\text{eff}} - \rho_v}{\rho_v - \rho_w} \right) \tag{8}
\]

In order to test the capabilities of AC for multidimensional characterization of core–shell NPs, CTAB-stabilized Au NPs were measured in the sector-shaped cells at 20 °C and at a rotor speed of 1000 rpm. The time for the Au NPs to sediment under these conditions was estimated to be 10 h, which equals a Pe number of 0.12. Thus, sufficiently large superposition of sedimentation and diffusional information was guaranteed for data analysis.

The partial specific volume of the particles was treated as a floating parameter during DBM analysis. The diffusion-corrected sedimentation coefficient was retrieved alongside an effective particle density of 6.7 g cm\(^{-3}\) (\( F = 0.1487 \text{ mL g}^{-1} \)). The apparent and diffusion-corrected sedimentation coefficient distributions are displayed in Figure 10A. The underlying experimental data and the best-fit profiles can be found in Figure S14, Supporting Information. It becomes evident that diffusion was the dominating effect during sedimentation of Au NPs under investigation, thus leading to a significant broadening of the apparent sedimentation coefficient distribution. Direct conversion of the apparent distribution to PSD would lead to an overestimation of the particles’ polydispersity. This underlines the necessity for correcting diffusive effects for small NP systems.

From a combination of the average sedimentation coefficient and the fitted effective particle density, the CTAB shell thickness was calculated to be 3.0 nm based on Equation (8). For this, the shell density was assumed to be equal to a CTAB density of 1.04 g cm\(^{-3}\), which was determined for to CTAB concentrations below the critical micelle concentration of \( 1 \times 10^{-3} \text{ M} \) in pure water.[59] Moreover, as the shell density is also influenced by water inclusions and surface effects, we estimated the shell density according to \( \rho_{\text{shell}} = (\rho_{\text{CTAB}} + \rho_{\text{water}})/2 \), which has been successfully applied in literature for the evaluation of sedimentation data of CTAB-stabilized gold nanorods.[6] It has to be noted that any potential contribution of water to the stabilizing shell has no countable effect on the estimated shell thickness as the CTAB density is very close to the density of water. Next, the core diameter distribution was calculated as indicated in Figure 10B. The
mean core diameter was determined to be 14 nm. Throughout the calculations, we further took into account that the effective particle density changes as a function of hydrodynamic diameter. Notably, minor peaks at higher particle diameters (>18 nm) can be attributed to aggregates. However, the small contributions of such signals can be safely neglected during analysis.

In order to assess the obtained data more closely, the retrieved shell thickness of 3.0 nm was compared with literature data. The CTAB shell thickness has been determined by Liz-Marzán and co-workers for Au nanorods by small angle X-ray and neutron scattering experiments to be 3.2 nm. This value is in good agreement with our hereby reported value, and confirms that shell thickness can be resolved with great accuracy. The differences can in part be attributed to small instabilities within the acquired sedimentation data, the influence of shape on the shell thickness, as well as minor aggregation effects throughout the sedimentation experiment. Moreover, Eisermann et al. determined the shell thickness of CTAB to be 2.2 nm on carbon black particles in aqueous media. However, as different core materials differ in chemical functionalization, the shell thickness is expected to vary compared to our investigation. Finally, for further validation of our results, we conducted a STEM analysis of the Au NPs. With this, it was possible to detect the Au cores only, as the shell of the NPs is not visible on the STEM picture. From these images, we calculated the respective core diameter distribution accordingly. One representative image from STEM analysis can be found in the left panel of Figure S15, Supporting Information. Moreover, the mean core diameters from AC and STEM match perfectly validating our approach, as can be seen in the right panel of Figure S15, Supporting Information. One further representative image and more details on particle counting are provided in the left and right panels of Figure S16, Supporting Information.

5. Conclusion

In order to assess the performance of AC for thorough particle characterization, the measurement range of AC was evaluated based on the dimensionless Pe number. For Pe numbers between 0.7 and 30, the simultaneous evaluation of diffusion and sedimentation coefficients is possible. For larger Pe numbers up to 4000, sedimentation properties can be accurately measured using AC. These results were translated into a 3D parameter space, marking the accessibility for AC and including particle sizes from 5 to 1500 nm, densities from 1.055 to 19 g cm⁻³, and rotor speeds from 300 to 5300 rpm. Moreover, we provided estimations for limiting particle mass concentrations based on Mie’s theory.

Sector-shaped cells have been developed and applied for pushing AC analysis to new frontiers. We demonstrated how the customized LUMiSizer(R) in combination with sector-shaped centerpieces improves the particle analysis of temperature-sensitive suspensions. Temperature instabilities are reduced and the sedimentation coefficient distribution of low-density particles could be determined at temperatures up to 45 °C. Rotor speeds up to 5300 rpm enlarge the measurement window of AC. This accessible region was evaluated carefully for polydisperse SiO₂ and PS NPs. Monomodal SiO₂ PSDs could be measured at various concentrations covering the whole detection range of AC. The retrieval as well as the measurement accuracy of the sedimentation coefficient distributions of monomodal and bimodal NP systems was validated by comparing the results from AUC and AC. We observed perfect agreement of determined sedimentation coefficient distributions with a deviation for the weight average sedimentation coefficient below 2%. For bimodal NPs, a signal extinction contribution limit of 10% for a large and polydisperse fraction was identified. Limiting particle mass concentrations have been determined for spherical particles by Mie simulations for different particle systems.

In the final part, a 2D analysis for AC was presented for the first time. For Au NPs, we successfully measured the shell thickness of the stabilizer. A CTAB layer thickness of 3.0 nm could be retrieved and our results have been validated through STEM analysis of the core-diameter distribution. With the core–shell analysis via AC now in place, the investigation of metallic or semiconducting NPs is now possible in future studies. Future directions will go towards enabling multiwavelength analysis in AC.

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.

Keywords

analytical centrifugation, core–shell nanoparticles, layering effects, nanoparticle characterization, sedimentation analysis

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