Chapter 4
Particle Sedimentation Behaviors in a Density Gradient

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Abstract Density gradient centrifugation, as an efficient separation method, is widely used in the purification of nanomaterials including zero, one-, and two-dimensional nanomaterials, such as FeCo@C nanoparticles, gold nanoparticles, gold nanobar, graphene, carbon nanotubes, hydrotalcite, zeolite nanometer sheet (the examples can be found in Chap. 5). Each system needs separation parameter optimization, which comes from tremendous research experiments. When particles are put on the top of density gradient medium, they will have a definite settling rate under centrifugal force (F_c) [1], which is influenced by their net density, size, and shape. In a sufficiently intense centrifugal field, the particle motion held quietly free from gravity and vibration [2]. This is the principle of the density gradient ultracentrifuge. Based on the above principle, we discussed the particle sedimentation behaviors and built the kinetic equation in a density gradient media. The kinetic equation could apply to zero, one-, and two-dimensional nanomaterials, within its variation form accordingly. We found that the separation parameters could be optimized based on the kinetic equation. A MATLAB program was further developed to simulate and optimize the separation parameters. The calculated best parameters could be deployed in practice to separate given nanoparticles successfully.

Keywords Density gradient centrifugation · Sedimentation mechanism
Sedimentation kinetics · Mathematical model · Separation parameters
Optimization calculation

4.1 Sedimentation Mechanism of a Nanoparticle in a Centrifugal Field

In the centrifugal system, the driving force of the particle movement is the centrifugal force (F_c).

$$F_c = mG \tag{4.1}$$

where m is the mass of the particle (the unit is g). G is the centrifugal acceleration.

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$$G = \omega^2 x \tag{4.2}$$

where ω is the angular velocity of the rotor, the unit is rad/s, x is the distance from the nanoparticle to the rotation center, and the unit is cm.

So

$$F_c = m\omega^2 x \tag{4.3}$$

The strength of the centrifugal force field can be described with the relative centrifugal field (RCF), namely the times of the gravitational acceleration (g).

$$\mathrm{RCF} = \frac{G}{g} = \frac{\omega^2 x}{g} \tag{4.4}$$

where the centrifugal angle is ω, and the distance from the particle to the rotation center is x. RCF can show the strength of the centrifugal force. For example, the rotor (P80AT) of Hitachi ultracentrifuge (CP80MX) can provide maximum RCF 615000 g with 80000 r/min.

One circle of the rotor is 2π radian. So, the rotational speed (n) of rotor can be described as follows:

$$n = \omega/2\pi \tag{4.5}$$

Rotational speed is an important factor of separation, providing a specific centrifugal field in a given centrifuge [3]. Hence, appropriate centrifuge should be chosen to separate nanomaterials according to the centrifugal rotational speed.

The characteristic of particle movement in liquid medium not only depends on the centrifugal force but also rely on density, size, and shape of the particle and the density and viscosity (reverse viscous resistance (Reverse Friction)) of the liquid medium. The gravity and intermolecular force can be usually ignored because the centrifugal field is two orders of magnitude higher than the acceleration of gravity in general. Force analysis of the particle in the separation process is shown in the following figure (Fig. 4.1).

So, the dynamics equation of the particle in the centrifugal process can be described by the following differential equation:

$$\mathrm{m}\frac{d^2x}{dt^2} = F_c - F_b - F_f \tag{4.6}$$

where t is the centrifugal time in units of s. F_b is the buoyancy, while F_f is the viscous resistance.

In different separation systems, the particle movement can be divided into three stages: The first one is an accelerative process with alterable positive acceleration; the second one is uniform motion without acceleration; and the last one is a

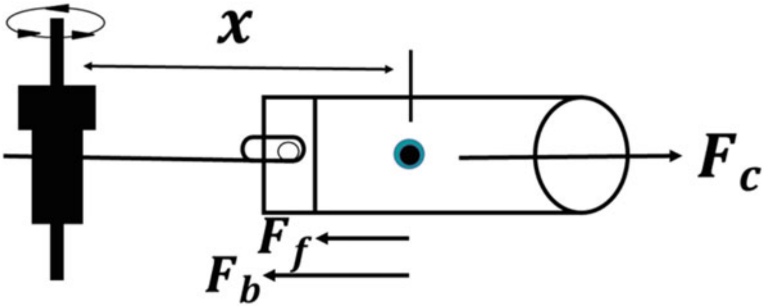

Fig. 4.1 Force analysis of the particle in a centrifugal field. Reprinted with permission from ref. [9]. Copyright 2016, American Chemical Society

decelerated stage with an alterable negative acceleration. When the particle achieves uniform motion [4], the force equilibrates ($d^2x/dt^2 = 0$) [1].

$$F_c = F_b + F_f \tag{4.7}$$

When the distance from the particle to the rotation center is x, and the mass of the particle is m, the centrifugal force can be calculated by Eq. (4.3).

According to Archimedes' principle, the buoyancy of the particle in the liquid medium is the weight of the displaced liquid.

$$F_b = V_p \rho_m g \tag{4.8}$$

where V_p is the volume of the particle, ρ_m is the density of the displaced liquid.

The buoyancy is proportional to particle volume but independent of particle shape. The volume of a particle is equal to the mass dividing a given density. So, Eq. (4.8) can be rewritten as:

$$F_b = \frac{m}{\rho_p} \rho_m g \tag{4.9}$$

This equation is set up in the gravitational field. While if a particle in the centrifugal field, the buoyancy can be considered as RCF times.

$$F_b = \frac{m}{\rho_p} \rho_m \omega^2 x \tag{4.10}$$

According to Stokes' Law, for a spherical rigid particle with a radius (r) which do not dissolve in the liquid medium, when the particle has a speed (dx/dt) under the centrifugal force field, it will be affected by the reverse viscous resistance (F_f).

$$F_f = 6\pi\eta r \times \frac{dx}{dt} \tag{4.11}$$

where η is the viscous coefficient of the liquid medium, r is the radius of the particle.

For non-spherical particles, they have much larger viscous resistance, and the frictional coefficient f is different from spherical particles f_0 (expression of $6\pi\eta r$). The relationship between f and f_0 can be described as $f = \theta f_0$, where the θ is the frictional ratio, and the θ value of other figurate nanostructures is usually in the range from 1 to 2.3, as shown in Fig. 4.2.

To accommodate particles of other shapes, one may apply the frictional ratio θ to Eq. (4.11),

$$F_f = 6\pi\eta r^* \times \frac{dx}{dt} \times \theta \tag{4.12}$$

where r^* is the radius of a sphere whose volume (V^*) is equal to that of the nanoparticles.

$$\mathrm{r}^* = \sqrt[3]{\frac{3V^*}{4\pi}} \tag{4.13}$$

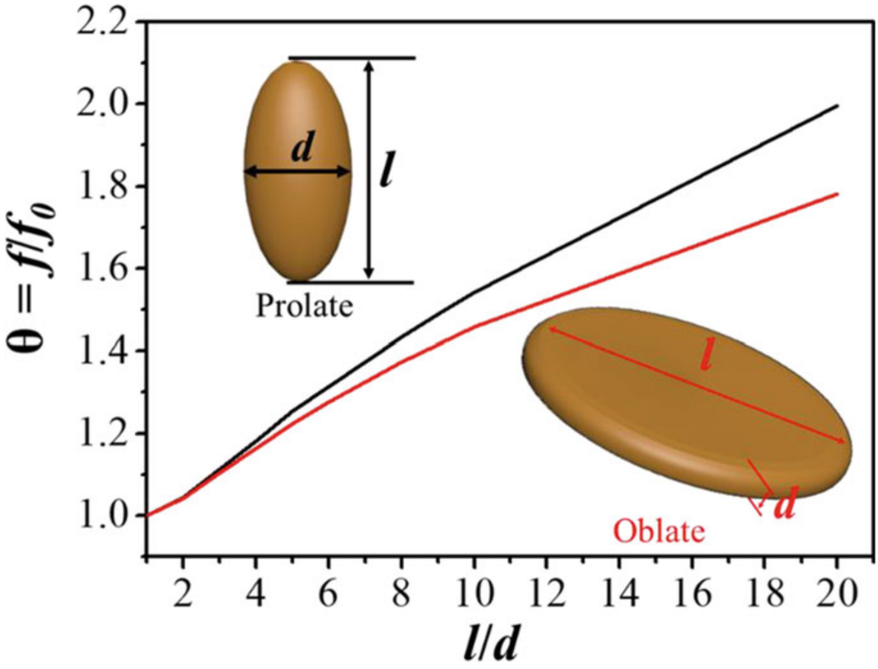

Fig. 4.2 Frictional coefficients for figurate non-spherical nanostructures [5]

For example, for specific rod-shaped CdS nanoparticles, the θ can be expressed as follows [1]:

$$\theta = 0.05213(L/D) + 0.954$$

where L is the length of the nanorod, D is the diameter of the nanorod.

Taking F_c, F_b, and F_f into Eq. (4.7), and using the product of volume and density to replace the mass, the sedimentation rate can describe as Eq. (4.14)

$$\frac{dx}{dt} = \frac{2r^{*2}(\rho_p - \rho_m)}{9\eta\theta}\omega^2 x \tag{4.14}$$

From the sedimentation rate formula, we should note:

1. r^* is the radius of a sphere whose volume (V^*) is equal to that of the nanoparticles (Eq. 4.14).
2. ρ_p is the net density of the particle, and ρ_p is the density of gradient media.
3. Theta (θ) is the frictional ratio.
4. For spherical particles: $\theta = 1$, $r^* = r$, r is the particle radius.

Some deductions can be drawn from above sedimentation rate formula:

1. The sedimentation rate of a particle is proportional to the square of the external particle diameter.
2. The sedimentation rate of a particle is proportional to the difference between particle density and medium density. When the difference is zero, the particle sedimentation will stop.
3. When the liquid medium viscosity increases, the particle sedimentation rate declines in proportion.
4. When the centrifugal field increases, the particle sedimentation rate increases in proportion to the centrifugal field.

In a given centrifuge system, the density and viscosity of the liquid medium are known quantities. For a certain particle, the r^*, ρ_p, η, and θ are also known quantity. So $\boldsymbol{2r^{*2}(\rho_p - \rho_m)/9\eta\theta}$ can be defined as sedimentation coefficient.

That is

$$s = \frac{2r^{*2}(\rho_p - \rho_m)}{9\eta\theta} \tag{4.15}$$

and

$$\frac{dx}{dt} = s\omega^2 x \tag{4.16}$$

For convenience, we mark the sedimentation coefficient as follows.

$$s = \frac{dx/dt}{\omega^2 x} \tag{4.17}$$

On the other hand, from Eq. (4.18),

$$F_c = F_b + F_f \tag{4.18}$$

where $F_{\mathrm{c}} = m\omega^2 x$, $F_{\mathrm{b}} = m_{\mathrm{diss}}\omega^2 x$, $F_f = fv$ and $m_{\mathrm{diss}} = m\ \rho_{\mathrm{media}}/\rho_{\mathrm{particle}}$, v is the speed of particle, f is the fractional coefficient of particle.

Sedimentation coefficient [69] (s) can also be defined as Eq. (4.19)

$$s = dx/dt/\omega^2 x = m[1 - (\rho_{\mathrm{media}}/\rho_{\mathrm{particle}})]/f \tag{4.19}$$

Thus, there are mainly three factors can affect the sedimentation coefficient (s); (1) Effect of mass (m); greater the mass of particle, greater the sedimentation coefficient (s), the particle with higher mass travels down the centrifuge tube rapidly. (2) Effect of shape of particle (f = fractional coefficient of particle); more spherical particle moves with high sedimentation speed because more spherical particle has lower fractional coefficient value. (3) Effect of ($\rho_{\mathrm{media}}/\rho_{\mathrm{particle}}$) value; generally, ($\rho_{\mathrm{media}}/\rho_{\mathrm{particle}}$) value decides the sign of sedimentation coefficient (s) and particles settling orientations during the centrifugation; (a) when ($\rho_{\mathrm{media}}/\rho_{\mathrm{particle}}$) = 1, the value of sedimentation coefficient (s) is equal to zero, particles locate in the certain position that mean $\rho_{\mathrm{media}} = \rho_{\mathrm{particle}}$, (b) when ($\rho_{\mathrm{media}}/\rho_{\mathrm{particle}}$) < 1, the value of sedimentation coefficient (s) is greater than zero, particles settle along the direction of centrifugal force that mean $\rho_{\mathrm{media}} < \rho_{\mathrm{particle}}$, and (c) when ($\rho_{\mathrm{media}}/\rho_{\mathrm{particle}}$) > 1, the value of sedimentation coefficient (s) is less than zero, the particles float against the direction of centrifugal force that mean $\rho_{\mathrm{media}} > \rho_{\mathrm{particle}}$.

In physics, sedimentation means the sedimentation velocity under unit centrifugal force. If we take the experimental value into Eq. (4.17), the sedimentation coefficient (s) can be calculated. When the time is t_1, the position of the particle is x_1, and when the time is t_2, the position of the particle is x_2. The equation can be rewritten as shown as follows.

$$sdt = \frac{1}{\omega^2} \times \frac{dx}{x}$$

Integral in the above range:

$$s\int_{t_1}^{t_2} dt = \frac{1}{\omega^2}\int_{x_1}^{x_2} \frac{dx}{x}$$

Namely,

$$s = \frac{\ln(x_2/x_1)}{\omega^2(t_2 - t_1)} = 2.303\frac{\lg(x_2/x_1)}{\omega^2(t_2 - t_1)} \tag{4.20}$$

Because of

$$\omega = \frac{2\pi n}{60} = 0.105n$$

We can get the relation between the sedimentation coefficient and the rotational speed (n):

$$s = \frac{2.1 \times 10^2 \lg(x_2/x_1)}{n^2(t_2 - t_1)} \tag{4.21}$$

where the unit of t_1 and t_2 is s, the unit of n is r/min.

We can choose centrifuges with different rotating speed according to the different sedimentation coefficients. In a certain separation system, we can estimate the time of centrifugal separation by Eq. (4.21).

4.2 Mathematical Description of Particle Sedimentation Kinetics

In the whole process of density gradient centrifugation, we can use Eq. 4.6 to describe the particle movement. In the liquid density gradient medium, the particle will have a solvation layer on the surface [6]. For ideal spherical particles with core density (ρ_c), radius (r), and solvation shell thickness (h) and the solvation shell density (ρ_h) (Fig. 4.3), the net density (ρ_p) can be estimated according to the following Equation,

$$\rho_p = \rho_h + (\rho_c - \rho_h)r^3/(r + h)^3 \tag{4.22}$$

It can be deduced from the above formula that the net density of a colloidal system would increase as the core size increases with respect to the hydration shell thickness, and the particle density will be close to the core material density when the nanoparticle is large enough (i.e., $r \gg h$).

Similarly, for the cylindrical particles with core density (ρ_c), radius (r), length (L), and hydrated shell thickness (h) in Fig. 4.4, the net density (ρ_p) can be estimated as:

$$\rho_p = \rho_h + \frac{(\rho_c - \rho_h)r^2L}{(r + h)^2(L + 2h)} \tag{4.23}$$

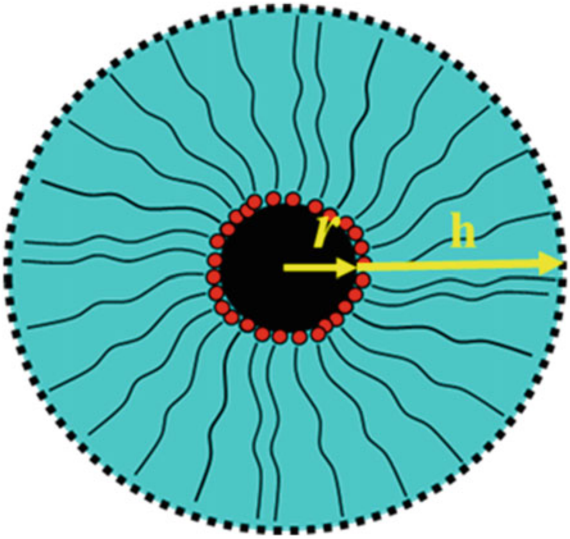

Fig. 4.3 Model of a hydrodynamic colloidal spherical nanoparticle. Reprinted with permission from ref. [9]. Copyright 2016, American Chemical Society

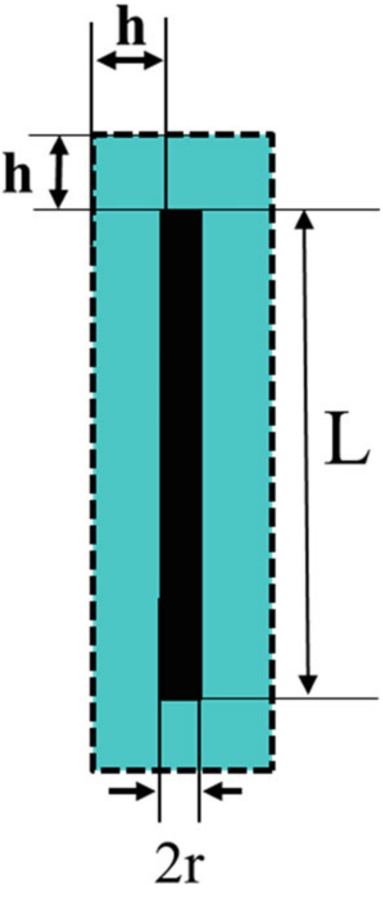

Fig. 4.4 Model of a hydrodynamic colloidal cylindrical nanoparticle

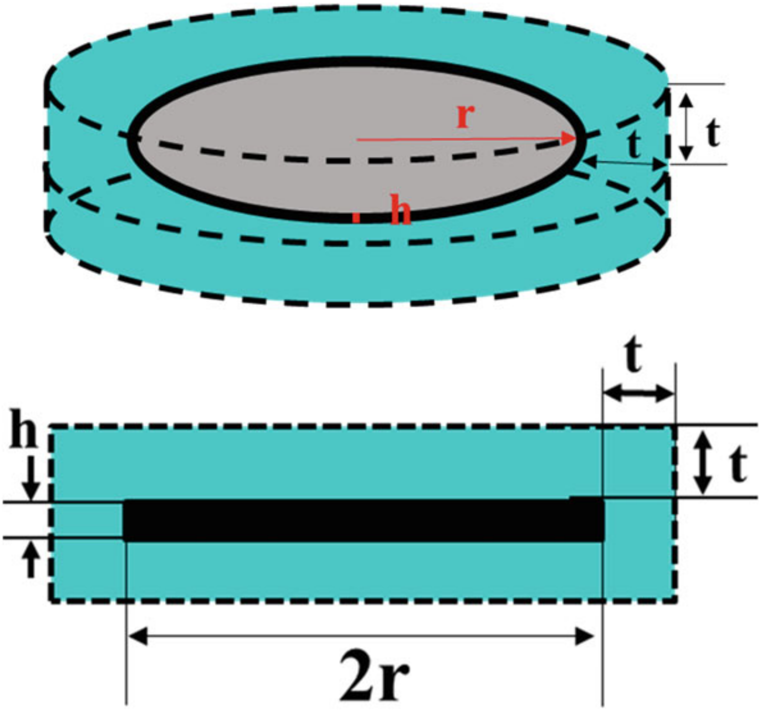

Fig. 4.5 A hydrodynamic colloidal nanosheets model

Similarly, for the two dimension nanosheet with core density (ρ_c), radius (r), thickness (h), and hydrated shell thickness (t) in Fig. 4.5, the net density (ρ_p) can be estimated as:

$$\rho_p = 1 + (\rho_c - 1)/[(1 + t/r)^2(1 + 2t/h)] \tag{4.24}$$

The particles with different morphologies have different net densities accordingly, and all have a sedimentation tendency as layered on top of the linear density gradient, driven by centripetal force. The movement behavior is determined by the particle size, net density, centrifugal force, buoyancy, and viscous resistance.

Hence, for spherical particles, we can describe their movement during the centrifugal process using the following equation,

$$\begin{cases} \mathrm{m}\frac{d^2x}{dt^2} = F_c - F_b - F_f \\ \mathrm{F}_c = m\omega^2 x \\ \mathrm{F}_b = \frac{m}{\rho_p}\rho_m\omega^2 x \\ \mathrm{F}_f = 6\pi\eta r * \frac{dx}{dt}\theta \end{cases} \Rightarrow \mathrm{m}\frac{d^2x}{dt^2} = m\omega^2 x - \frac{m}{\rho_p}\rho_m\omega^2 x - 6\pi\eta r * \frac{dx}{dt}\theta$$

The nanoparticle mass can be represented by apparent density, the above equation can be rearranged to get the universal kinetic equation:

$$\frac{d^2x}{dt^2} + \frac{9\eta\theta}{2\rho_p r{*}^2}\frac{dx}{dt} + \frac{\rho_m - \rho_p}{\rho_p}\omega^2 x = 0 \tag{4.25}$$

All particle motion in the density gradient media during the centrifugal process can be described using Eq. 4.25. Let's discuss the formula in three different situations: applying to zero-, one-, and two-dimensional nanostructures.

Firstly, for zero-dimensional nanostructures: $\theta = 1$, $r* = r + h$, r is the particle radius, h is the hydrated shell thickness, the kinetic equation can be simplified as:

$$\frac{d^2x}{dt^2} + \frac{9\eta}{2\rho_p (r+h)^2}\frac{dx}{dt} + \frac{\rho_m - \rho_p}{\rho_p}\omega^2 x = 0 \tag{4.26}$$

Secondly, for one-dimensional nanostructures: $r^* = \sqrt[3]{3/4(r+h)^2(l+2h)}$, r is the radius of cylindrical nanostructure, l is the cylindrical nanostructure length, and h is the hydrated shell thickness. The kinetic equation can be simplified as:

$$\frac{d^2x}{dt^2} + [\frac{3}{4}(r+h)^2(l+2h)]^{-\frac{2}{3}}\frac{9\eta\theta}{2\rho_p}\frac{dx}{dt} + \frac{\rho_m - \rho_p}{\rho_p}\omega^2 x = 0 \tag{4.27}$$

Lastly, for two-dimensional nanostructures: $r^* = \sqrt[3]{3/4(r+t)^2(h+2t)}$, r is the nanosheets radius, h is the nanosheets thickness, t is the hydrated shell thickness, then the kinetic equation can be simplified as:

$$\frac{d^2x}{dt^2} + [\frac{3}{4}(r+t)^2(h+2t)]^{-\frac{2}{3}}\frac{9\eta\theta}{2\rho_p}\frac{dx}{dt} + \frac{\rho_m - \rho_p}{\rho_p}\omega^2 x = 0 \tag{4.28}$$

The multifunctional, universal kinetic equation can be used in not only rate zonal separation but also isopycnic separation.

For rate zonal separation, which mainly uses the different settling rate to sort the nanoparticles, the max density of density gradient media is smaller than that of nanostructures ($\rho_\mathrm{m} < \rho_\mathrm{p}$) in general, and Eq. 4.25 can record the whole motion of nanoparticles.

For isopycnic separation, which mainly uses tiny differences of net density to sort the nanoparticles, the density of nanoparticles should locate in the range of the density of gradient media. When the nanoparticles reach the isopycnic state ($\rho_\mathrm{p} = \rho_\mathrm{m}$), the settling rate will become zero, and the nanoparticles will stay at that position even prolonging the time. The centrifugal time can also be calculated through Eq. 4.25, as well as the sedimentation coefficient (Eq. 4.20).

It can be deduced from the above formula (Eq. 4.25 for all nanostructures; Eq. 4.26 for zero-dimensional nanostructures; Eq. 4.27 for one-dimensional nanostructures; Eq. 4.28 for two-dimensional nanostructures) that the positions of the particles in the centrifugal tube after separation are determined by centrifugal time, centrifugal rotational speed, density gradient range, and medium viscosity, etc. Therefore, there will be a lot of factors affecting the separation effect.

4.3 The Influence of Separation Parameters

For optimized separation, various parameters should be considered: centrifugal rotational speed, centrifugal time, density gradient range, and medium viscosity and so on. An ideal separation should be described as: 1. The spatial distribution should be the longest, with the smallest and biggest NPs located at the top and bottom of the centrifugal tube. 2. The size distribution of the as separated particles along the centrifugal tube is linear. We will analyze the above factors in detail.

4.3.1 Influence of the Centrifugal Rotational Speed (ω)

At insufficient centrifugal rotational speed, the separated nanoparticles mainly distributed in the top half of the centrifuge tube, while excessive centrifugal rotational speed made the fractions concentrated in the bottom of the centrifuge tube. The two cases could not make the particles dispersed in the whole centrifugal tube. An appropriate centrifugal speed could fractionate particles very well, indicating better separation efficiency (Fig. 4.6).

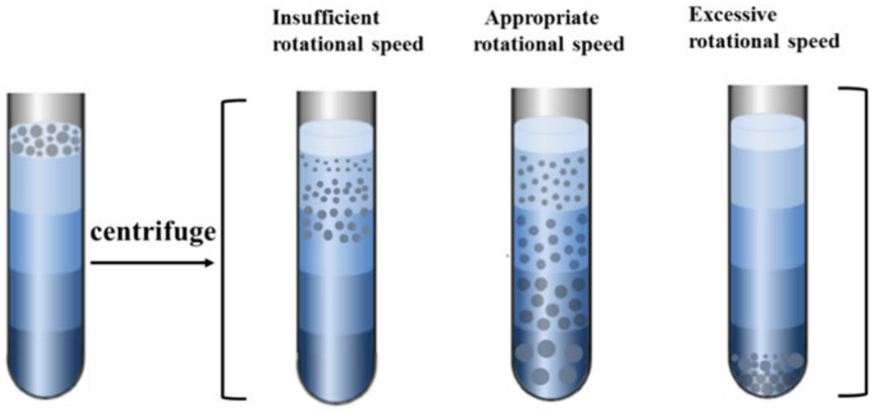

Fig. 4.6 Influence of centrifugal rotational speed at rate zonal separation

4.3.2 Influence of the Centrifugal Time (T)

The centrifugal time is another important factor in density gradient separation. For low-density materials, such as carbon nanotubes and graphene nanosheets with surfactant wrapping, their net densities are ~1.1 g/cm^3, and the gradient density should be tailored to cover particle net densities. Under centrifugal force, the low-density particles would move to and stay at the layers where the medium density equal to their net densities (isopycnic separation). No matter how long the centrifuge time is, the particles will not sediment down (Fig. 4.7a). While if the net densities of particles are higher than the highest density that the gradient media can reach (rate zonal separation), the particles will sediment through the gradient unless the external centrifugal force is removed [7]. Therefore, the particles with different sizes and net densities have different sedimentation velocities (Eq. 4.14), and they will stop at different locations after a given centrifugal time. When time is long

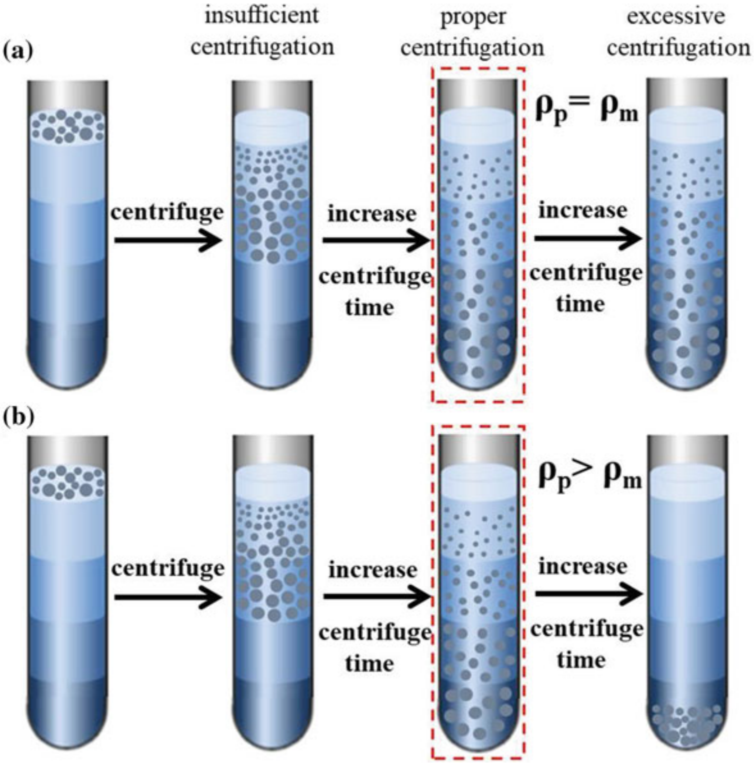

Fig. 4.7 Schematic illustration of typical isopycnic separation **a** and rate zonal separation **b**. The optimized separation states as shown in red boxes. Reprinted with permission from ref. [7]. Copyright 2014, Elsevier Inc. All rights reserved

enough, all the particles will sink to the bottom of the centrifuge tube (Fig. 4.7b). At a given centrifugal system, we should choose an appropriate centrifugal time for rate zonal separation.

4.3.3 *Influence of the Density Gradient Range (ρ_m)*

The choice of gradient media will also affect the separation efficiency. For a given nanoparticle, its net density is a constant. If the net density is relatively small, the isopycnic separation can be selected to sort the nanoparticles. It is worth noting that the range of the gradient media should cover the net density of nanoparticles. If the distribution of net densities is very narrow, a more precise density gradient should be chosen. For example, when the distribution of net densities is from 0.85 to 0.90 g/cm^3, we can use the density gradient with 0.84–0.91 g/cm^3, which can make the fractions fully distributed along the centrifugal tube; while the larger range (such as 0.7−1.0 g/cm^3) would not get such separation effect.

For high-density materials, such as metal, metallic oxide, and metallic selenide, the rate zonal separation is a better choice, and the density gradient should be also chosen accordingly. For instance, the net density of cadmium selenide quantum dots with the size range from 3 to 7 nm synthesized in 1-octadecene can be calculated by Eq. 4.22, which is ranged from 0.82 to 1.26 g/cm^3. For the separation of those CdSe quantum dots, cyclohexane/carbon tetrachloride density gradient can be chosen. Here, the density of cyclohexane is 0.78 g/cm^3 and carbon tetrachloride is 1.59 g/cm^3, so cyclohexane/carbon tetrachloride system can provide the density gradient range from 0.78 to 1.59 g/cm^3, which can be tuned to match the net density range of CdSe quantum dots. Moreover, if the density gradient range (e.g., 0.8–1.0 g/cm^3) leads the fractions enriching in bottom layers or the density gradient (e.g. 1.3–1.5 g/cm^3) leads the fractions accumulating in the upper layers of the centrifuge tube, the density gradient range can be further tuned to make the fractions fully distributed. Thus, whatever it is isopycnic separation or rate zonal separation, a suitable choice of density gradient range depends on the net density of nanoparticles.

To separate the particles synthesized in water should choose an aqueous gradient medium, such as cesium chloride aqueous solution, sodium chloride aqueous solution, and sucrose solution. While to separate the particles synthesized in oil should choose an organic gradient medium, such as cyclohexane/carbon tetrachloride, ethanol/ethylene glycol, and acetone/chloroform.

4.3.4 *Influence of the Medium Viscosity (η)*

Viscosity can affect the viscous resistance and also affect the stability of the density gradient. For the separation of particles with small net density, high viscosity liquid medium (e.g., ethylene glycol), will cost long time to get a good separation effect.

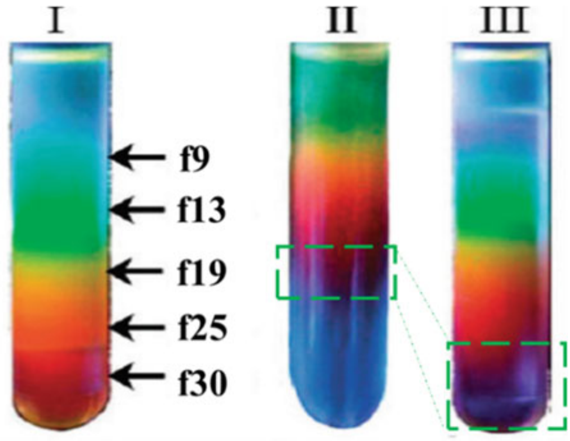

Fig. 4.8 Images of ultracentrifuge vessels containing cadmium selenide nanoparticles under UV irradiation at 365 nm: (Vessel I) polystyrene-free gradient, 60 min centrifugation at 50000 rpm, (Vessel II) polystyrene-containing gradient, 60 min centrifugation at 50000 rpm, and (Vessel III) polystyrene-containing gradient, 110 min centrifugation at 50000 rpm. Reprinted with permission from ref. [8]. Copyright 2010, American Chemical Society

In high viscosity density gradient medium system, larger centrifugal force is needed to make the particles move.

While in some conditions, the viscosity can be modified by introducing polymer without influencing density. When Bai et al. [8] used the cyclohexane/carbon tetrachloride density gradient to separate the cadmium selenide nanoparticles, and they studied the influence of viscosity on the separation effect by adding the polystyrene (PS) in the density gradient medium. As the introduction of PS into the organic gradient layers, it can significantly increase their viscosity; it should slow down the sedimentation of nanoparticles. As expected, PS-containing gradient (vessel II) showed a limited separation compared to PS-free gradient (vessel I), and only by applying longer centrifugation time, the separation can be completed (vessel III), and thus a finer separation can be achieved (Fig. 4.8). This work demonstrated the possibility to separate by tailoring media viscosity.

When using two or three kinds of liquid medium or a substance solution to prepare the density gradient, the viscosity of gradient mainly depends on the density. So we can build a relation between the viscosity and the density as follows:

$$\eta = \eta(\rho_m) \tag{4.29}$$

For sucrose as an example, we can find the following data in the *Chemical Property Manual: Organic Volume* (Table 4.1).

Table 4.1 Density and viscosity coefficient of different concentration sucrose solutions at 20 °C

Density of sucrose solution (g/cm^3)	Viscosity coefficient (mPa.s)
1.2	1.957
1.25	2.463
1.3	3.208
1.35	4.352
1.4	6.21
1.45	9.449

We analyze the experimental data of sucrose solution on density and viscosity coefficient and find the relationship between viscosity coefficient and density is similar to the quadratic polynomial relation. Therefore, we can assume that

$$\eta = \alpha\rho_m^2 + \beta\rho_m + \delta \tag{4.30}$$

Using the least square method to fit, we can obtain:

$$\alpha = 129.41; \beta = -314.45; \delta = 193.11$$

Experimental data fitting method can provide the relationships between the viscosity coefficient and the gradient medium in other separation systems.

Above analysis indicates that centrifugal parameters including centrifugal forces, centrifugation time, density range, and medium viscosity would influence the final efficiency of density gradient separation. Thus, in the succeeding section, we will discuss the mathematical optimization of density gradient separation.

4.4 Optimization Model for Best Separation Parameters

Based on above discussion, many factors (centrifugal rotational speed, centrifugal time medium viscosity, density gradient range, and hydrated shell thickness) would influence the final density gradient separation effect. Moreover, the factors are highly connected. For example, higher rotational speed needs shorter time, or lower rotational speed takes longer time. Therefore, a large number of control experiments are needed to explore the best separation parameters for a perfect separation result, while it will be time consuming and inefficiency.

To address this problem, we develop a mathematical optimization method to study the kinetic equation in the centrifugal process [9]. Although there are a lot of variables of the kinetic equation, and it is a nonlinear differential equation without exact solution, we can briefly consider the equation as the relation expression between nanoparticle size (r) and its position (x). After the simulation of these variables, we can get a good linear distribution between r and x, and find the optimized separation parameters.

In detail, we consider the centrifugal accelerating and de-accelerating process, and the following functions are used to describe angular speed.

$$\omega(\mathrm{t}) = \begin{cases} \frac{\omega t}{T_0} & (0 < t \leq T_0) \\ \omega & (T_0 < t \leq T_0 + T_1) \\ \omega\left(\frac{T_0 + T_1 + T_2 - t}{T_2}\right) & (T_0 + T_1 < t \leq T_0 + T_1 + T_2) \end{cases} \tag{4.31}$$

where ω is the stable angular speed of the centrifuge after accelerating process, T_0, T_1, and T_2 are the durations of accelerating, separating, and moderating processes.

In order to further simplify the optimization model, we assume that the ideal distribution of the fractions after density gradient separation is linear:

$$X(r) = ar + b \tag{4.32}$$

where X is the position of the particle with diameter of r, a and b are linear constants.

As illustrated above, the gradient interfaces would influence the sedimentation resistance a little, thus in order to get better separation and simplify the calculation, we assume that the ideal density gradient is linear:

$$\rho_m(x) = dx + c \tag{4.33}$$

where x is the distance between rotation center and the gradient with a density of ρ_m, c, and d are linear constants.

Therefore, based on above modeling, the location of a nanoparticle with a diameter of r is the function of separation time T_0, T_1, T_2, angular speed ω (i.e., centrifugal force), gradient ρ_m, and media viscosity η. The location of a particle with a diameter of r could be described as X (T_0, T_1, T_2, ω, d, c, a, b, η). The position X of a particle at the moment t can be calculated by the following differential equation as the initial states are given. There are two initial conditions when the separation starts: the particle's initial position is x_0 and initial velocity is 0. So we can get the following conditional equation:

$$\begin{cases} \frac{d^2x}{dt^2} + \frac{9\eta(\rho_m(x))}{2\rho_p(r+h)^2}\frac{dx}{dt} + \frac{\rho_m(x) - \rho_p(r)}{\rho_p(r)}\omega(t)^2 x = 0 \\ x(0) = x_0, x'(0) = 0 \end{cases} \tag{4.34}$$

Because the viscosity of the density gradient is related to the density of the gradient medium, the influence of the viscosity can be attributed to the density of the gradient. Then, the optimization could be carried out by using above X value. The objective function of the least square optimization model is then set up as:

$$G(T_0, T_1, T_2, w, d, c, a, b) = c_1^2[x_0 - (ar_0 + b)]^2 + c_2^2[x_m - (ar_m + b)]^2 + \sum_{i=1}^{m-1} [X(T_0, T_1, T_2, w, d, c, a, b) - (ar_i + b)]^2 \tag{4.35}$$

where c_1 and c_2 are the weighting factors, which contributing to the size distribution of the fractions. x_o and x_m are the upper and lower positions of the tube. For a setting parameter a, b, d, c_1, c_2, and c, when objective function G reach the minimal value, the gradient function $\rho_m(x)$ will give the ideal gradient.

A set of estimated separation parameters are given as initial values, by using the nonlinear least square method mentioned above, we could get the minimum value of the objective function G using a MATLAB program. After calculation, the MATLAB program output the optimized size distribution, which almost coincides with the ideal size distribution, as shown in the comparison chart in Fig. 4.9. It should be noted that the optimized distribution is just a calculated distribution other than the real distribution of particles. Nevertheless, the computer program will output the best separation parameters after calculation, and then we can use those separation parameters to separate our nanoparticles.

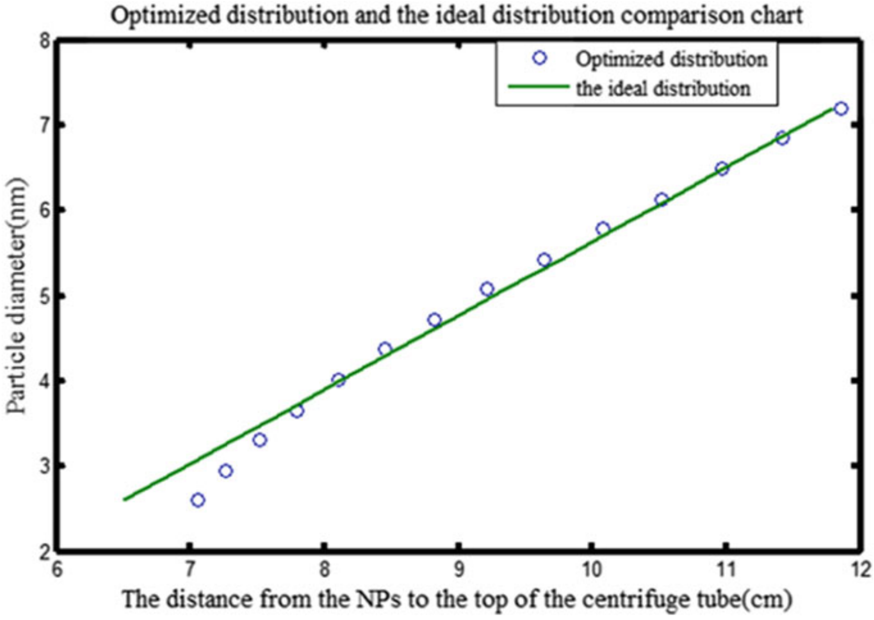

Fig. 4.9 Ideal size distribution and optimized size distribution calculated by MATLAB program using optimization model. Reprinted with permission from ref. [9]. Copyright 2016, American Chemical Society

For example, in the separation system of cadmium selenide nanoparticles using the cyclohexane and carbon tetrachloride density gradient, we can optimize the model to get the best separation parameters. According to the cadmium selenide nanoparticles with the size of 3–7 nm, the calculated separation conditions are 51012 rpm of centrifugal rotational speed, 1.65 h of centrifugal time within a 5–60% (cyclohexane/carbon tetrachloride) density range. Using those separation parameters, we can get ideal separation effect.

In addition, for the separation of other morphological nanostructures, a physical quantity of the shape factor can be introduced to correct the effect of morphology on the dynamics equation. As a result, the same optimization simulation method can output a series of suitable centrifugal parameters.

Appendix: MATLAB Program for the Computational Mathematical Optimization of Spherical Nanoparticles

% model assumptions:

%1. All the nanoparticles are sphere; if not, use morphology factor f to modify the model;

%2. The nanoparticles have a solvation layer;

%3. Using linear density gradient; if not, modify the gradient function;

%4. The ideal diameter distribution of nanoparticles is linear distribution; the objective function is G;

%5. Nanoparticles do not react with the medium;

%6. Optimization variables: linear acceleration time (T_0), the time of constant speed (T_1), linear deceleration time T_2, (the total time is $T = T_0 + T_1 + T_2$), the angular velocity in constant speed (omega), c and d are the coefficient of linear density gradient; a and b are the coefficient of the ideal linear distribution; the objective function is $G(T_0, T_1, T_2, \text{omega}, c, d, a, b) = c_1 \wedge 2 * (x_0-(a * r_0 + b)) \wedge 2 + \text{sum}((x(r_j,T)-(a * r_j + b)) \wedge 2)(j = 0{:}m) + c_2 \wedge 2*(x_m-(a * r_m + b)) \wedge 2$, ($c_1$ and c_2 are appropriate constant); among them, $r_j = r_0 + j*(r_m-r_0)/m$, r_0, r_m are the minimum and the maximum radius of particles, respectively; x_0 is the distance between the top of the centrifuge tube and the center of rotation, x_m is the distance between the bottom of the centrifuge tube and the center of rotation.

%7. The movement of NPs (X(t)) follows the following equation: $x'' + 9 * \text{ita} (p_m(x))/(2 *p_p(r) * r \wedge 2) * x' + (p_m(x) - p_p(r))*\text{omega(t)}/p_p(r) * x = 0$; ita is the viscosity of the medium solution, the ideal density gradient: $p_m(x) = c + d * x$, the density of nanoparticle: $p_p(r)$; the angular velocity: $\text{omega(t)} = \text{omega} * t/T_0$ $(0 < t<T_0)$; omega $(T_0 < t<T_0 + T_1)$; $\text{omega}*(T_0 + T_1 + T_2-t)/T_2$ $(T_0 + T_1 < t< T_0 + T_1 + T_2)$;

% Using the Lsqnonlin in MATLAB to solve the optimization problem

global r x_0 x_m

$m = 10$; % the number of output dots

```
x0 = 6.5; xm = 11.8; r0 = 1.3e-7; rm = 3.6e-7; % the unit is centimeter, might be different for different rotors; x0 and xm is the distances between center of rotation and the top and bottom of the centrifuge tube, respectively; r0 and rm is the size of particles.
h = (rm–r0) / m; r = r0:h:rm;
y0 = [60,3600,60,4000,0.6,0.5,2e7,4]; % the initial value of the optimization calculation
lb = [30,1200,30,1000,0,0,0,−1e8]; % the minimum bounds of optimization variables
ub = [1000,100000,1000,10000,5,5,1e10,1e10]; % the maximum bounds of optimization variables
options = optimset(‘LargeScale’,’on’,’Display’,’iter’,’TolX’,1e-30, ‘MaxIter’, 200, ‘MaxFunEvals’, 5000, ‘TolFun’, 1e-10);
[y, resnorm] = lsqnonlin(@objfun, y0, lb, ub,options) % optimization calculation
a = y(7); b = y(8);
arb = a * r + b; % the ideal linear distribution
Fm = objfun(y); xt = Fm(2:end-1)’ + arb;
plot(xt, 2 * r,’o’, arb, 2 * r) % output comparison chart
title(‘Optimized distribution and the ideal distribution comparison chart’)
xlabel(‘The distance from the NPs to the top of the centrifuge tube(cm)’)
ylabel(‘Particle diameter(cm)’)
legend(‘Optimized distribution’,’the ideal distribution’)
function F = objfun(y) % the objective function
global r x0 xm
T0 = y(1); T1 = y(2); T2 = y(3); omega = y(4); c = y(5); d = y(6); a = y(7); b = y(8);
T = T0 + T1 + T2;
tspan = [0,T];
xt = [];
m = length(r);
for i = 1:m
ri = r(i); [t,x] = ode15s(@odefun,tspan,[x0,0],[],y,ri);
xti = x(end,1);
xt = [xt,xti];
end
arb = a * r + b;
c1 = 10000; c2 = 10000;
F = [c1 * (x0-arb(1)), xt−arb, c2 * (xm−arb(end))];
F = F’;
function xp = odefun(t,x,y,ri)
pc = 6; h = 2e-7; pm = 0.9; % pc is the density of core, pm is the density of shell, the unit is g/cm3; h is the thickness of the shell, the unit is centimeter.
pp = pm + (pc−pm) * (1−h/ri) ^ 3;
T0 = y(1); T1 = y(2); T2 = y(3);
c = y(5); d = y(6); omega = y(4);
```

```
if t >= 0 & t < T0
omegat = omega * t / T0;
elseif t >= T0 & t <= T0 + T1
omegat = omega;
elseif t > T0 + T1 & t <= T0 + T1 + T2
omegat = omega * (T0 + T1 + T2−t)/T2;
else
omegat = 0;
end
(c + d * x(1)) > 0.5 & (c + d * x(1)) < 2; % the minimum and maximum bounds of the density gradient
ita = ita(pm); % viscosity is relate with the density of liquid medium, the unit of viscosity is mPa.s
xp = [x(2);-9 * ita * x(2) / (2 * ri * ri * pp) + (pp−(c + d * x(1))) * omega * omega * x(1) / pp]; % the kinetic equation of spherical nanoparticles
```

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