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New possibilities of accurate particle characterisation by applying direct boundary models to analytical centrifugation

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Analytical centrifugation (AC) is a powerful technique for the characterisation of nanoparticles in colloidal systems. As a direct and absolute technique it requires no calibration or measurements of standards. Moreover, it offers simple experimental design and handling, high sample throughput as well as moderate investment costs. However, the full potential of AC for nanoparticle size analysis requires the development of powerful data analysis techniques. In this study we show how the application of direct boundary models to AC data opens up new possibilities in particle characterisation. An accurate analysis method, successfully applied to sedimentation data obtained by analytical ultracentrifugation (AUC) in the past, was used for the first time in analysing AC data. Unlike traditional data evaluation routines for AC using a designated number of radial positions or scans, direct boundary models consider the complete sedimentation boundary, which results in significantly better statistics. We demonstrate that meniscus fitting, as well as the correction of radius and time invariant noise significantly improves the signal-to-noise ratio and prevents the occurrence of false positives due to optical artefacts. Moreover, hydrodynamic non-ideality can be assessed by the residuals obtained from the analysis. The sedimentation coefficient distributions obtained by AC are in excellent agreement with the results from AUC. Brownian dynamics simulations were used to generate numerical sedimentation data to study the influence of diffusion on the obtained distributions. Our approach is further validated using polystyrene and silica nanoparticles. In particular, we demonstrate the strength of AC for analysing multimodal distributions by means of gold nanoparticles.

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1. Introduction

Recent studies have shown that highly accurate information on the dispersion properties of nanoparticles (NPs) in colloidal systems can be obtained by means of analytical ultracentrifugation (AUC), where the measured sedimentation properties of particles can be directly linked to particle size distributions (PSDs).^{1–4} In addition, information on the particle shape as well as interaction between particles can be extracted.^{5–7} In contrast to characterization techniques based on light scattering, the measurement of even broad size distributions is possible *via* centrifugation as different size fractions become differentially accelerated in the gravitational field. Moreover, compared to transmission or scanning electron microscopy (SEM), the statistics obtained are much better due to the fact that counting individual particles is not necessary.

One of the most commonly used centrifugation based techniques is disc centrifugation. It allows the dynamic increase of the rotor speed during the course of the experiment, which enables the characterisation of particles with broad size distributions.⁸ Measurement times can be kept short owing to the varying rotor speed. As this technique involves the use of a density gradient medium, reference measurements and calibration are required. The density gradient solvent can also interact with the particles, often resulting in colloidal instabilities. AUC provides by far the most accurate information on NP characterisation by measuring the temporal and spatial progress of the particle concentration during centrifugation.^{9,10} Due to very high rotor speeds (up to 60 000 rpm) AUC can detect particles less than one nanometre in size with Ångström resolution.¹¹ It is non-invasive and requires only a small amount of sample (~0.5 mL). Furthermore, no density gradient is required and therefore no reference systems or calibration experiments are necessary. Numerous data evaluation procedures were developed within the last few decades, providing highly accurate information on sedimentation coefficient distribution, shape anisotropy and interaction parameters.

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Even optical information on NPs can be accessed *via* multi-wavelength absorbance detection.³ However, AUC requires considerably more skill and experimental effort than disc centrifugation and was also limited in the dynamic range of the particle size measurement due to the fixed rotor speed. Measurements at multiple speeds and dynamic rotor speed gradients have recently overcome the limitation of fixed rotor speeds.^{3,12}

NPs are widely used due to their outstanding product properties in many applications. The product properties are often strongly dependent on the underlying PSDs. For obtaining PSDs of fast sedimenting NPs with sedimentation coefficients typically higher than 10 000 sved using AUC, it is required to run the instrument at very low rotor speeds, which often makes the process unstable and less accurate. Analytical centrifugation (AC), such as the instrument LUMiSizer® provided by the L.U.M. GmbH (Berlin, Germany) is optimised for lower rotor speeds and serves as a perfect supplement to AUC as it is based on the same physical measurement concept. In principle, the main difference between AUC and AC is the magnitude of the gravitational field that can be achieved. The AUC can generate fields as strong as 250 000g, whereas the AC is currently limited to 2300g. However, so far only one manufacturer exists for each of these techniques, and widely differing hardware concepts are used for the AUC and AC due to the specific requirements. For example, the AUC uses a vacuum chamber and complex cells to ensure thermal and mechanical stability for all rotor speeds. This makes the daily usage also considerably more complicated than for the AC, which uses air conditioning and single-use plastic cells. Moreover, reusable standard laboratory cuvettes made out of plastic or glass can be used and up to 12 cells can be run in parallel so that even fast screening experiments are easily realised with the LUMiSizer.

Unfortunately, possibilities of data evaluation for AC data are very limited compared to AUC. AC is predominantly used as a tool to analyse dispersion stability, even though a simple particle size analysis is implemented.¹³ AC is more often used for the former and is specifically developed to provide stability analysis at lower rotor speeds (less than 4000 rpm). Besides, AC has been sporadically used in the past to obtain PSDs by correlating it to the measured sedimentation velocity distributions.^{14,15} An AC-based sedimentation equilibrium technique has also been used to gather information on the osmotic pressure *versus* particle concentration.^{6,16}

We believe that AC experiments coupled with a more elaborate data analysis technique can serve as a perfect supplement to particle characterisation by AUC, due to the fact that size analysis can be significantly simplified with the AC without renouncing the sophisticated data analysis techniques developed for AUC. The current data analysis technique employed in the LUMiSizer utilises only a small portion of the experimental data measured by the optical system, with no option for noise decomposition. Utilising most of the available experimental data and incorporating noise decomposition to the data analysis system would result in significantly better statistics.

Hence, in this study we extend the data analysis method successfully used in the field of AUC to the analysis of AC data. AUC analysis is a more robust technique and is better developed for quantifying the noises which are commonplace for optical data acquisition techniques in the centrifuge. The method described here also enables fitting of the meniscus position, which is highly desirable because the meniscus position is an important boundary condition in determining the particle size.

2. Theory

2.1 Fundamentals of sedimentation

In a centrifugation experiment, particles are accelerated due to an applied gravitational field, which can be up to 2300 (AC) or 250 000 (AUC) times higher than the gravitational field of the earth. Particles move by overcoming the buoyancy and frictional effects depending on their mass, size and shape. Based on the densities of the particle and the fluid, centrifugation can result in either sedimentation or flotation of the particles. In an analytical experiment, the temporal and spatial movements of the sedimentation boundary from the meniscus to the cell bottom are measured using an optical detector. The shape and progress of the sedimentation boundary with time are defined by the sedimentation and diffusion properties of the particles. The AC uses an optical setup, which allows the acquisition of the Space- and Time-resolved Extinction Profiles (STEP™), whereas a scanning type data acquisition is normally employed in AUC. A schematic drawing of the STEP technology is shown in Fig. 1.¹³ This design allows a very fast detection of

Fig. 1 Measurement scheme of the multi-sample analytical photo-centrifuge. The light source (1) sends out parallel NIR-light (2) which is passed through the sample cells (3) lying on the rotor (4). The distribution of local transmission is recorded over the entire sample length by the CCD-line detector (5). Figure reproduced from ref. 17 (Fig. 1) with kind permission from Springer Science and Business Media.

full radial intensity profiles with time intervals as short as one second. Thus, with its resolution in time and space, AC provides much more data than AUC equipped with an absorbance detector.

The sedimentation coefficient s of a particle is defined as the ratio of its sedimentation velocity u to the gravitational field:

$$s = \frac{u}{\omega^2 r} \quad (1)$$

The centrifugal acceleration of the gravitational field is defined by the squared angular velocity ω^2 and the distance from the axis of rotation r .

The sedimentation coefficient is a unique measure of the hydrodynamic properties of a particle in a given solvent of viscosity η and density ρ_s and can be linked to the particle diameter x and particle density ρ_p using Stokes' law:

$$x = \sqrt{\frac{18\eta s}{\rho_p - \rho_s}} \quad (2)$$

Employing this relationship, the PSD can be calculated from the sedimentation coefficient distribution obtained from a sedimentation velocity (SV) experiment. SV experiments are typically run for longer times until almost all the particles completely sediment to the bottom of the loading cell.

The change in concentration c due to sedimentation and diffusion can be described by a mass conservation approach. The Lamm equation (3) provides an accurate description of the sedimentation of individual non-interacting particles in a sector shaped cell and can thus be used to determine the intrinsic sedimentation and diffusion coefficient (D) distributions:

$$\frac{\partial c}{\partial t} = D \underbrace{\left(\frac{\partial^2 c}{\partial r^2} + \frac{1}{r} \frac{\partial c}{\partial r} \right)}_{\text{diffusion}} - \underbrace{\omega^2 s \left(r \frac{\partial c}{\partial r} + 2c \right)}_{\text{sedimentation}} \quad (3)$$

2.2 Optical detection

For an absorbance based measurement, which is done by the AC as well as the absorbance detector in the AUC, the measured quantity is the attenuation of light given by the intensities of the incident light I_0 and the transmitted light I . It can be expressed by Lambert Beer's law:

$$\log_{10} \frac{I}{I_0} = -\epsilon c d = -\tau c \quad (4)$$

For a given optical path length d , the extinction is dependent upon the molar mass concentration c as well as the extinction coefficient ϵ of the solute/particle. Alternatively, the ratio of the extinction coefficient to the optical path length gives the turbidity. The attenuation of light depends on the absorbance as well as the scattering of light. Dependent upon the refractive indices of the solvent and the particle as well as the density, shape and size of the particle, the extinction of light is dominated by the contribution of either the absorbance or the scattering. Hence, the distribution is extinction

weighted but can be converted to a mass weighted distribution as long as the extinction coefficients of the particles (or solutes) are known. For this, Mie's theory can be applied under the assumption of spherical particles, using the complex refractive indices of the materials to calculate the extinction coefficient based on the size of the particle. It has to be noted that the linear dependence between the extinction and the concentration is only true in the linear regime, where multiple scattering can be neglected. This can be accomplished by reducing the particle concentration. Usually, it is a good rule of thumb to keep the optical density (OD) below 1. Mie's theory was not used in this study, however, it was verified that the particle concentration is in the linear regime, so that all data could be represented as extinction weighted.

2.3 Data analysis

2.3.1 Traditional approach used for analytical centrifugation. The software utilised in LUMiSizer uses a simple correlation to determine the sedimentation velocity distribution of NPs in solution. For this either the variation of the extinction at a fixed radial position over the measurement time (constant position) is evaluated or the dispersion concentration over the entire sample length for a given time is analysed (constant time).¹³ Noteworthy, the AC measurements discussed here provide snapshots of the extinction profiles along the whole cuvette at different times. In both cases, the sedimentation velocity u can be linked to space and time using:

$$u = \frac{r_m \ln \frac{r_m}{r_0}}{t_m} \quad (5)$$

where r_m designates the measurement position, r_0 the meniscus position and t_m the measurement time. The constant position approach typically requires larger experimental times because all particles have to pass the measurement position to be considered for the evaluation. However, a lot of information obtained from an AC experiment is not utilised as either the radial or the temporal information is discarded for the data analysis. Even though numerous discrete data points can be averaged, lots of information gets lost with this approach. In any case, the meniscus position has to be known for an accurate solution of eqn (5). However, due to the low gravitational fields in the AC and possible optical effects arising from the refractive indices, the meniscus is very often not well defined. Since the traditional approach does not include any fit of the meniscus position, the solution can be impaired in the case of an incorrectly set meniscus.

2.3.2 Apparent sedimentation coefficient distribution based on direct boundary modelling. In contrast to the approach used in LUMiSizer, direct boundary models (DBM) include the optical data at all radial positions and different experimental times. Schuck *et al.* developed a model to determine the apparent, not diffusion corrected, sedimentation coefficient distribution by least-squares boundary modelling of the sedimentation boundary ($ls-g^*(s)$).¹⁸ Their approach is based on the model-free dc/dt method developed by Stafford

($g(s^*)$), which has the advantage that time-invariant noise is eliminated during data analysis.¹⁹ In contrast to the dc/dt method, the $ls-g^*(s)$ model is not limited to a designated number of sedimentation scans and can utilise all experimental data for data analysis. However, artefacts can occur in the case of improper analysis due to an incorrect model or experimental noise. A detailed description of both models is given in the works of Schuck *et al.* and Stafford *et al.*^{18,19} Therefore, only a short description of the $ls-g^*(s)$ model is given in this section.

A set of experimental sedimentation profiles $a(r, t)$, at radial position r and time t , can be described by the apparent sedimentation coefficient distribution $g^*(s)$ using the subsequent optimisation problem:

$$a(r, t) \cong \int g^*(s) U(s, r, t) ds \quad (6)$$

$U(s, r, t)$ can be calculated as the solution of Lamm's equation for non-diffusing particles in cells of any defined geometry. For a cell geometry defined by the cross sectional width $W(r)$ and flux $j(r)$, the Lamm equation (3) can be rewritten as:

$$\frac{\partial U}{\partial t} = \frac{-1}{W(r)} \frac{\partial (W(r)j(r))}{\partial r} \quad (7)$$

In the case of linear Lamm equation where the force is radius dependent, the flux $j(r)$ can be defined as:

$$j(r) = s\omega^2 r U - D \frac{\partial U}{\partial r} \quad (8)$$

For a sector shaped cell, the width varies with the radial position and $W(r) = \theta r$ (where θ is the sector angle). Combining eqn (7) and (8) along with the assumption that particles are non-diffusing ($D = 0$), one arrives at the expression for U , given by:

$$U(s, r, t) = e^{-2\omega^2 st} \times \begin{cases} 0 & \text{for } r < r_0 e^{\omega^2 st} \\ 1 & \text{else} \end{cases} \quad (9)$$

Eqn (9) describes the behaviour of an initially equally distributed non-interacting sample with the sedimentation coefficient s , in a sector shaped cell, during sedimentation. Thereby the effect of radial dilution is taken into account while influences of diffusion are neglected.¹⁹ This can also be extended to other cell geometries after accounting for the radial dependent force on the particles and different values for $W(r)$. Although the Lamm equation is valid only for sector shaped cells in a strict sense, this analysis can be extended to rectangular cells for low rotor speed cases, where the wall effects are negligible due to very small lateral diffusion. In the case of AC the cells for loading samples are rectangular and not sector shaped wherein there are no effects of radial dilution ($W(r) = \text{constant}$). This results in a change in the solution to the sedimentation boundary step function for non-diffusing particles and can be calculated by solving eqn (6) and (8) which leads to:

$$U(s, r, t) = e^{-\omega^2 st} \times \begin{cases} 0 & \text{for } r < r_0 e^{\omega^2 st} \\ 1 & \text{else} \end{cases} \quad (10)$$

The problem can be discretised in good approximation for a desired range and number of sedimentation coefficients, which is then solved *via* a least-squares approach to minimise the function $f(g^*)$:

$$f(g^*) = \sum_r \sum_t \left[a(r, t) - \sum_{i=1}^N g_i^* U(s_i, r, t) \right]^2 + \alpha \sum_{i=2}^{N-1} (g_{i-1}^* + g_{i+1}^* - 2g_i^*)^2 \quad (11)$$

The last term in this equation is needed to stabilise the solution and to generate a smooth distribution based on Tikhonov–Phillips regularisation. α is the regularisation parameter, which can be set during data analysis using the F -test, which ensures that the regularised solution is not too different statistically from the least squares fit.²⁰ This procedure ensures that a smooth distribution consistent with the data is obtained, where $ls-g^*(s)$ is the extinction weighted distribution for the apparent sedimentation coefficient with no correction for diffusion.

A considerable advantage of this approach is the possibility of noise fitting (radial and time invariant). Moreover, the meniscus position can be determined by optimising the analysis for the smallest root mean square deviation (RMSD). This is of major importance for AC data because the meniscus position is often not well observable at low rotor speeds. A drawback of the $ls-g^*(s)$ approach is the inability to correct for diffusional broadening. As shown later in the manuscript, for the predominant particle systems considered in AC this is not crucial due to their large mass where the particle motion due to diffusion can be negligible compared to the particle motion by sedimentation.

2.3.3 Decomposition of time and radial invariant noise. It is hard to completely eliminate experimental errors in optical data measurement systems. These data contain time-invariant (TI) noise components as well as radial-invariant (RI) noise components in addition to random noises, which can affect the accuracy of the results obtained. Simple algebraic techniques are available to decompose the noise components from experimental data which have already been implemented successfully in centrifugation analysis software.

In this study, we use the same technique as described in detail by Schuck and Demeler as well as in the manual of Sedfit software.²¹ In our software we have incorporated the possibility of calculating the RI and TI noises, which enables better treatment of the experimental AC data. In AC analysis artefacts on the loading cell such as dirt and scratches are the prime reasons for TI noises while changes in the intensity of the flash lamp during the course of the experiments represent RI noise. As explained before, TI and RI noise components are vital for sedimentation analysis based on optical data. Noise components need to be subtracted from the optical data to obtain acceptable results from the sedimentation data.

2.3.4 Diffusion deconvoluted sedimentation coefficient distribution based on direct boundary modelling. For small particles (typically <20 nm) diffusional broadening of the sedimentation boundary is observed. To obtain the correct PSD,

the effect of diffusion has to be accounted for. In principle, the sedimentation problem can be formulated in the same manner as shown before for the $1s\text{-}g^*(s)$ approach, with the difference that the step function is replaced by the solution of the Lamm equation $L(s, D(s), r, t)$.²² However, the particles used in this study have very low diffusion coefficients and hence diffusion broadening can be neglected in the data analysis. Another aspect to be noted here is the influence of the cell geometry. As explained in the section on DBM, the cell geometry has an effect in altering the equations involved, which is reflected in the solution $L(s, D(s), r, t)$.

2.4 Numerical simulation of the centrifugation process

Numerical prediction of sedimentation concentration profiles is helpful in testing and studying the retrieval model systematically. Numerical solution to Lamm's equation (3) is employed in the analysis of sedimentation concentration profiles, and the finite element method is the most extensively used simulation technique. A microscopic description of the particle motion in an (ultra)centrifuge can also be considered in order to calculate the concentration profiles. A simple approach in this direction has been adopted by Diez *et al.* where the motion of the particles is monitored by Brownian Dynamics (BD) simulation, taking into account a deterministic force which simulates the centrifugal force as well as a random force that is characteristic of the Brownian motion.²³ The particle motion can be tracked *via*:

$$r(t + \Delta t) = r(t) + \Delta r_{\text{Sed}} + \Delta r_{\text{Brow}} \quad (12)$$

where $r(t)$ is the position of a particle at time t and $r(t + \Delta t)$ is its position at time $t + \Delta t$. Δr_{Sed} represents the change in the radial position of the particle due to the sedimentation and Δr_{Brow} represents the change in position due to Brownian motion. This is valid for larger time steps as well and the deterministic change in the position due to sedimentation is dependent on the rotor speed and the sedimentation coefficient and is given by:

$$\Delta r_{\text{Sed}} = r(t) \{ \exp(s\omega^2 \Delta t) - 1 \} \quad (13)$$

The displacement due to Brownian motion can be defined using the diffusion coefficient and is represented by a normal variable of mean zero and variance, for a one dimensional case, given by:

$$\langle \Delta r_{\text{Brow}}^2 \rangle = 2D\Delta t \quad (14)$$

Diez *et al.* proposed this method as a substitute of the forward model solution of Lamm's equation for AUC analysis. For our application, this approach is more versatile compared to the Lamm equation solver because it is not restricted to any specific cell geometry. BD simulations can be easily modified to obtain the sedimentation profiles for AC experiments as well with the only difference being the absence of radial dilution. Moreover, the influence of diffusional broadening of the sedimentation boundary can be considered or neglected by removing the last term in eqn (12).

In the simulation, the solute is represented by a large number of particles, N_{part} , which is divided into certain number of bins with each bin representing a radial position between the meniscus and the bottom of the cell. As the simulation proceeds the particles are moved based on eqn (12) and the concentration at each bin is calculated based on the number of particles in that particular bin. The initial distribution of particles into bins is not straightforward for sector shaped cells as the radial dilution needs to be accounted for, such that the number concentration and not the absolute number of particles is constant. As we are modelling the cells for AC analysis, the number of particles in each bin can be kept constant for the initial distribution as the volume of all the bins are the same. Compared to the idea put forward by Diez *et al.*, changes are made in our simulations such that the particle sizes (and thereby sedimentation coefficients) are chosen from a PSD which ensures that the numerical simulations resemble the centrifugation experiments closely. In order to calculate the particle concentration at a radial position, the total volume of particles in the respective bin is calculated instead of the total number of particles. Even though this technique is slower on a serial processor compared to finite element solutions, there is the added advantage of incorporating the interactions between particles on a microscopic scale and studying the effect on the centrifugation experiments. Once the concentration profiles are obtained *via* BD simulations, it is trivial to add random and systematic noise to the data to mimic real experimental data. This procedure can be used to check the robustness of the AC evaluation method used in this study.

BD simulations present a viable and powerful technique for calculating the forward models or simulating experiments that would help as a tool in fixing experimental parameters. The geometry of the measurement cells can be easily adapted and diffusion can be considered as desired. This makes BD simulations much more viable than any other forward model. Combining the BD simulations for A(U)C analysis with other simulations to obtain the hydrodynamic radius presents an effective tool in finalising experimental parameters. In addition, it is an option of being utilised as a forward model for data analysis techniques, especially for non-spherical particles and in case of pronounced interactions between the particles. BD algorithms have been successfully employed in calculating the hydrodynamic radii of non-spherical particles that can be used in conjunction with this algorithm in order to study their sedimentation behaviour.^{24–26}

3. Methods and materials

3.1 Materials and sample preparation

3.1.1 Reference particles. A spherical polystyrene 203 nm diameter standard was purchased from Fisher Scientific (3000 series, cat 3200A, Lot 39030) and was used as received. The stock solution was 10 g L⁻¹ and concentrations were prepared *via* dilution with ultrapure water (18.2 MΩ cm⁻¹; total oxidis-

able carbon <10 ppb). Silica NPs 479 nm in diameter were purchased from L.U.M. GmbH (product no. 600-61041, batch SiO₂-494-08-0.15-01) and were used as received. The stock solution had a particle concentration of 1.5 g L⁻¹ and was used without further dilution. For titration experiments to adjust controlled bimodality, silica NPs with diameters of 100 nm (#43-00-102) and 200 nm (#43-00-202) were purchased from Micromod Partikeltechnologie GmbH, type sicastar®. Stock-solutions with equal extinction at a wavelength of 470 nm were prepared *via* dilution and were then mixed as needed to obtain the desired mixing ratios.

3.1.2 Gold nanoparticles. Gold NPs were synthesised according to the method of Turkevich *et al.*^{27–30} 7.3 mL of an aqueous solution of 0.2 wt% NaAuCl₄ was added to 100 mL of boiling H₂O. After further boiling, 12.5 mL of an aqueous solution of 0.5 wt% tri-sodium citrate was added that reduced the gold salt and induced the formation of gold NPs which finally grew to 10 to 20 nm (SEM).

Gold NPs of different sizes for the NP mixture were produced *via* seed-growth synthesis. Au seeds were synthesised in the first step by adding 1 mL of 25 mM NaAuCl₄ into 150 mL of 2.2 mM tri-sodium citrate under boiling conditions. After the synthesis of the Au seeds for 10 min, the reaction solution was cooled down to 90 ± 1 °C. Then, 1 mL of 60 mM tri-sodium citrate and 1 mL of 25 mM NaAuCl₄ were added to the solution one after the other with a time delay of ~2 min. After 30 min at 90 °C, the reaction was completed and a sample of 2 mL was extracted. This growth process is repeated in order to get different sizes of Au NPs. Mean particle sizes were measured by means of either SEM or dynamic light scattering (DLS). For the preparation of the 3-component mixture of gold particles with arbitrary concentration but similar extinction we used fractions with mean diameters of about 26.6 ± 1.5 nm (SEM), 47.1 ± 3.3 nm (SEM) and 63.2 ± 11.9 nm (DLS).

3.2 Methods and measurements

3.2.1 Analytical ultracentrifugation. A modified preparative centrifuge, type Optima L-90 K, available from Beckman Coulter has been used for the sedimentation velocity experiments. Further information regarding the applied multiwavelength optics as well as the data acquisition can be found in the literature.³ Titanium centrepieces with path length of 12 mm were used for all experiments. The rotor speed was set to 5500 rpm and the temperature was 20 °C for the polystyrene NPs experiments.

3.2.2 Analytical centrifugation. AC experiments of dispersions were carried out according to ISO 13318-2 using the LUMiSizer LS611 (L.U.M. GmbH, Berlin, Germany). A wavelength of 470 nm was used and the temperature was set to 25 °C for all measurements. Polycarbonate cells with path lengths of 2 and 10 mm were used. The rotor speeds were set to 1000 rpm for gold NP, 4000 rpm for polystyrene NP and 2000 rpm for silica NP measurements.

3.2.3 Dynamic light scattering. The size distributions of the gold NPs were measured using a Zetasizer instrument (Nano ZS, Malvern Instruments GmbH, Germany). Measure-

ments were carried out in backscattering mode at 173° to achieve the highest resolution for smaller particles.

3.3 Brownian dynamics simulations

BD simulations were carried out for particles whose properties are similar to those of the silica NPs and gold NPs used for the experiments in this study. As common to all BD simulations, the noise from the simulations reduces as the number of particles used in the simulations is higher. It is an optimisation between the numerical errors and computational time. As the objective was not the optimisation of the computational time, simulations were carried out with 2.5e7 particles to minimise the numerical error involved. For each of the simulations, the meniscus was positioned at 10.5 cm and the cell bottom was fixed at 13.0 cm. The cell length was divided into 1250 bins, which is equivalent to getting concentration profiles at 1250 radial positions for each scan or getting the concentration profile for every 20 µm. Reflective boundary conditions were invoked at the meniscus as well as the cell bottom which is slightly different from the method proposed by Diez *et al.*²³

BD simulations were done for silica NPs with a mean size of 479 nm and a standard deviation of 20 nm, for a total time of 800 s at 2000 rpm, with the data obtained for every second. Simulations were performed for rectangular cell geometry as given in the AC and sector shaped geometry as given for AUC experiments.

For fast sedimenting systems as they are in AC experiments, broadening of the distribution due to diffusion seems unlikely for most cases. To check the influence of diffusion on the sedimentation boundary and distribution, we simulated sedimentation profiles of gold NPs with a mean size of 15 nm and a standard deviation of 0.75 nm in a rectangular cell. The rotor speed was kept low at 1000 rpm leading to a slow sedimentation, which results in a total time of the corresponding experiment of around 16 hours, which would magnify the effect of diffusion on the simulated data.

3.4 Data evaluation

Particle size analysis in the LUMiSizer software was used as implemented. Three radius positions were used as proposed by the software and were averaged for calculating the PSD. The meniscus positions were set either by the software or manually in case of a false detection. Sedimentation velocity or PSDs with intensity weighting were exported and converted to sedimentation coefficient distributions to be comparable with the results of the DBM.

DBM analysis was used as presented in this manuscript. For this, the $\omega^2 t$ values had to be calculated after the experiment based on the measured times and rotor speeds because the AC does not include an inherent $\omega^2 t$ calculator at the hardware level. Thus, dynamic changes in the rotor speed such as the acceleration phase cannot be considered for the $\omega^2 t$ values. For all evaluations the resolution in the sedimentation coefficient was set to 100 or 150 grid points depending on the width of the distributions. Time and radius invariant noise as well as meniscus positions were fitted, wherever needed. DBM analy-

sis was used with and without taking into consideration the effect of radial dilution depending on the evaluated data. All sedimentation coefficient distributions were regularised using Tikhonov–Phillips regularisation with an α -value of 0.99 so as to obtain smooth distributions.

4. Results and discussion

4.1 Brownian dynamics data

BD simulations were carried out mainly for two reasons. It is important to understand the effect of the cell geometry on the concentration data as well as the effect on the data evaluation. It is equally necessary to study the influence of the diffusion on the sedimentation and the retrieved distribution.

As can be clearly seen in Fig. 2a and b, a sector shaped cell leads to a faster decay of the plateau concentration due to the effect of radial dilution. In contrast, the influence of diffusion on the sedimentation data is hardly visible from Fig. 2c and d

because broadening of the sedimentation boundary occurs not only due to diffusion but also due to the intrinsic PSD.

As shown in Fig. 3a, the different cell geometries are correctly represented by our data evaluation procedure. The mean value of the distribution used for the forward simulation is retrieved with an accuracy of 99.99%. The intrinsic width of the distribution is also nicely represented in both evaluations. In Fig. 3b, the influence of diffusion on the retrieved distributions is shown. As expected, the effect of diffusion has resulted in a broader distribution for the sedimentation coefficients. However, there is a negligible change in the peak of the distribution as well as the mean sedimentation coefficient retrieved from the distribution. It has to be emphasised that the properties of the particles and the experimental parameters were chosen in a way that would test the validity of the proposed method.

In a typical AC study, the effect of diffusion will not be as pronounced as the case shown in Fig. 3b as the chosen conditions represent a worst case scenario for the applicability of the proposed method. Hence it is reasonable to infer that the influence of diffusion can be neglected for the majority of AC experiments. This in turn simplifies data evaluation consider-

Fig. 2 Data obtained for BD simulations of silica (479 nm) and gold (15 nm) NPs. Data shown is for silica NPs in a rectangular (a) and sector shaped (b) cell, both including diffusion. Concentration profiles of gold NPs in a rectangular cell were simulated with (c) and without diffusion (d). Data for a designated number of scans is shown for better clarity.

Fig. 3 Sedimentation coefficient distribution for the given distributions used as input parameters in the BD simulations and distributions retrieved for the sector and rectangular shaped cell geometries. Silica (a) and gold NPs (b) were simulated and evaluated.

ably since an adaptation of Lamm's equation to rectangular cells would be complex and computationally expensive.

However, for small particles it would be beneficial to ascertain as to when the effects of diffusion become non-negligible, since this will broaden the retrieved PSD. A commonly used method is the order of magnitude comparison between the sedimentation and the Brownian force.³¹ Here, we attempt to compare the possible displacements caused by the sedimentation and the Brownian forces. The standard deviation of the Brownian motion is used to represent the displacement caused by diffusion and the ratio, b is given as:

$$b = \frac{r_{\text{meas}} \left[\exp \left\{ \frac{x^2}{18\eta} (\rho_p - \rho_s) n^2 \Delta t \right\} - 1 \right] \sqrt{3\pi\eta x}}{\sqrt{2kT\Delta t}} \quad (15)$$

where r_{meas} is the radial position from the axis at which the ratio is calculated, n is the rotor speed, k is the Boltzmann constant, T is the absolute temperature, Δt is the time step used in the calculation and the other symbols having their typical meaning. Fig. 4 shows a plot of the ratio b as a function of the sedimentation coefficients of gold NPs at a radial distance of 11.5 cm from the axis of rotation at a rotor speed of 2000 rpm.

As given by eqn (15) the ratio is dependent on the radial position, time steps (a time step of 60 s is used in the calculations here), the rotor speed and the properties of the particle and the solvent. However, this simple calculation can be used to provide an approximate limit beyond which diffusional effects could be neglected in the data analysis. In fact, further BD simulations and data retrieval revealed that the diffusional effects could be neglected when the ratio roughly approaches a value of 10. This corresponds to a mean particle size of 30 nm and a sedimentation coefficient of 10 271 sved for the gold NPs.

For any other case the lowest size which can be accurately characterised by our methodology can be calculated by apply-

Fig. 4 The ratio of displacements by sedimentation to the standard deviation of the displacement by Brownian diffusion as a function of sedimentation coefficient values for gold nanoparticles.

Fig. 5 Original and retrieved noise vectors for TI (a) and RI (b) noise. (c) Meniscus fit including polynomial fit to calculate the radius position for the lowest RMSD value.

ing the corresponding particle, solvent and experimental parameters and the aforementioned ratio of 10. Besides, for a given set of experimental parameters BD simulations with and without taking into account diffusion can also be conducted prior to the analysis of the experimental data. This allows for further checking of how pronounced the influence of diffusion on the retrieved PSD will be. As stated in the theoretical section, our approach is also able to consider TI and RI noise. We added both types of noise to the simulated silica NPs (including diffusion) and compared the retrieved noise vectors. Moreover, we set the meniscus position to an arbitrary value and chose the exact position by utilising the meniscus fitting option. The results of the noise decomposition and the meniscus fitting are shown in Fig. 5. Both noise contributions are perfectly represented by our evaluation, and the obtained sedimentation coefficient distribution fits the original distribution as well as the noise-free simulation. The correct meniscus position of 10.5 cm is retrieved with an accuracy of 99.996%.

4.2 Correlation of analytical centrifugation with analytical ultracentrifugation

Next, we performed measurements with the same sample on the AC and AUC to assess the accuracy of the AC compared to the well validated AUC. We used NIST certified polystyrene NPs 203 nm in diameter and conducted the experiments at similar gravitational forces to ensure a fair comparison. For this, we

reduced the rotor speeds for the AC measurements because of the larger distance from the axis of rotation (about 11.5 cm compared to 6.5 cm). The raw data of the 203 nm particles and the residuals for the best fit $ls-g^*(s)$ model are provided for the AUC and AC measurements in Fig. 6a and b. It is clearly recognisable that the evaluation of the AUC experiment provides better randomly distributed noise than the analysis of the AC data. The reason for this is the steeper sedimentation boundary in the AC experiment. Since we were not able to perform the AC in the 10 mm cells, presumably due to effects of convection, we had to use the 2 mm cells. Lower polystyrene particle concentrations comparable to the AUC experiments were also tested but resulted in an unstable sedimentation boundary. We attribute this to the low density of these par-

ticles and the unstable temperature control of the AC. Hence, we used a dispersion with a concentration of about 0.1 vol%, which is 20-fold higher than that employed for the AUC experiment, which then provided a stable boundary.

However, self-sharpening of the sedimentation boundary could occur at this concentration, which can explain the steeper boundary in the later scans.¹¹ Nevertheless, strong hydrodynamic non-ideality was not observed herein since both PSDs agree very well, as seen in Fig. 6c. Moreover, non-ideality in the optical detection system due to multiple scattering could also have a slight effect at the beginning of the experiment, since it would further smooth out the apparent sedimentation boundary during the first scans.³² However, due to the comparable low volume concentration it was expected to have a very small effect. Even though both effects are expected to play only minor roles, a combination can result in higher residuals. The mean sedimentation coefficients were 1160 sved for the AUC and 1164 sved for the AC measurement, which translates to particle sizes of 200.8 nm and 201.0 nm, respectively. Hence, a very good agreement with the specifications provided by the manufacturer is found for both measurements. It has to be mentioned that slight variations are to be expected, since the A(U)C provides the sedimentation equivalent particle size, whereas the specification is based on transmission electron microscopy measurements. Slight variations in the particle density and the stabilising shell can affect the obtained particle size by A(U)C. The width of the distribution obtained by AC is slightly narrower, which agrees with the raw data. Besides these observations, the results found by AC agree very well with the results found by the highly accurate AUC.

Thus, the AC in combination with DBM provides excellent results comparable to the AUC analysis. For fast sedimenting species, the AC also has some advantages over the AUC equipped with absorbance detection. AC can capture full radial concentration profiles in less than one second even with full loading (up to 12 samples). It offers a much higher radial resolution, which is almost in the range of the interference detector of the AUC. The optical setup shows much less noise contribution. It can go to lower rotor speeds without influencing the rotor stability. Disadvantages are the fixed wavelength (470 nm or 865 nm), square shaped cells, which could lead to wall effects and hydrodynamic non-ideality, inaccurate temperature control and the $\omega^2 t$ calculations with missing acceleration monitoring on the hardware level, which leads to inaccuracies for all particle sizes because it has to be done after the experiment.

4.3 Comparison of traditional data evaluation and direct boundary modelling

4.3.1 DBM versus LUM software. To compare the traditional evaluation implemented in the LUMiSizer software with our model, we conducted an AC experiment on silica NPs and converted the sedimentation coefficient distributions obtained by the AUC models to PSDs using eqn (2) with suitable changes in the abscissa. For best comparison, we applied

Fig. 6 Raw data and data for the best fit $ls-g^*(s)$ model with residuals for the AUC (a) and AC (b) measurement. (c) Normalised PSDs of the 203 nm polystyrene NPs obtained by AUC and AC. The mean value specified by the manufacturer is indicated by a vertical line.

the same material parameters and chose extinction weighting for both distributions.

In Fig. 7, the raw data of the silica NPs experiment after subtracting TI and RI noise as well as the distribution of the best direct boundary fit are presented. The fitted profile nicely matches the experimental data, even though some systematic error can be identified, which could not be removed by the TI and RI noise decomposition. Both noise vectors are given in Fig. 7b and c. Since also the first scan does not show a horizontal and thus constant concentration, we do attribute this to imperfections of the measurement cell. Slight variations in the cell thickness and hence optical path length will cause a deviation in the measured absorbance as a function of the

radius and local analyte concentration. Since the meniscus position was well observable, the traditional approach was not hampered and both evaluations provided the same mean particle size. However, the DBM revealed a broader PSD with an additional size fraction around 550 nm, which was not observable with the traditional approach. We attribute this to the better statistics of our model and the resulting higher sensitivity. Interestingly, this species resembles a dimer formed from two individual particles having a primary particle diameter of about 470 nm.

To showcase the increased limit of detection by the DBM, titration experiments were performed. For this, two samples of differently sized silica NPs with diameters of 100 nm and 200 nm respectively were mixed. Different mixing ratios were obtained and it was checked to what extent the relative amount of the smaller NPs could be correctly reproduced by the DBM and by the traditional approach. The results of the experiments are given in Table 1. The DBM allowed the correct retrieval of the relative amount down to 1% (extinction weighted) due to the superior utilisation of the measurement data, whereas the traditional analysis clearly failed to retrieve appropriate amounts. Whether the concentration is under- or overestimated by the traditional analysis depends on the PSD and the type of noise in the experimental data.

The relative amount of the 100 nm NPs was overestimated by the traditional analysis, presumably due to contributions of systematic noise. However, systematic and in particular random noise can also lead to an underestimation of the amount of low concentrated NPs, such as in the case of the results shown in Fig. 7c.

4.3.2 AC versus DLS. Additionally, we compared AC results evaluated with DBM as well as the LUM software with DLS. DLS was chosen due to the fact that it is the usually used technique for particle size analysis found in nearly every laboratory. In contrast to scattering techniques, AC does not rely on ensemble measurements, and multiple species can thus be easily distinguished, such as shown for a mixture of differently sized gold NPs in Fig. 8.

Even though the main size fractions at about 25, 50 and 65 nm, are also provided by the LUM analysis, DBM offers

Fig. 7 (a) Concentration data of AC experiment on silica NPs after noise subtraction (black) and best direct boundary fit (red). (b) Time invariant noise vector. (c) Radius invariant noise vector. (d) PSDs of the silica NPs obtained by the traditional constant radius approach and the ls-g*(s) DBM.

Table 1 Results of the titration experiments with the relative amounts of the 100 nm NPs as retrieved by the direct boundary model and the traditional data analysis as provided by LUM

Mixing ratio ^a of 100 : 200 nm NPs	Relative amount of 100 nm silica NPs		
	Given	Found by DBM ^b	Found by LUM ^b
1 : 1	0.50	0.50 (0%)	0.54 (8%)
1 : 4	0.20	0.20 (0%)	0.23 (15%)
1 : 9	0.10	0.11 (10%)	0.13 (30%)
1 : 49	0.02	0.02 (0%)	0.03 (50%)
1 : 99	0.01	0.01 (0%)	0.04 (300%)

^a Volumetric mixing ratios were adjusted based on the particles' extinction at 470 nm. ^b Values in brackets give the relative deviation from the given amount.

much better statistics and an increased signal-to-noise ratio. Agglomerates at around 70–80 nm could also be detected by the AC experiment in combination with the direct boundary approach. Besides, peaks found by the traditional LUM analysis were much narrower due to sharpening of the sedimentation boundary during the experiment. This effect is induced by convection in the measurement cell, which is considered in detail in the next section. Hence, only data obtained over 30 minutes were used for the DBM because data obtained later could not be fitted well due to hydrodynamic non-idealities. The individual sizes of the three fractions are nicely reproduced by AC. The largest fraction measured by AC was found to be slightly smaller than the corresponding DLS measurement. We attribute this to a small amount of larger particles or agglomerates, which shifted the mean value obtained by the DLS measurement. As expected, DLS failed to resolve the individual fractions for the mixture due to its insufficient resolution as an integral technique. Moreover, the larger fractions and the small amount of agglomerates found by AC hamper the detection of the smallest particles in the DLS.

4.4 Assessment of particle size distributions and monitoring of hydrodynamic ideality

As found in Fig. 8, the width of the size distributions were different for the LUM analysis and the DBM. We attribute this to convection in the measurement cell at about 30 minutes, followed by sharpening of the sedimentation boundary. Hence, the width of the distribution was considerably underestimated for the smallest species by the traditional constant radius approach because only data from later scans could be

Fig. 8 Extinction weighted PSD of a mixture of three differently sized gold NP fractions as found by AC with LUM software and DBM. The distribution values of the LUM evaluation were multiplied by 0.5 for the sake of clarity. Besides the main diameters of about 25, 50 and 65 nm, some agglomerates could also be detected with our data analysis approach. No individual fractions were found in the volume weighted PSD as obtained by DLS due to insufficient resolution. The mean values measured for the individual fractions by SEM or DLS is indicated by a vertical line.

used for the smaller species at the designated radius positions. With our approach we were able to identify and exclude those biased scans from the data analysis (bad fits), which led to a distribution not hampered by any non-ideality effects.

The herein observed phenomena of non-ideality in AC experiments is not new and was recently discussed in detail by Butenko *et al.*³³ It was shown that temperature gradients can induce such convection, which can be followed by layering in the measurement cell.³⁴ This in turn will affect the width of distributions or can even result in false peaks. Hydrodynamic non-ideality seems to play a crucial role in many AC experiments and can lead to wrong results, which can hardly be identified without complementary methods. However, with DBM we have a powerful tool, which is able to distinguish between actual polydispersity and convectional effects. This strengthens the results obtained by AC and makes supplementary experiments unnecessary in most cases.

To study the phenomena of non-ideality and DBM in detail, we investigated an example of gold NPs measured in a 2 mm cell. Herein, we demonstrate how the effect of layering is identified, assessed and excluded from the data analysis. The considered gold NPs are expected to have a monomodal distribution.²⁸ However, the AC measurement revealed a bimodal distribution for both data analysis approaches due to layering happening in the later scans (see Fig. 9c). Since the algorithm seeks the fit with the lowest RMSD value, it fits the best for the intermediate scans, whereas the residuals are the highest for the early and later scans (Fig. 9a). Unfortunately, just by looking at the experimental data it is often quite hard to determine whether the obtained polydispersity is true or not. This is because even a multimodal distribution will just show one sedimentation boundary at the initial stages of the experiment. Nevertheless, with our approach non-ideality can easily be discovered by looking at the noise map. The noise map gives a grey scaled map of the residuals, with the abscissa being the radial position and the ordinate representing the subsequent scans (top to down). Positive and negative residuals are given by different shades of grey. For a perfect fit, the map would not show any patterns inside. However, in this experiment we can distinguish between three different stages. In the first stage, the model does not describe the experimental data well, which is recognisable by a white diagonal line. Just one line is observed, which supports the fact that there is only one sedimentation boundary. In the second stage, convection and hydrodynamic non-ideality occur, which can be detected by the wavelike patterns in the residuals along the diagonal line. Moreover, the diagonal line splits into two individual lines, which represents the formation of two sedimentation boundaries due to layering in the cell. In the third stage, these lines/boundaries propagate through the cell.

These patterns are only observable because the model cannot fit the experimental data, which makes it very likely that non-ideality has occurred during the course of the experiment. Hence, we evaluated the data again with the DBM but took into account only scans of the first stage (1–150), where non-ideality effects had not yet set in (before peak splitting in

Fig. 9 (a) Raw data and data for the best fit $ls-g^*(s)$ model of the AC experiment on gold NPs. (b) Residuals bitmap of the first direct boundary analysis showing a stepped sedimentation profile occurring during the middle of the AC experiment due to convection. (c) Normalised extinction weighted PSDs as obtained by LUM-software with traditional constant radius approach and the $ls-g^*(s)$ DBM. Data has been evaluated twice with the DBM to address the hydrodynamic non-ideality.

the noise map). As expected, a monomodal distribution is found, resulting in a much more reasonable representation of the PSD. Herein it has to be mentioned that the RMSD value cannot be used *a priori* without considering the noise map to assess non-ideality because a different number of scans is used for both analyses. Thus, the RMSD value for the first analysis is smaller (0.0031) than for the second analysis (0.0044) because more scans were considered for the first fit, leading to better statistics.

Only with the DBM it is possible to assess the quality of data analyses (RMSD values and noise maps), which is crucial in obtaining accurate PSDs. Thus, we believe that our approach is a powerful tool for particle size analysis because it also serves as a perfect method to determine data quality and reliability. The latter aspect is important as AC could become a widely applied technique for particle size analysis that is not restricted to be used by a few experts but is accessible as a robust and versatile standard method.

4.5 Summary

As we have shown, the DBM of AC data possesses great potential for enhanced particle characterisation. This method combines a versatile analysis technique with an experimental procedure that is easily manageable even for novice users. Even though many analyses can also be done in principle by AUC as well, AC experiments can be performed with much less experimental effort, less costs, and higher throughput, and do not require as much know-how. Moreover, AC extends the accessible size range to larger particle sizes owing to the lower rotor speeds. For fast sedimenting particles this makes it superior to AUC. AC in combination with our novel data analysis approach can access PSDs with high accuracy and resolution for particles larger than approximately 15 nm (with a density of 2000 kg m⁻³). For example, multiple species can be distinguished with high resolution in contrast to most standard scattering techniques such as DLS. We believe that this opens up manifold possibilities in (nano-)particle analysis for colloidal systems, especially when multimodalities need to be addressed with a maximum efficiency. The latter is expected to become more and more important within the near future.

In summary, DBM of AC data provides many advantages compared to the traditional approach based on the constant time or constant position analysis. It is therefore an important step in establishing centrifugation techniques as the gold standard for particle characterisation which is found in nearly every lab that is working in the field of colloidal systems.

(a) Data representation: sedimentation analysis, as presented in this work, yields the sedimentation coefficient distribution, which is the primary quantity that can be measured in a centrifugation experiment. Despite the sedimentation velocity used in the LUMiSizer software being dependent on the radial positions and rotor speeds, the sedimentation coefficient is independent of the radial position and rotor speeds, which makes it easily comparable even for different experimental settings. Sedimentation coefficients can also be converted to particle sizes using eqn (2).

(b) Better statistics and increased dynamic range: in the direct boundary approach almost every data point is utilised for the data analysis, which results in significantly better statistics. Thus, the limit of detection with DBM is enhanced and a higher statistical confidence can be achieved for less concentrated species.

(c) Noise handling: experimental sedimentation data invariably contains time and radius invariant noise, which can be fitted by this approach. This leads to better results from the data analysis.

(d) Meniscus fitting: determination of boundary parameters, such as the meniscus position, becomes possible as RMSD values can be calculated for each set of fitting parameters. This is of considerable advantage since the meniscus position is often not well determinable due to low gravitational fields at these low rotor speeds. This is a huge disadvantage in the current LUMiSizer software because an imperfect menis-

cus position directly affects the accuracy of the measurement with respect to sedimentation coefficients and particle sizes.

(e) Evaluation of measurement quality: as residuals are obtained for each scan and radial position, it is possible to assess the quality of the fit compared to the experimental data. Recent studies have revealed that temperature gradients in the AC can result in convection and false peaks in the derived distributions. With DBM such phenomena can be easily noticed and distinguished from *e.g.* multimodal size distributions. The latter is possible since the residuals will become larger with noticeable bias. This also allows for optimisation and quality control of the hardware by using a well understood particle system.

5. Conclusions

In this study we have shown that DBM originally developed for AUC can also be applied successfully to AC data. Our studies confirm that the results by AC can be directly compared to AUC. For this, we developed a new data evaluation technique, which is capable of dealing with the AC data. DBM provides much better possibilities for particle characterisation. Due to enhanced statistics and the possibility of noise fitting and meniscus modelling, better results in terms of accuracy and sensitivity can be observed compared to the traditional approach of data analysis that takes into account just a few radial positions or scans. Residuals obtained by DBM allow for evaluation of the measurements, which is of high importance because quality and reliability of the experimental data can be assessed.

NIST certified particles were measured with AC and the results agreed well with the values specified by the manufacturer. Our comparison of both techniques showed that AUC is slightly more accurate and has more random residuals due to better temperature control and optimised data acquisition of the data evaluation. However, the detection capability and accuracy of AC in combination with AUC evaluation techniques is still superior to most other techniques based on scattering or particle counting. In our opinion, the combination of AC and AUC provides novel and highly accurate access to particles ranging from a few Ångström to several microns. In principle, AC can capture sedimentation coefficients ranging from about 10^2 to 10^8 sved resulting in particle sizes of about 3 nm to 2.5 μm for gold and 50 nm to 60 μm for polystyrene, respectively. Since AC requires much less experimental effort and user experience compared to AUC, it should be applied to any system where the sedimentation of the particles is large enough to be detected on a feasible time scale (<1 day). Due to its high resolution and absolute measurement principle AC is superior to integral techniques such as DLS. Moreover, no density gradient media are required such as for disc centrifugation, which gives a much higher degree of freedom with respect to the experimental design.

It is to be noted that many other data evaluation methods for AUC, which were for the time being not considered in this

work, can also be applied to AC. This includes not only the model free approaches, such as the van Holde–Weischet or the dc/dt analysis,^{19,35} but also high-performance analysis including correction for diffusion such as the $c(s)$ analysis in Sedfit or the 2DSA and PCSA models implemented in Ultrascan3.^{36–39} This will provide the opportunity to take into account the effect of diffusion where it is significant and will in turn allow for the simultaneous analysis of the size, density and shape of NPs using AC.

Software performing Mie correction is currently under development. It will allow the determination of mass and number weighted PSDs of spherical particles *via* DBM and will thus further extend the functionality of the technique.

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