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Probing sedimentation non-ideality of particulate systems using analytical centrifugation†

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Analytical centrifugation is a versatile technique for the quantitative characterization of colloidal systems including colloidal stability. The recent developments in data acquisition and evaluation allow the accurate determination of particle size, shape anisotropy and particle density. High precision analytical centrifugation is in particular suited for the study of particle interactions and concentration-dependent sedimentation coefficients. We present a holistic approach for the quantitative determination of sedimentation non-ideality via analytical centrifugation for polydisperse, plain and amino-functionalized silica particles spanning over one order of magnitude in particle size between 100 nm and 1200 nm. These systems typically behave as neutral hard spheres as predicted by auxiliary lattice Boltzmann simulations. The extent of electrostatic interactions and their impact on sedimentation non-ideality can be quantified by the repulsion range, which is the ratio of the Debye length and the average interparticle distance. Experimental access to the repulsion range is provided through conductivity measurements. With the experimental repulsion range at hand, we estimate the effect of polydispersity on concentration-dependent sedimentation properties through a combination of lattice Boltzmann and Brownian dynamics simulations. Finally, we determine the concentration-dependent sedimentation properties of charge-stabilized, fluorescently-labeled silica particles with a nominal particle size of 30 nm and reduced interparticle distance, hence an elevated repulsion range. Overall, our results demonstrate how the influence of hard-sphere type and electrostatic interactions can be quantified when probing sedimentation non-ideality of particulate systems using analytical centrifugation even for systems exhibiting moderate sample heterogeneity and complex interactions.

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Introduction

Particle interactions are of utmost importance for the understanding of colloidal stability and mass transport of particles and are therefore a key parameter for any technical application.¹ In this respect, centrifugation constitutes a promising technique for the experimental investigation of concentration-dependent interactions in colloidal systems.^{2–4} The method offers the possibility to systematically investigate hydrodynamic and thermodynamic interaction parameters, which is key^{2,4–7} for the comprehensive description of mass transport phenomena beyond the limit of infinite dilution where Stokes' law prevails.^{8–10} Sedimentation non-ideality originates from a concentration dependency of the particles' translational friction coefficient.^{4,7,11–14} Firstly, the particles' sedimentation behavior is influenced by a volume flux of solvent in opposing direction, which is caused by solvent filling the space created by the sedimenting particles.¹¹ Furthermore, the viscosity and density of the dispersion increase with particle concentration, which leads to a further decrease in the sedimentation rate.¹⁵ Based on theoretical considerations of rigid spheres, Batchelor derived a

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dependence for the settling velocity based on the particles' volume fraction.^{16,17} He demonstrated that the relation of the settling velocity u with the particle volume fraction can be described by a linear expression according to $u_0(1 - 6.55c_v)$, where u_0 is the settling velocity of a single neutral hard/rigid sphere in a fluid and c_v is the associated volume fraction.¹⁶

Electrostatic interactions can further alter sedimentation properties as long as the interparticle distance is sufficiently small compared to the Debye screening length.^{18,19} At volume fractions exceeding typically 5–10%, coupling of the motion of particles can be observed and linear relationships between the particle volume fraction and the frictional coefficient can no longer be confirmed.^{20,21} In this context, Batchelor and Wen derived a correction for the sedimentation velocity, which was shown to scale with $u_0[1 - (6.55 - 0.44\Omega)c_v]$.¹⁷ The parameter Ω is derived from a general interaction potential φ according to:

$$\Omega = 3 \int_0^\infty \left(e^{-\frac{\varphi(\hat{s})}{k_B T}} - 1 \right) \hat{s}^2 d\hat{s} \quad (1)$$

The temperature and Boltzmann constant are denoted as T and k_B , respectively. The mean interparticle spacing is $\hat{s}$, which can be calculated from the particle volume concentration c_v , as will be discussed subsequently.

Analytical ultracentrifugation (AUC) has been established in the last century as a tool for comprehensive hydrodynamic characterization of macromolecules^{22–25} and particles^{26–29} including the investigation of interactions between macromolecules.^{7,13,15,18,19} In this context, it was recently demonstrated how interaction parameters for perfectly monodisperse species, in particular the second virial coefficient, from AUC experiments are in line with theoretical predictions from DLVO theory, which are based on interaction potentials.^{12–14,30,31} Besides AUC, analytical centrifugation (AC) has emerged as a useful technique for the characterization of particles as it provides access to full sedimentation coefficient distributions with significantly less experimental effort.^{2,32,33}

Despite of these recent advances in studying non-ideal sedimentation phenomena using AUC and AC, there is still a significant lack of understanding how mass transport of polydisperse particle systems is affected by particle size and electrostatic interactions. In particular, software tools available for the analysis of sedimentation non-ideality are limited to particles systems with low polydispersity targeting the interactions of discrete species in a narrow size range. However, the complex interplay between non-specific particle interactions and the effect of particle size in general on sedimentation properties still needs to be further investigated.

Recently, we used lattice Boltzmann (LB) simulations to study the sedimentation of particles which interact according to a DLVO interaction potential.³⁴ The simulations revealed that the sedimentation behavior is strongly influenced by electrostatic interactions, if the extent of electrostatic interactions is in the order of the mean interparticle spacing. The ratio of the mean interparticle spacing and the extent of

electrostatic interactions can be quantified through the so-called repulsion range.³⁴

While the latter was established using LB simulations,³⁴ in this study, our aim is to establish a characterization framework for the investigation of interparticle interactions by probing sedimentation non-ideality. From an experimental point of view, access to the average repulsion range is provided *via* the Debye screening length, which was determined by means of conductivity measurements of the dispersions. With the experimentally determined repulsion range at hand, we demonstrate that the effect of polydispersity on concentration-dependent sedimentation can be assessed through a combination of LB and Brownian dynamics (BD) simulations. We study particle systems with moderate polydispersity and vary the particle size over a large range, spanning one order of magnitude and thus a variety of repulsion ranges. These experiments require the use of AC at low rotor speeds because of the high settling velocities of the larger particles.

Theoretical background

Fundamentals of sedimentation

In AC experiments, particles are subject to centrifugal fields amounting up to $4000 \times g$.^{32,33,35} The drag of particles in a fluid depends on the sedimentation velocity of the particle and the translational and orientation-averaged friction coefficient f ,³⁶ which can be calculated in the limit of infinite dilution as:

$$f = 3\pi\eta x_h \quad (2)$$

whereby η denotes the viscosity of the solvent and x_h is the hydrodynamic diameter of the particle. The sedimentation coefficient s is related to the orientation-averaged friction coefficient by:²⁶

$$s = \frac{u}{\omega^2 r} = \frac{m_p \left(1 - \frac{\rho_s}{\rho_p} \right)}{f} \quad (3)$$

Here, the particle mass is m_p , the solvent density is ρ_s and the particle density is ρ_p . The angular velocity and the radial position within the measurement cell are denoted as ω and r , respectively.

For small particles, sedimentation is significantly overlaid by stochastic Brownian motion leading to band broadening due to diffusional effects.³⁷ Sedimentation and diffusion in sector-shaped cells are thereby described by Lamm's equation.³⁸ However, for particles larger than typically 20 nm, which are the focus of this work, the effect of diffusion on sedimentation boundaries can be safely neglected.³³ In such cases, the sedimentation coefficient can be directly converted to a sedimentation-equivalent particle size x :²⁶

$$x = \sqrt{\frac{18\eta s}{(\rho_p - \rho_s)}} \quad (4)$$

Experimentally, the temporal and spatial evolution of the particle concentration is measured in AC using an optical

detection system tracking the extinction of the sedimenting particles.³² The sedimentation coefficient is given by the position of sedimentation boundaries and typically assessed through direct boundary modelling or so-called parameter-free approaches, which directly analyze measured sedimentation data without any prior knowledge of the sedimenting species.^{32,39}

Description of non-ideal sedimentation

As noted before, Stokes' law loses its validity beyond the limit of infinite dilution because the particles' sedimentation is influenced by a volume flux of solvent in opposing direction.⁸ In addition, the viscosity and density of the dispersion increase with particle volume fraction and electrostatic interactions can alter the sedimentation properties further.¹⁶ Hydrodynamic non-ideality effects are typically taken into account by a first-order approximation of the friction coefficient as function of particle mass concentration c :^{15,40}

$$\frac{f}{f_{c=0}} = 1 + k_s c \quad (5)$$

The friction coefficient at infinite dilution is denoted as $f_{c=0}$. Following this concept, the Gralen coefficient k_s was introduced as an empirical constant describing the linear dependence of the friction coefficient with mass concentration. As the friction coefficient is inversely proportional to the sedimentation coefficient, eqn (5) can be rewritten as:

$$s = \frac{s_0}{1 + k_s c} \quad (6)$$

The sedimentation coefficient at infinite dilution is denoted as s_0 . The sedimentation coefficient can also be expressed in terms of the volume fraction c_v of the particles instead of their mass concentration:¹¹

$$s = \frac{s_0}{1 + K_s c_v} \quad \text{with } K_s = \frac{k_s}{\nu_s} \quad (7)$$

The swollen specific volume of the particles ν_s accounts for entrained solvent dragged along with the particles. A linear dependence of k_s or K_s on particle volume fraction is observable at moderate volume fractions below 5–10 vol%.^{20,21} At higher concentrations, coupling effects occur and lead to non-linear relationships for the friction coefficient. Fig. 1 depicts a graphic representation of eqn (6).

Concentration-dependent sedimentation of neutral hard spheres

Based on the fundamental exploratory work on hydrodynamic non-ideality by Schachman⁸ and Burgers,⁹ Batchelor considered the sedimentation behavior of neutral hard/rigid spheres in a surrounding fluid.^{16,17} Such interactions are termed hard-sphere type interactions throughout this manuscript. He obtained the following term for the sedimentation coefficient at low volume fractions:

$$s = s_0(1 - 6.55c_v) \quad (8)$$

Several contributions must be considered to the overall non-ideality effect, *i.e.* solvent backflow, hydrodynamic

Fig. 1 Reciprocal measured sedimentation coefficients versus volume fractions for plain silica particles with a nominal diameter of 100 nm. The sedimentation coefficient at infinite dilution is obtained from extrapolation to zero concentration. The apparent slope is retrieved from a linear fit of the data as schematically indicated.

particle–particle interactions and particle–solvent interactions. Notably, eqn (8) can be derived from eqn (7) by means of a Taylor series approximation at low concentrations, as is shown in Fig. S8 (ESI†).

The concentration dependency of the sedimentation coefficient for neutral hard spheres was shown to be independent of the sedimentation equivalent particle size for spherical particles.^{16,17} Conclusively, hard-sphere type interparticle interactions can be described by a K_s value of 6.55. In addition to the considerations of Batchelor, interparticle interactions caused by electrostatic repulsion and shape anisotropy can result in a more pronounced concentration dependence of the sedimentation coefficient.^{14,15,34}

Approaches to describe sedimentation non-ideality

The Gralen coefficient. The Gralen coefficient has been widely applied to describe the concentration dependence of the sedimentation coefficient of macromolecules or to account for specific and non-specific protein–protein interactions.^{15,18,19} It is typically retrieved by measuring the apparent sedimentation coefficient at various particle volume fractions as shown in Fig. 1. Johnson and Harding discussed the discrepancies between the extracted values for K_s from different theoretical studies and showed that they can be matched if the respective sedimentation coefficients are correctly correlated to the solution density.¹¹ Moreover, Harding and Johnson studied the turnip-yellow mosaic virus as a suitable spherical model substance to test hydrodynamic theories of transport.⁴¹ Pavlov *et al.* measured Gralen coefficients of polymer macromolecules and determined values between 14 mL g^{−1} and 260 mL g^{−1} depending on the molar mass and other polymer particle parameters.⁴² Planken *et al.* measured K_s via AUC for sufficiently strong particle attraction and showed an enhancement of the sedimentation rate.⁴³ Jansen *et al.* measured the sedimentation velocity of silica spheres in AUC experiments.⁴⁴ Isotropic

attraction between the particles resulted in a K_s value of less than 6.55. Thus, isotropic attraction between the particles accelerates the sedimentation rate.⁴⁴ Only recently, it has been shown by Schuck and co-workers that k_s can be derived for discrete species under certain boundary conditions from a single SV experiment.^{7,12} Using BD simulations, we could further demonstrate that this works well for AC experiments.^{4,37}

Hindrance function. Besides the Gralen coefficient, there are several other approaches to account for the reduction of the particles' sedimentation rate. Only recently, a mathematical model has been developed by Antonopoulou *et al.*, which predicts the reduction of the sedimentation velocity by calculating a theoretical maximal volume fraction from interparticle spacing.³ Given the case that the sedimentation velocity gradually decreases with the volume fraction, the concentration dependency of the sedimentation coefficient can be described by the hindrance function h_{hind} ,^{2,45} which is defined in the ESI† in eqn (S1)–(S3).

Interparticle interactions according to DLVO theory. The DLVO theory describes interparticle interactions based on an overall interaction potential,⁴⁶ which includes van der Waals and electrostatic interactions. The latter is described as a function of the interparticle distance a :⁴⁷

$$\varphi_{\text{el}}(a) = \pi x^2 \varepsilon_0 \varepsilon_r \zeta^2 \frac{\exp\left(-\frac{1}{\lambda_D}(a-x)\right)}{a} \quad (9)$$

ζ is the zeta potential and a is the distance of the centers of two particles. The electrostatic potential scales with the Debye screening length λ_D which can be calculated for known ionic strength I and dielectric constants ε_0 and ε_r :

$$\lambda_D = \sqrt{\frac{\varepsilon_0 \varepsilon_r k_B T}{2 N_A I e Z^2}} \quad (10)$$

Here, the elementary charge and Avogadro's constant are denoted as e_Z and N_A , respectively. The approximation employed for the repulsive part of the DLVO interaction in eqn (9) assumes spherical particles of a size similar to or smaller than the Debye length and is independent of whether constant charge or constant potential boundary conditions are chosen on the particle surface.⁴⁸

For the purpose of this work, DLVO interaction potentials were applied in order to describe interparticle interactions *via* LB simulations. The Debye length as well as the zeta potential were measured experimentally and used throughout the simulations. In principle, this would provide the opportunity to derive interaction potentials directly from sedimentation studies.

Materials and methods

Synthesis and preparation of the particle dispersions

All chemicals were used as received without further purification. The solid content of the synthesized particle dispersions was determined by measuring the weight loss of evaporated solvent. Synthesized or commercial particle dispersions were diluted

using ultrapure water (18.2 MΩ cm; total oxidizable carbon <10 ppb) to the desired concentrations.

Silica particle synthesis. Silica particles with diameters ranging from 100 nm to 500 nm were synthesized according to the Stöber process.^{49,50} In short, 150 mL of ethanol (99.9%, Sigma Aldrich) was mixed with 32.4 g ultrapure water. Ammonium hydroxide (28–30% NH₃ basis, Sigma Aldrich) in concentrations ranging from 0.1 M (2.2 g) to 0.7 M (10.0 g) was added under constant stirring. Next, 17.5 g (0.28 M) tetraethylorthosilicate (TEOS; 98%, Sigma Aldrich) in 50 g ethanol was added to the reaction mixture and kept under constant stirring at 800 rpm. The reaction proceeded for at least 24 h at room temperature. The resulting plain silica particles were purified by centrifugation and redispersion in deionized water.

Commercial silica particles. Commercial plain silica particles and amino-functionalized silica particles with particle sizes ranging from 200 nm to 1200 nm were purchased from Micromod Partikeltechnologie GmbH, Rostock. For each sample, a stock solution of 50 g L^{−1} was obtained and ultra-sonicated for 10 min prior to analysis or dilution. Representative particle size distributions for various nominal diameters have been measured *via* sedimentation velocity AC (SV-AC) experiments at low concentrations and are provided in the ESI† in the left panel of Fig S2.

Fluorescently labelled silica particles. Commercial fluorescently labelled silica nanoparticles, sicastar[®]-greenF, with a nominal diameter of 30 nm were obtained from Micromod GmbH, Rostock. The stock solution had a particle mass concentration of 25 g L^{−1} and was ultra-sonicated for 10 min prior to analysis.

Polystyrene particles. Polystyrene particles with diameters ranging from 150 nm to 800 nm were synthesized by a surfactant-free emulsion polymerization.^{51,52} In short, double deionized water was heated to 70 °C in a three-neck round bottom flask under constant purging with nitrogen. The monomer styrene (ReagentPlus with 4-*tert*-butyl catechol as a stabilizer, ≥99%, Sigma Aldrich) was cleaned by shaking with 0.1 M sodium hydroxide solution and subsequent passing over an aluminium oxide column to remove the inhibitor. Subsequently, it was added to the water phase under constant stirring. After 10 min equilibration time, the co-monomer acrylic acid (Sigma Aldrich, 99%) dissolved in 5 mL deionized water was added and the reaction mixture was left to equilibrate for another 10 min. After 30 min, the polymerization was started by addition of the initiator ammonium persulfate (APS, 98% Sigma Aldrich) dissolved in deionized water. The radical polymerization was continued for 24 h under permanent stirring at 70 °C under nitrogen atmosphere. Representative particle size distributions for various nominal diameters have been measured *via* SV-AC at low concentrations and are provided in the ESI† in the right panel of Fig S2.

Analytical centrifugation

AC experiments were carried out according to ISO 13318-2 using the LUMiSizer LS611 (LUM GmbH, Berlin, Germany). The temperature was set to 20 °C throughout all measurements. Sedimentation data was acquired at wavelengths of

470 nm and 865 nm. The wavelength was chosen such that extinction properties were in the order of unity or less for each sample. Polycarbonate cells with path lengths of 2 mm were used. For all particle dispersions, the rotor speeds were chosen such that all particles sedimented within 1.5 h.

Analytical centrifugation with sector-shaped centerpieces

For AC measurements with the sector-shaped cells, a custom-made LUMiSizer from L.U.M. GmbH was used.³⁵ Data acquisition with this instrument was conducted at wavelengths of 470 nm and 865 nm. The temperature was set to 20 °C and kept constant. Unless otherwise stated, the rotor speed was varied such that all NP systems sedimented within 2 h.

Analysis of sedimentation data

Evaluation of sedimentation data was carried out by the parameter-free approach³² implemented in the SEPView software, referred to as 'constant position', which tracks the optical signal in the AC at a fixed radial position. From this, the sedimentation velocity distribution was determined. In the analysis procedure, averaging over three radial positions was performed for better statistics.³²

Furthermore, data from BD simulations was evaluated by means of a modified direct boundary model developed by Walter *et al.*³³ in order to study the effect of sample polydispersity. Unless otherwise stated, a set of 100 sedimentation coefficients was used for each analysis, while the limits were adjusted based on the sedimentation properties of the individual samples.

Zeta potential measurements

Zeta potential measurements were carried out using a Nano ZS Zetasizer (Malvern Instruments GmbH, Germany). All samples were measured at a fixed particle volume concentration of approximately 5.5×10^{-3} .

Conductivity measurements

Conductivity measurements were performed using the conducting meter from the Wissenschaftlich-Technische Werkstätten, Weilheim. For each sample, a dilution series was prepared at six different volume concentrations and interpolation of the data was performed to extract the conductivity at a specific particle volume fraction by applying a linear fit. Prior to each measurement, ultrapure water was measured as a reference. The conversion of the measured conductivity to ionic concentration is achieved by means of the molar conductivity as shown in eqn (S4) provided in the ESI.† For ions with a low ion concentration, which is the case for all silica and polystyrene dispersions, the molar conductivity can be assumed to be equal to the limiting conductivity. The exact ionic species are unknown, but their nature can safely be assumed based on the chemicals that are used in the synthesis. The molar limiting conductivity of ionic species at a temperature of 20 °C can thus be assumed to be equal to $6 \text{ mS m}^2 \text{ mol}^{-1}$ for a single charged species, and $12 \text{ mS m}^2 \text{ mol}^{-1}$ for an ionic pair, which closely matches the molar limiting conductivity of several species, such as NaCl.⁵³

Estimation of concentration-dependent sedimentation coefficients

The calculation of the hard-sphere model and the respective apparent slopes throughout the result section was performed as follows. For each sedimentation equivalent particle size, a sedimentation coefficient at infinite dilution was calculated based on eqn (3) using the density of silica and solvent properties of water. Moreover, the apparent slope was calculated based on eqn (6) using a K_s of 6.55 for particle volume concentrations of 1×10^{-4} and 1×10^{-2} .

Lattice Boltzmann simulations

The LB simulations were conducted according to the method as presented previously by Jung *et al.*³⁴ In short, a discretized simulation box is filled with fluid and particles under periodic boundary conditions. The dynamics of the fluid are simulated with the LB method. Particle positions in each spatial dimension evolve from Langevin dynamics. Pairwise interparticle forces are taken into account by DLVO interactions according to eqn (9) using $\zeta = 50 \text{ mV}$, the dielectric permittivity of water and variable Debye lengths corresponding to the measured conductivity. Interparticle interactions at distances much larger than the Debye length where DLVO interactions become negligibly weak are cut off for numerical efficiency. The particles and the fluid are coupled by interpolating the fluid flow *via* a frictional force according to the method of Ahlrichs and Dünweg.⁵⁴ Further details are provided in the work by Jung *et al.*³⁴

Results and discussion

Determination of the extent of electrostatic interactions

Our recent theoretical studies on non-ideal sedimentation of particles *via* LB simulations revealed that, in addition to hard-sphere type interactions, particle interactions governed by electrostatics, which can be quantified by the Debye screening length and the particles' zeta potential, directly influence their concentration-dependent sedimentation properties.³⁴ In particular, electrostatic interactions significantly affect sedimentation as long as the Debye screening length is in the order of the interparticle distance.

In our theoretical description, a general measure for the impact of electrostatic interactions on sedimentation non-ideality is the repulsion range α , which is defined according to:

$$\alpha = \frac{\zeta}{\hat{s}_{cv}} \quad (11)$$

The parameter α relates ζ as the Debye screening length λ_D normalized by the particle radius to $\hat{s}_{cv}$ as the surface-to-surface distance $\hat{s}$ normalized by the particle diameter, indicated by the subscript c_v . In other words, α relates the extent of electrostatic interactions to the mean interparticle distance. The normalized surface-to-surface distance of two particles $\hat{s}_{cv}$ is defined as:

$$\hat{s}_{cv} = \frac{2}{x} \sqrt[3]{\frac{V_p}{c_v}} - 2 = \sqrt[3]{\frac{4\pi}{3c_v}} - 2 \quad (12)$$

V_p is the volume of a particle with diameter x . Notably, both the Debye screening length and the surface-to-surface distance are herein normalized to the particle size.³⁴ However, the normalization changes neither the meaning of the repulsion range nor the conclusions that can be drawn from its interpretation. Through LB simulations with varying particle size, Debye screening lengths and zeta potentials, it was demonstrated how electrostatic interactions have significant influence on the sedimentation behavior, which translates into a decrease of the sedimentation velocity at elevated particle volume fractions at repulsion ranges beyond 0.05.³⁴ In the case of α approaching zero, the sedimentation behavior follows a hard-sphere behavior according to Batchelor's law.^{16,17,34}

The mean distance is retrieved when averaging over all particle distances and it was shown by LB simulations that the average distance accounts for the effect of electrostatic interactions when comparing the results from several simulations.

From an experimental point of view, we have access to the repulsion range through the particle volume fraction according to eqn (12) and conductivity measurements, as the conductivity of the solution is directly related to the ionic strength, which is the only unknown in eqn (10).

Therefore, in order to assess the extent of electrostatic forces within our measurements, we measured the conductivity of the silica dispersions with various nominal diameters, which is directly related to the salt content of the dispersions and hence the Debye length of the system. Our conductivity measurements revealed that the Debye length of all plain silica particle dispersions with various nominal diameters is approximately (12.1 ± 2.0) nm for a fixed particle volume fraction of 5.5×10^{-3} . The electrolyte concentration and hence the Debye screening length is a function of the particle volume fraction due to dilution with ultrapure water, as shown in Fig. S3 (ESI†). Moreover, slight variations in the Debye screening length were observed when comparing different samples which is visualized in Fig. S4 (ESI†). However, these changes are marginal with respect to the changes in the interparticle distance and can thus safely be neglected herein. Subsequently, the repulsion range α can be calculated for several sedimentation equivalent diameters at a fixed volume concentration.

Our experimental results are provided in Fig. 2, which can be divided into two parts. At elevated values for α , particle interactions are governed by electrostatic effects. Below a threshold of 0.05,³⁴ the interparticle distance exceeds the extent of the electrostatic forces. Consequently, the sedimentation behavior in this region should follow a hard-sphere behavior. As can be seen in Fig. 2, the experimentally determined α -values for all silica particles with a sedimentation equivalent diameter above 100 nm indicate that the particles behave like hard spheres.

Based on these findings, LB simulation were performed with parameters corresponding to the experimental conditions. We mimicked the electrostatic properties of silica particles with nominal sizes larger than 100 nm by inserting the experimentally determined zeta potentials (see Fig. S9, ESI†) in our simulations. Notably, the LB simulations take local changes of

Fig. 2 Repulsion range α , which is the ratio of the normalized Debye screening length ξ and the calculated normalized interparticle distance δ_{c_v} , as a function of the mean sedimentation equivalent diameter of the silica particles. The Debye length was experimentally determined based on conductivity measurements to be (12.1 ± 2.0) nm. The mean interparticle distance is calculated for a fixed volume concentration of 5.5×10^{-3} . The error bars correspond to the variation of the Debye screening length from the different conductivity measurements for different nominal particle sizes.

the interparticle distances into account as these are a statistical quantity and thus not necessarily equivalent to the mean distance calculated from eqn (12). As a first validation step, hard spheres were simulated for comparison and respective data analysis showed perfect reproduction of Batchelor's predictions where $K_s = 6.55$ as shown by Jung *et al.*³⁴ Next, the electrostatic interaction potential was calculated based on the DLVO potentials. The LB simulations mimicking our experimental conditions from Fig. 2 confirmed that the apparent slopes of all silica particles that are larger than 100 nm are practically identical to the theoretical predictions from Batchelor's theory, as is shown in Fig. S5 (ESI†). This result is perfectly in line with the experimentally determined repulsion ranges in Fig. 2.

Moreover, we mimicked the sedimentation behavior of 30 nm silica particles with a repulsion range of 0.114 in our LB simulations and confirmed a significant increase of the apparent slope of the normalized sedimentation velocity to 18.1.

Sedimentation non-ideality of polydisperse particles

However, as colloidal particles are associated with a polydispersity in size, the interparticle distance is also a function of the particle size distributions. Moreover, it is known from literature that the presence of one species significantly impacts the sedimentation behavior of other species in solution,³¹ which is typically referred to as the Johnston–Ogsten effect.⁵⁵ Both effects were not accounted for in our previous LB simulations as we considered perfectly monodisperse particles to keep computational costs moderate. Conclusively, we studied the effect of polydispersity on the sedimentation non-ideality through auxiliary BD simulations to preclude any effect of polydispersity in our experimental framework and our results.

In principle, the whole sedimentation coefficient distribution and thus also particle size distributions is accessible from SV-AUC experiments.^{32,33} The width of the particle size distribution is a measure for polydispersity. From respective analysis of sedimentation data at high dilution, we determined a polydispersity in the order of 15–25% for the silica particles and 5% for our polystyrene particles (see Fig. S2, ESI†). Therefore, in order to account for polydispersity, we calculated the repulsion range with an increase or decrease in the average interparticle distance assuming a deviation in size of $\pm 25\%$ while keeping the Debye screening length constant (see Fig. S6, ESI†).

In order to preclude any effect of polydispersity on our experimental study, we conducted BD simulations^{37,56} for silica particles with a nominal diameter of 600 nm and a standard deviation of 15%, mimicking the experimentally determined polydispersity. We considered the particle–particle interactions through BD simulations by using a K_S of 6.55, which was predicted by LB simulations for this particle size based on the experimentally determined repulsion range. We further simulated one case with a K_S of 21.6, representing a strongly interacting case for this particle size.³⁴ The particle volume fraction was varied between 2×10^{-3} and 1×10^{-2} .

For the first case, a $K_{S,\text{retrieved}}$ of 6.4 (deviation of 2.3% from the input parameter) was retrieved from a linear fit of the analyzed apparent median sedimentation coefficients at various particle volume fractions as shown in Fig. 3. A deviation of 3.4% was found for the case of $K_{S,\text{BD}} = 21.6$ (data are shown in Fig. S14, ESI†). Due to this very small effect, we conclude that the retrieval of K_S from median sedimentation coefficients is not affected by the polydispersity at moderate particle volume fractions. Conclusively, our BD simulations show that the particle size distributions of the silica dispersions are sufficiently narrow, hence the sedimentation non-ideality is not affected. Moreover, it has to be noted that we observe a linear dependence of the normalized sedimentation rate on the particle volume fraction, as can be seen

Fig. 3 Apparent median sedimentation coefficients s normalized by the sedimentation coefficient at infinite dilution s_0 as retrieved from BD simulations (blue data points and dashed line) and normalized apparent sedimentation coefficients from eqn (7) (black data points and straight line).

in Fig. 3. These observations from simulated data are key to experimentally study interparticle interactions of polydisperse particles at moderate concentrations, which is subsequently carried out.

Data analysis further revealed that the apparent sedimentation coefficient distributions are self-sharpened at increasing particle volume fraction, as depicted in Fig. S15 (ESI†). The extent of self-sharpening increases at higher particle volume fractions. This observation is in line with theoretical expectations and can be explained as follows. The effect of polydispersity results in a broadening of the sedimentation boundaries due to the slight differences in the sedimentation velocity of the particles with a different size (see Fig. S7, ESI†). In addition, the boundaries are affected by sedimentation non-ideality as the concentration increases within the sedimentation boundary. These mutual effects are well described by the BD simulations and reflect upon the apparent sedimentation coefficient distributions.⁴ It has to be noted that only the effect of local dispersion and hence varying particle concentration is considered by BD, while at the same time a size-independent K_S value was used. To obtain a full picture of the sedimentation of polydisperse systems including the cross-correlation of interactions of differently sized particles, specifically tailored LB simulations would be required. This will be in particular indispensable for polydisperse multimodal particle systems. However, for the monomodal systems with moderate polydispersity studied in this work, the conducted simulations have proven to be sufficient.

In summary, our theoretical predictions from both LB simulations and BD simulations pave the way for studying colloidal particles over a broad size range *via* sedimentation analysis in order to establish a holistic analytical tool to study interparticle interactions at varying repulsion ranges.

Particle interactions for plain and amino-functionalized silica particles

Motivated by our theoretical predictions, we herein aim to develop a comprehensive approach to experimentally investigate sedimentation non-ideality for colloidal systems to retrieve the effect of hard-sphere type and electrostatic interactions over a broad particle size range. In this context, silica particles are chosen as a model system as Stöber silica has been thoroughly investigated^{57–61} and is used in various applications.^{62–64} Plain silica particles are available over a broad size range and silica bulk suspensions contain high volume concentrations, which is an ideal prerequisite to study the effect of particle size on sedimentation non-ideality.

As a first test case, we measured in a single AC experiment the sedimentation properties of silica particles with a nominal diameter of 100 nm spanning over a broad range of particle volume fractions. For each particle volume fraction, we determined the sedimentation coefficient distribution, from which the median sedimentation coefficient was extracted.

Fig. 1 shows that the measured inverse median sedimentation coefficient scales linearly with the particle volume fraction, which confirms the applicability of eqn (7). From a linear

regression of the data, the apparent slope and the y-intercept is retrieved as exemplarily shown in Fig. 1 and Fig. S1 (ESI†). The latter provides the sedimentation coefficient at infinite dilution, which is converted into a sedimentation equivalent diameter using eqn (4). We evaluated the apparent slopes for plain silica particles of various nominal diameters ranging from 100 nm to 1200 nm. Furthermore, as silica particles can carry different surface functionalization that affects colloidal stability and particle interactions,^{65,66} we further determined the apparent slopes for amino-functionalized silica particles for various nominal diameters. The stability of all samples was confirmed by auxiliary zeta potential measurements, which are shown in the ESI† in Fig. S9.

For each silica dispersion, a dilution series with twelve different particle volume fractions was prepared and analyzed within a single SV-AC experiment. We made sure that the range of volume fractions varied at least by a factor of three in order to properly resolve the impact of sedimentation non-ideality. From every dilution series, the apparent slope and the sedimentation equivalent diameter at infinite dilution were extracted which are presented in Fig. 4. The linear regression model yielded statistical uncertainties of the individual linear fits, which provided the error bars for the retrieved apparent slopes. The reproducibility of the apparent slope was confirmed by determining the sedimentation properties of silica particles at various particle volume fractions in two separate AC runs (see Fig. S10, ESI†).

The apparent slopes for hard spheres were further calculated based on Batchelor's theory with a value for $K_s = 6.55$ in order to compare the experimental observations with theoretical predictions.^{16,17} Our results depicted in Fig. 4 show that the concentration dependencies of the investigated plain silica particles such as the amino-functionalized silica particles follow a hard-sphere behavior.

Fig. 4 Apparent slope as a function of the sedimentation equivalent diameters for commercial plain silica particles (grey circles), plain silica particles from own synthesis (red triangles) and amino-functionalized (orange symbols) silica particles. The results from LB simulations mimicking the experimental conditions are further shown (open quadratic symbols). Moreover, the prediction from Batchelor's hard-sphere model is depicted (dashed black line).

It has to be noted that we herein choose to discuss the apparent slope of the inverse sedimentation coefficients as a function of the sedimentation equivalent diameters. From simulated data, the Gralen coefficient as well as the apparent slope can be reproduced,⁴ even for the case of polydisperse particles, as established by BD simulations and shown in Fig. 3. However, when determining it experimentally, the Gralen coefficient or K_s value was shown to be associated with minor uncertainties in the case of polydisperse particles. While the Gralen coefficient is established in the field of biomolecules or synthetic polymers, application to particle technology revealed challenges for our systems. The main reason for these challenges is attributed to the fact that the silica particles varied by about one order of magnitude in size, leading to variations in the sedimentation coefficient at infinite dilution of at least two orders of magnitude (see Fig. 4). In consequence, small uncertainties for the determination of the sedimentation coefficients at infinite dilution due to slight temperature instabilities^{67,68} and different ranges of particle volume fractions for different nominal particle sizes can result in significant variations of the Gralen coefficient or K_s values. In contrast, the apparent slope was found to be much more robust against such minor uncertainties. In that context, the reproducibility of the apparent slope was found to be excellent when conducting the concentration series several times, while the retrieved K_s values showed higher variation (see Fig. S11, ESI† for the case of silica particles with varying nominal diameters between 100 nm and 500 nm). We estimated the error propagation for k_s in eqn (S10) (ESI†) and for the apparent slope in eqn (S16) (ESI†) and further visualized the effect of small changes in K_s on the apparent slope in the right panel of Fig. S11 (ESI†).

As a further proof-of-principle experiment, we dried and washed silica particles with a nominal diameter of 500 nm and dispersed them in an acetate buffer solution at pH 5. This way, perfect control over the ionic strength and hence the Debye length was achieved. Consequently, we determined in two independent AC runs apparent slopes of $(4.97 \pm 0.31) \times 10^{-5} \text{ Sv}^{-1}$ and $(6.12 \pm 0.67) \times 10^{-5} \text{ Sv}^{-1}$. These experimental results correspond to hard-sphere type interparticle interactions as is shown in Fig. S12 (ESI†). These results further agree with our results in Fig. 4, thus confirming the validity of our approach. Moreover, our observation for the silica particles with respect to interparticle interactions are in line with findings in literature.^{69–71}

For validation of our studies in rectangular cells, we determined the apparent slopes for plain silica particles with a nominal particle size of 200 nm and 600 nm using AC cells with sector-shaped centerpieces and a redesigned centrifugal rotor.³⁵ We found perfect agreement with our previous results as can be seen in Fig. 4. This observation is in line with the theoretical expectations as the sedimentation properties of non-diffusing particles can be accurately determined in the common setup.³³

So far, our investigations at moderate concentrations below $\sim 2 \text{ vol}\%$ indicate that the concentration dependence of the

silica particles between 100 nm and 1200 nm can be explained solely by hard-sphere type interactions.

As a proof-of-principle we further repeated the same investigation for polydisperse polystyrene particles, which are well-characterized and are available over a broad size range.^{52,72,73} In addition, they show reduced polydispersity ($\sim 5\%$) as can be seen in both panels of Fig. S13 (ESI†). We determined median sedimentation coefficients for particle volume concentration ranging from 1×10^{-3} and 5×10^{-3} for nominal particle diameters ranging from 200 to 800 nm. The apparent slopes of polystyrene particles ranging from 200 nm to 800 nm further agree well with the predictions for hard-sphere type interactions. In summary, it is possible to measure sedimentation non-ideality for various repulsion ranges for particle dispersions with low and moderate polydispersity.

Outlook on tailoring interactions

So far, our results provide experimental evidence for the prediction of sedimentation non-ideality of differently sized particles from theoretical studies.³⁴ Throughout all our experimental investigations, the sedimentation properties were dominated by hard-sphere type interactions, which is explained by repulsion ranges below 0.05. However, based on our findings in Fig. 2 for the experimentally determined repulsion range, the sedimentation non-ideality of 30 nm silica nanoparticles must be significantly influenced by electrostatic interactions.

From an experimental perspective, there are several strategies to investigate systems at larger repulsion ranges. The most common approach is to study particles at elevated particle volume fractions or to study smaller nominal particles sizes as indicated in Fig. 2.³⁴ Both approaches scale the interparticle distances towards the extent of electrostatic interactions, hence the Debye screening length. However, this is a challenging task from an experimental point of view. On the one hand, silica or polystyrene particles with nominal diameters beyond 100 nm exhibit a large extinction cross-section which makes the optical detection throughout AC experiments at large volume fractions nearly impossible without further technical modifications of the detection system or measurement cells.⁷⁴ On the other hand, smaller particles, *i.e.* with nominal particle sizes below 100 nm, show a significantly reduced extinction cross-sections and hence smaller mass extinction coefficients. Therefore, the concentration range that can be optically detected within an AC run in the current setup is also limited.

Here, we aim to overcome these challenges by studying the sedimentation non-ideality of fluorescently labelled silica nanoparticles with a nominal size of 30 nm *via* SV-AC. This is of particular importance as their sedimentation behaviour is strongly influenced by the effect of electrostatic interactions. Their fluorescent nature makes these particles optically detectable even though their size is small, while the interparticle distance is significantly reduced. First, we confirmed a linear dependence of the median sedimentation coefficient on the particle volume fraction, which is shown in Fig. 5. The linear relationship of the inverse median proves the applicability of eqn (7) even in the presence of electrostatic interactions as long as the concentrations are not too high. Second, the apparent slope was determined to be $(7.75 \pm 0.71) \times 10^{-2} \text{ Sved}^{-1}$, which is 4.6 times larger than the

Fig. 5 Measured reciprocal apparent sedimentation coefficients for fluorescent silica particles with a nominal diameter of 30 nm at various particle volume fractions. A linear fit was applied to the data for the determination of the apparent slope and the x-intercept, which provides the sedimentation coefficient at infinite dilution.

theoretical value for hard-sphere type interactions (apparent slope = $1.60 \times 10^{-2} \text{ Sved}^{-1}$ for $K_s = 6.55$).

For a comparison with our theoretical results, we converted the inverse sedimentation coefficients to sedimentation velocity normalized to the value at infinite dilution (see Fig. S16, ESI†). From a linear fit of the data, we analyzed sedimentation non-ideality by extracting a slope of 22.2. Remarkably, this result is well in line with our previous predictions from LB simulations,³⁴ which predict a slope for the normalized sedimentation velocity of 18.1 for silica particles with a nominal diameter of 30 nm and a repulsion range of 0.114. With this particular result at hand, we believe to have demonstrated how SV-AC experiments can be utilized to quantify the effect of electrostatic interactions for particulate systems.

In summary, our theoretical predictions agree very well with the results from our experimental sedimentation analysis *via* AC. In future investigations, our promising results will not only allow for the comparison of hydrodynamic modelling and sedimentation measurements, but will further provide the opportunity to probe electrostatic interactions on a particulate level *via* macroscopic sedimentation analytics.

Conclusions

The sedimentation non-ideality of particles was recently investigated through lattice Boltzmann simulations. It was demonstrated how the effect of electrostatic interactions can be quantified through the repulsion range, which is given by the ratio of the normalized Debye screening length and the normalized mean interparticle spacing. For repulsion ranges above 0.05, electrostatic interactions impact sedimentation. Below this threshold, the electrostatic potentials do not overlap, hence interactions are of hard-sphere type, which is mainly caused by solvent backflow as predicted by Batchelor's theory.

From an experimental point of view, access to the repulsion range is provided through conductivity measurements, which

yield the salt concentration and hence the Debye screening length of the systems. We experimentally measured the repulsion range for silica dispersion with various nominal particle sizes from 30 nm to 1200 nm. We determined repulsion ranges below the threshold of 0.05 at moderate particle concentrations for silica dispersions with sizes exceeding 100 nm. In contrast, a considerably larger repulsion range of 0.114 was obtained for silica particles with a size of 30 nm.

As all technical dispersions are associated with a particle size distribution, we estimated the effect of polydispersity through a combination of lattice Boltzmann and Brownian dynamics simulations, which can be extended to the case of particles with moderate polydispersity. We excluded any significant distortions of the retrieved sedimentation data due to moderate polydispersity and demonstrated how the concentration-dependence of the sedimentation behaviour can be described well by median sedimentation properties.

Next, we applied a comprehensive experimental methodology for the investigation of sedimentation non-ideality caused by hard-sphere type and electrostatic interactions of colloidal systems spanning over one order of magnitude in size *via* analytical centrifugation. For plain and amino-functionalized silica particles with nominal diameters ranging from 100 nm to 1200 nm, our studies revealed that the nature of the interactions is of hard-sphere type at moderate concentrations below 2 vol%, which is perfectly in line with our the measured repulsion ranges. Conclusively, there is no influence of electrostatic interaction on sedimentation non-ideality for these particles and the given experimental conditions. Moreover, we applied our approach to polystyrene particles with nominal particle sizes ranging from 200 nm to 800 nm and arrived at the same conclusions.

Finally, we demonstrated that particle systems that are influenced by electrostatic interactions can be investigated by analytical centrifugation as well. To this end, the interparticle distance was reduced by investigating fluorescently labelled silica nanoparticles with a nominal size of 30 nm. The retrieved sedimentation non-ideality was found to be significantly influenced by electrostatic interactions. The apparent slope was further shown to be in line with the prediction from lattice Boltzmann simulations. This promising result in particular paves the way towards the determination of electrostatic interactions using sedimentation analytics.

An important prerequisite for future investigations is that particle systems can be studied at volume concentrations up to 10%, which requires the application of measurement cells with very short path lengths (0.1–1 mm). Such developments are currently targeted.

Overall, it is shown that analytical centrifugation serves as an important tool for gaining in-depth understanding of interparticle interactions, which can be described through the repulsion range for the experimental results. Follow-up studies will in particular focus on dispersions with reduced ionic strength, hence large Debye lengths and greater control over the repulsion range, extended sample heterogeneity resulting from multimodality and surface charge, including colloidal systems with long-range electrostatic interactions.

Conflicts of interest

There are no conflicts to declare.

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