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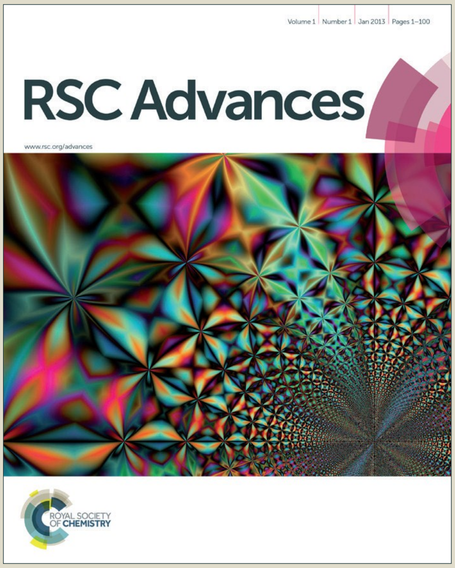

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Experimental determination and analysis of gold nanorods settlement by differential centrifugal sedimentation

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Direct measurement of the sedimentation coefficients of gold nanorods with controlled shape factor was performed using differential centrifugal sedimentation (DCS). The results serve as a testing ground for various existing models describing non-spherical nanoparticles moving in a centrifugal field based on sphericity and force analysis, and point to further refinement.

Sorting polydispersed colloidal nanoparticles into fractions utilizing their differential movement dictated by their size and shape has made significant progress in recent years[1-4] . Among various methods for this purpose, centrifugation-based separation has gained popularity due to its convenient operation and post-treatment. It is also thought to be an efficient approach to maximize the yield of the monodisperse samples[5,6]. Good separation performance using density gradient centrifugation, either in aqueous or organic media, has been reported[7] [8-11]. More recently, Sun *et al* reported the separation of one-dimensional nanostructures self-assembled by gold nanoparticles using ultracentrifugation [12]. To support these efforts and design more quantitative strategy of purification in terms of selectivity and efficiency, better understanding of nanoparticle sedimentation in a centrifugal field is highly desired.

The challenges in analysing non-spherical nanoparticle sedimentation stem from the orientation-dependent drag force under centrifugal field. For example, inconsistent observations on sedimentation have been reported by many groups when they tried to separate and purify colloidal gold nanorods (GNRs). Murphy et al have reported that long GNRs settled preferentially down to bottom because of their larger mass when they used high aspect ratio cylindrical GNRs [13]. Similar phenomenon has been observed in Sun's work where ultrashort single-walled carbon nanotube mixture was separated by centrifugation, which is consistent with Murphy's theoretical assumption [14]. A conclusion has been drawn based on these experimental results that the mass of GNRs is the key factor for GNRs settlement. However, Srinivasarao et al have observed that the GNRs settled faster with shorter length and wider diameter than those with comparable volume or mass but larger aspect ratio [15]. Similar results have also been seen when separating gold nanoparticles (GNPs) from GNRs using traditional centrifugation techniques[6,16], where the sedimentation of GNRs depends more on their diameter than their mass or aspect ratio [15].

Several groups have laid the foundation towards a comprehensive model for analysing nanorod sedimentation under centrifugation. Sharma et al [15] first put forward a model for the GNRs sedimentation coefficient. However, as later pointed out[17], predicting sedimentation velocity of the nanorods with given size and aspect ratio using their equations is difficult since the calculation involves solving several indeterminate equations. He et al later modelled GNRs as Brownian ellipsoidal particles and derived an orientation-averaged sedimentation coefficient along the vertical direction

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of apparent movement [17], but it is lack of experimental data to verify and support his theory. The suitability of the model to real nanorod samples has yet to be confirmed. Moreover, the experimental validation of these works was mostly based on preparative separation using benchtop centrifuges, which could not provide precisely-measured sedimentation coefficients. Therefore, the relationship among the measurable settlement time, fluid properties including viscosity and density, and the feature size, e.g. aspect ratio and diameter of the GNRs need to be established and refined by additional experimental measurement.

Directly measuring the sedimentation coefficients of nanoparticles using DCS simplifies the procedure and avoids solving complicated Lamn equation in analytical ultracentrifugation (AUC). The magnitude of the sedimentation coefficient for gold nanoparticle (nanorods) is about 10^3-10^4 S which is out of the upper limit of AUC (~10^2 S) for precise measuremnts. We carried out differential centrifugal sedimentation (DCS) in a disc centrifuge configuration, which is equipped with a high speed centrifugation motor (up to 24000 rpm) and an optical detector with a detectable size limit down to 10 nm (CPS Instruments, Inc.)[19]. DCS has usually been used to analyse particle size and density, where the size resolution of 0.2 nm has been achieved in ligand-passivated Au clusters [19]. Recently, Dawson *et al* have employed DCS to study the biomolecular-nanoparticle interaction by comparing the difference of the stokes diameter of GNPs obtained before and after the formation of the protein corona[20]. These cases have demonstrated that CPS is an efficient technique to obtain time-space dependent parameters such as size and density. Furthermore, our studies indicate that the technique can be used to measure time-dependent parameter directly e.g. sedimentation coefficient assisted by the use of reference materials.

Gold nanorods with either equal volume or diameter were synthesized by a seeds-mediated method [18]. The UV–visible spectra of the GNRs used in this study are shown in Fig.1, where Fig.1a is for GNRs with equal mass/volume, and Figure. 1b is for GNRs with equal diameter. In Fig.1b, an obvious red-shift of the longitudal surface Plasmon resonance (SPR) peak from 600 to 750nm is observed while the transverse SPR peak keep unchanged. The aspect ratio of the GNRs calculated using the wavelength of their longitudinal SPR peak λ_{LSPR} according to Equation 1 are listed in Table S1 [21].

$$\beta = \frac{\lambda_{LSPR} - 420}{95} \qquad (1)$$

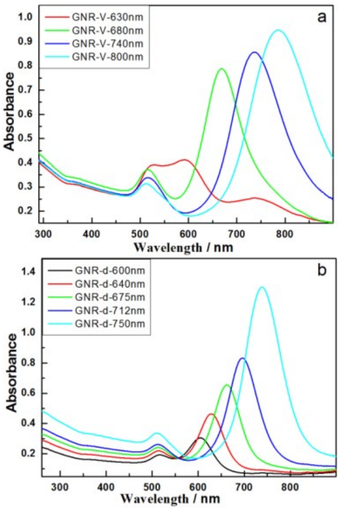
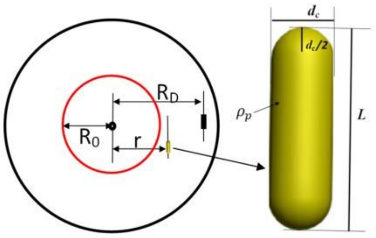


Figure 1. UV-vis spectra of the GNRs with equal mass (a) and equal diameter (b).

As stated above, it is relatively straightforward to obtain sedimentation coefficient in DCS. According its definition ($s=v/\omega^2r$), since $v=dr/dt$, s can be rewritten as $s=dr/\omega^2rdt$ or $d\ln r/\omega^2dt$. After integration, s becomes $\ln(R_D/R_0)/\omega^2t$, where t is the settling time of the particles moving from the starting point R_0 to the detector position R_D as shown in Scheme 1. $\ln(R_D/R_0)$ is difficult to be experimentally determined but it can be obtained by the use of the reference materials (RM), *e.g.* monodisperse SiO_2 nanoparticles with a certified diameter of 164 nm, and with s calculated from Stokes law.



Scheme 1. Schematic diagram of the disc in DCS and the geometry of gold nanorods.

For spherical particles, the settling velocity can be calculated by Stokes law based on the drag-centrifugal force balance and expressed as

$$v = \frac{dr}{dt} = \frac{(\rho_{RM} - \rho_f)\omega^2 r\, d_{RM}^2}{18\eta} \qquad (2)$$

where d_{RM}, ρ_{RM} is the diameter and density of the reference particle, v is its settling velocity. After integration, it can be rewritten as

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$$\ln\frac{R_D}{R_0}=\frac{d_{RM}{}^2(\rho_{RM}-\rho_f)\omega^2 t_{RM}}{18\eta}=\omega^2 k \qquad (3)$$

where t_{RM}, the movement time of the reference particles, can be precisely measured by DCS, $k=\frac{d_{RM}{}^2(\rho_{RM}-\rho_f)t_{RM}}{18\eta}$ while all other parameters such as d_{RM}, ρ_{RM}, ρ_f, ω and η are known under the experimental conditions. Therefore, the sedimentation coefficient (s_m) of nanorods can be obtained using known $\ln(R_D/R_0)$ by:

$$s_m=\frac{\ln(\frac{R_D}{R_0})}{w^2 t}=\frac{k}{t} \qquad (4)$$

In this work, special attention will be paid on the influence of two factors *i.e.* mass and aspect ratio of the GNRs on their sedimentation behaviour.

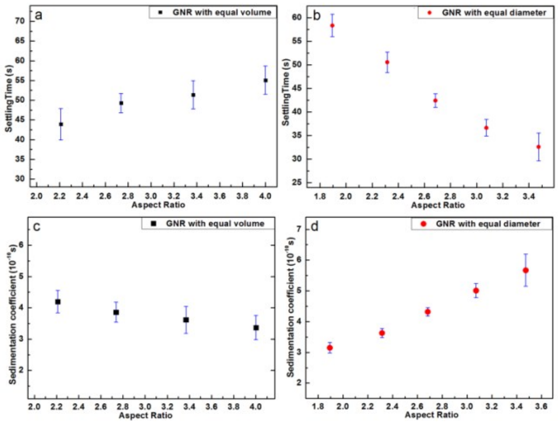

Figure 2 The relationship between the aspect ratio and the settling time (a, b) and the measured sedimentation coefficient (c, b) for the GNRs with either equal volume or diameter.

A series of gold nanorods with either equal volume or equal diameter but different aspect ratio have been interrogated using DCS. As shown in Fig.2a, the settling time of GNRs with equal volume increases gradually with the increase of their aspect ratio. It suggests that nearly-spherical GNRs (with smaller aspect ratio) move faster than their rod-like counterparts. The corresponding sedimentation coefficient (s_m) is displayed in Fig.2c, where s_m decreases with the increase of the aspect ratio. On the other hand, as shown in Fig.2b, for GNRs with equal diameter and different length (aspect ratio), their settling time decreases with the increase of the aspect ratio. As a consequence, the measured sedimentation coefficient increases with the increase of the aspect ratio as shown in Fig.2d. That is consistent with the usual observation that particles would sediment faster with larger mass or smaller drag area (longitudinally paralleling area).

The sedimentation coefficient for a given sample with monodisperse size and known density is generally considered to be a constant under fixed fluid viscosity, density and rotation speed. For GNRs, when the centrifugal force F_c, buoyant force F_b and drag force F_d acting on the particle are transiently balanced, Eq.5 applies, where V is the volume of the particle, ρ_p and ρ_f is the density of the particle and fluid, ω is the revolution speed of the disc, r is the distance from the rotation axis to the location of the particle.

$$V(\rho_p-\rho_f)\omega^2 r=F_d=\frac{1}{2}C_D S_{\parallel}\rho_f v^2 \qquad (5)$$

A few assumptions are made here for the nanorod samples used in this study. First, the influence of the ligand on the surface of the GNRs on the sedimentation is ignored, where the aspect ratio of a nanorod is expressed as $\beta=L/d_c$. Second, the ends of the nanorod are considered as two hemispheres on top of one cylinder with same diameter (d_c) as shown in Scheme 1 and, therefore, its volume can be written as $V=\left(\frac{\pi}{4}\beta-\frac{\pi}{12}\right)d_c^3$. Finally, the motion of the GNRs follows the swirling direction of the fluid inside the chamber, *i.e.* a spiral movement of the nanorods whose longitudinal direction is aligned along the tangent of the disc-concentric circle at any time.

Various empirical formula based on particle shape factors (ratio between particle surface area and that of equivalent volume) exist in the literature which are quite successful in fitting experimental sedimentation velocities for different particle shapes, including prolate ellipsoidal, cylinders, prisms, spheroids, double conical, and chains of particles. For the drag force in Eq.5, $F_d=\frac{1}{2}C_D S_{\parallel}\rho_f v^2$, $S_{\parallel}=d_c^2$ $(\frac{\pi}{4}+\beta-1)$ is the projected area of the nanorods in the settlement direction. Drag coefficient C_D can be correlated with the Reynolds number, Re, the overall sphericity, the lengthwise sphericity and the transverse sphericity, following Leith[22] and Sommerfeld et al's formula[23]. Note Sommerfeld et al[23]modified the Leith expression (Eq.6) using lengthwise sphericity (Eq. 7) and better predicted the drag coefficient of spherical particles[24] and cylindrical particles[25] with the consideration of the particle orientation.

$$C_D=\frac{8}{Re}\frac{1}{\sqrt{\Phi_{\perp}}}+\frac{16}{Re}\frac{1}{\sqrt{\Phi}} \qquad (6)$$

$$C_D=\frac{8}{Re}\frac{1}{\sqrt{\Phi_{\parallel}}}+\frac{16}{Re}\frac{1}{\sqrt{\Phi}} \qquad (7)$$

For nanorods considered in this study, the sphericity Φ is expressed as the ratio between the surface area ($S_e = 4\pi(\frac{d_e}{2})^2 = \pi(\frac{3}{2}\beta - \frac{1}{2})^{2/3} d_c^2$) of the volume equivalent sphere with a diameter of $d_e = (\frac{3}{2}\beta - \frac{1}{2})^{1/3} d_c$ and that (S_{NR}) of the considered nanorod in Eq.8. The crosswise sphericity $\Phi_\perp$ is defined as the ratio between the cross-sectional area (A_e) and the laterally projected cross-sectional area ($S_\perp$) as shown in Eq.9. The lengthwise sphericity $\Phi_\parallel$ is the ratio between the cross-sectional area ($A_e = \frac{\pi}{4} d_e^2 = \frac{\pi}{4}(\frac{3}{2}\beta - \frac{1}{2})^{2/3} d_c^2$) of the volume equivalent sphere and the difference between half the surface area and the longitudinally projected cross-sectional area ($S_\parallel$) of the considered particle as proposed by Sommerfeld and shown in Eq.10. In order to examine the effect of the orientation-based sphericity and drag area on the sedimentation coefficient, several orientations from paralleling to vertical are used to calculate the s. The experimental results show that the calculated *s* value is closest to the measured one when the GNR is perpendicular to its displacement direction. The Reynolds number R_e is expressed as $R_e = \frac{\rho_f d_e v}{\eta} = \frac{\rho_f (\frac{3}{2}\beta - \frac{1}{2})^{1/3} d_c v}{\eta}$, where η is the averaged viscosity of the fluid at the temperature of measurement.

$$\Phi = \frac{S_e}{S_{NR}} = \frac{(\frac{3}{2}\beta - \frac{1}{2})^{2/3}}{\beta} \tag{8}$$

$$\Phi_\perp = \frac{\mathrm{A_e}}{\mathrm{S_\perp}} = \frac{\frac{\pi}{4}\mathrm{d_c^2}\left(\frac{3}{2}\beta - \frac{1}{2}\right)^{\frac{2}{3}}}{\frac{\pi}{4}\mathrm{d_c^2}} = \left(\frac{3}{2}\beta - \frac{1}{2}\right)^{\frac{2}{3}} \tag{9}$$

$$\Phi_\parallel = \frac{A_e}{\frac{S_{NR}}{2} - S_\parallel} = \frac{\pi\left(\frac{3}{2}\beta - \frac{1}{2}\right)^{\frac{2}{3}}}{(2\pi - 4)\beta - \pi + 4} \tag{10}$$

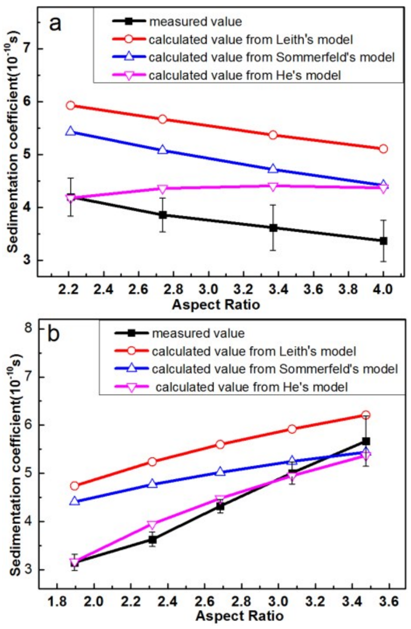

Figure 3 Sedimentation coefficient of the GNRs with equal volume/mass (a) and equal diameter (b) determined by different model (See Supporting Information).

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The sedimentation coefficients calculated from different literature models (for calculation details see Supporting Information S3) are summarized in Fig.3 and compared with our experimental data. For both 3a and 3b, calculated results using Leith's formula (overall and crosswise sphericity) are consistent with experiment in terms of slope, but differ the most in absolute values. Sommerfeld's modified formula appears to provide values closer to the experiments, implying the lengthwise sphericity does give better description for nanorods in this case. For GNRs with equal volume, He's formula provides a close result in values but trend in a reverse way[17]. For GNRs with equal diameter, He's work fits experimental results quite well. In short, He's model is ideal for nanorods with equal diameter but not for nanorods with equal volume (mass), which indicates this model more sensitive to the mass rather than shape factor, i.e. the nanorods with ellipsoidal or cylindrical shape are not much different in the calculated *s* but quite different with different mass. However, Sommerfeld's model provides a better description on the influence of the shape factor of non-spherical particles on their settlement behaviour based on sphericity analysis, but it only applies to the particles in either macro- or micro-scale[26, 27]. Therefore, new development and improvement is required to calculate the particles sedimentation coefficient in nanoscale based on this model. Moreover, the direct measurement on the sedimentation coefficient of the nanorods has been achieved for the first time, which provides a comparable ground for further theoretical modelling and quantitative analysis.

There are several factors that may cause the difference between the measured and calculated values 1) measurement error in nanorods dimensions; 2) the effect of the ligand layer nanorod surface, which increases the cross-sectional area perpendicular to movement direction, and lower the effective density of the particles; 3) the deterioration of the pre-filled gradient fluid caused by the temperature fluctuation and the inter-layer sucrose diffusion, which transfers to inconsistency between reference material calibration and actual measurement on nanorods. To improve the accuracy and precision for both the measurement and theoretical

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description, analyzing these influencing factors will be the main task in future.

Conclusions

In this work, using high speed DCS and high quality gold nanorods samples synthesized with either equal volume or diameter, the influence of the size and aspect ratio on the settlement behaviour of the gold nanorods has been systemically investigated. The experimental results show that the settling time becomes longer with the increase of the aspect ratio for the GNRs with equal volume (mass) and the decrease of the length (aspect ratio) for those with equal diameter. Experimentally, it is the first attempt to measure the sedimentation coefficient of non-spherical particles directly and the results provide a comparable base to evaluate the *s* value predicted by other models. Insightful trends have been observed suggesting this is a reliable way to measure and predict the sedimentation behaviour of the rod-like particles moved under a vertically mounted centrifugal field, *i.e.* CPS disc centrifugation. Further study on analysing influencing factors and determining the sedimentation coefficient of other non-spherical particles is underway.

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