Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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To cite this Article Christov, K. , Todorova, G. , Kenderov, P. and Kenderova, J.(1991) 'Mathematical Modeling of Sedimentation Processes in a Centrifuge', Separation Science and Technology, 26: 9, 1257 — 1265
To link to this Article: DOI: 10.1080/01496399108050528
URL: http://dx.doi.org/10.1080/01496399108050528

SEPARATION SCIENCE AND TECHNOLOGY, 26(9), pp. 1257–1265, 1991

Mathematical Modeling of Sedimentation Processes in a Centrifuge

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Abstract

A mathematical model describing concentration-dependent sedimentation in a centrifuge is suggested. The present model contains the already existing models as partial cases, and it has some advantages. An interactive system for numerical solution of the model equations was created. This system simulates the sedimentation processes in a centrifuge. The special inverse problem module of the system determines particle parameters from the experimental data.

In this paper we suggest a mathematical model describing the concentration-dependent sedimentation in the rotor of a centrifuge. This model contains the already existing models as partial cases.

It is known (*8*, *9*) that the process of sedimentation is described by the following parabolic equation:

$$\frac{\partial c}{\partial t} = \frac{1}{r}\frac{\partial}{\partial r}\left(r\left(D\frac{\partial c}{\partial r} - \vartheta c\right)\right) \tag{1.1}$$

with boundary condition

$$D\frac{\partial c}{\partial r} - \vartheta c = 0, \qquad \text{for } r = r_1 > 0 \text{ and } r = r_2 > r_1 \tag{1.2}$$

and initial condition

$$c(r,0) = c_0(r) \tag{1.3}$$

Here $c(r,t)$ represents the weight concentration of the sedimenting particles as a function of time t and radial distance r. D is the diffusion coefficient and ϑ is the average velocity of the sedimenting particles. Usually ϑ has the form

$$\vartheta = A\frac{1 - \bar{v}_2\rho}{\eta}\omega^2 r = S\omega^2 r \tag{2}$$

where ω is the angular velocity of the centrifuge rotor, A is a constant characteristic for the particle, and $\bar{v}_2$ is the specific volume of the particle, which means the deviation of the solvent volume for an infinitely small increase in particle concentration (*3*). ρ and η are the density and the viscosity of the surrounding solution, respectively. The Svedberg coefficient S can be viewed as the velocity generated by a unit centrifugal force. S depends on many factors, especially on the shape of the sedimenting particles as well as on the viscosity and density of the surrounding solution in which the sedimentation takes place. What is important for us in this article is that S depends on the concentration $c(r,t)$ as well. Experiments carried out by many authors have shown that, in general, S decreases when particle concentration increases. Empirically, this relation is described either by

$$S = S_0(1 - k_1 c) \tag{3}$$

or by

$$S = \frac{S_0}{1 + k_2 c} \tag{4}$$

for different types of particles (*3*, *8*, *13*). Here S_0 is the value of the Svedberg coefficient for an infinitely diluted solution and k_1 and k_2 are empirically adapted constants. The experimental data show that Eq. (4) is adequate for particles with a clearly expressed ellipsoid form, while Eq. (3) better approximates the data for ball-shaped particles.

This situation cannot be considered as satisfactory for at least three reasons. First, it is desirable to have a unified formula which "works" in all cases, including the partial cases mentioned above. Second, the coefficients k_1 and k_2 in Eqs. (3) and (4) do not have easily understandable physical meanings and have to be determined experimentally in every

particular case. The third reason is that one of the models predicts a physically impossible evolution of the process. When Eq. (3) or (4) is substituted in Eqs. (1.1)–(1.3) (via Eq. 2), we get two mathematical models which have different behaviors. The model with Eq. (3) adequately describes the process only when the initial concentration $c_0(r)$ is less than $1/k_1$. When c_0 is greater than $1/k_1$, the concentration $c(r,t)$ "blows up," i.e., increases to infinity for finite times (*15*). However, for physical reasons, concentration $c(r,t)$ is always smaller than 1. The mathematical model with Eq. (4) does not manifest this effect (*15*).

To remove these difficulties, we suggest the following formula which generalizes Eqs. (3) and (4):

$$S = S_0 \frac{1 - \left(\bar{v}_2 + \dfrac{[\eta]}{\nu}\right)c}{1 + [\eta]c} \tag{5}$$

Here $[\eta]$ is the intrinsic viscosity of the particles (*3*), ν is the Simha coefficient (*3*), which is a well-known function of the axis ratio of the equivalent hydrated ellipsoid, and $\bar{v}_2$ is the specific volume of the particle.

To derive this formula we take into account the following effects which occur when the particle concentration increases: the effect due to the change of the density and viscosity of the solution (which is now the mixture of the solvent and the particles) and the so-called backflow effect. The sedimenting particles replace the surrounding solution and make it flow backward (*1*). The aggregation-like effects are not taken into account.

We now proceed to prove Eq. (5). The volume V_s of the solution with g_2 grams of particles in it is

$$V_s = V_0 + \bar{v}_2 g_2$$

where V_0 is the initial volume of the pure solvent.

Then

$$\frac{V_0}{V_s} = 1 - \bar{v}_2 \frac{g_2}{V_s} = 1 - \bar{v}_2 c$$

If ρ_0 denotes the density of the pure solvent, the density of the solution, ρ, is

$$\rho = \frac{\rho_0 V_0 + g_2}{V_s} = \rho_0 \frac{V_0}{V_s} + c = \rho_0(1 - \bar{v}_2 c) + c$$

and therefore

$$1 - \bar{v}_2\rho = (1 - \bar{v}_2 c) - v_2\rho_0(1 - \bar{v}_2 c) = (1 - \bar{v}_2 c)(1 - \bar{v}_2\rho_0) \tag{6}$$

On the other hand, the viscosity of the solution is given as a function of the particle concentration c as follows (*3*):

$$\eta = \eta_0(1 + [\eta]c + bc^2 + \cdots) \approx \eta_0(1 + [\eta]c) \tag{7}$$

where $[\eta]$ is the intrinsic viscosity of the particle and η_0 is the viscosity of the pure solvent.

Substituting Eqs. (6) and (7) in Svedberg Eq. (2), we get for the relative velocity of one particle in the solution

$$\vartheta = A\frac{(1 - \bar{v}_2\rho_0)(1 - \bar{v}_2c)}{\eta_0(1 + [\eta]c)}\omega^2 r = S_0\frac{(1 - \bar{v}_2c)}{1 + [\eta]c}\omega^2 r$$

where

$$S_0 = A\frac{(1 - \bar{v}_2\rho_0)}{\eta_0}$$

Now we take into account the backflow effect. Denote by ϑ_p and ϑ_m the actual velocity of the hydrated particles and the solution, respectively. Then the equilibrium between the transfer of the hydrated particles with partial volume φ and the transfer in the opposite direction of the solution volume, $1 - \varphi$, takes place:

$$\vartheta_p\varphi = \vartheta_m(1 - \varphi)$$

Therefore

$$\vartheta_p = \vartheta - \vartheta_m = \vartheta(1 - \varphi)$$

The partial volume can be expressed as

$$\varphi = V_{\text{hydr}}\frac{N_0}{M}c$$

where V_{hydr} is the volume of the hydrated molecule, N_0 is Avogadro's number, and M is the molecular weight of the particle.

On the other hand,

$$V_{\text{hydr}}\frac{N_0}{M} = \frac{[\eta]}{\nu}$$

where ν is the so-called Simha coefficient, which is a well-known function of the axis ratio of the equivalent hydrated ellipsoid. Finally, we have

$$\vartheta_p = \vartheta(1 - \varphi) = S_0\frac{(1 - \bar{v}_2 c)}{(1 + [\eta]c)}\left(1 - \frac{[\eta]}{\nu}c\right)\omega^2 r \approx S_0\frac{1 - \left(\bar{v}_2 + \dfrac{[\eta]}{\nu}\right)c}{1 + [\eta]c}\omega^2 r$$

In this way, Eq. (5) is obtained. It should be noted that for small concentrations, Eq. (5) could be replaced by both

$$S \approx S_0\left(1 - \left(\bar{v}_2 + [\eta] + \frac{[\eta]}{\nu}\right)c\right) = \frac{S_0}{1 + \left(\bar{v}_2 + [\eta] + \dfrac{[\eta]}{\nu}\right)c}$$

This model contains both of the existing models mentioned above as partial cases. The experimental data (*3*, *10*) indicate that the model $S = S_0/(1 + k_2 c)$ is adequate for synthetic polymers of high molecular weight and for various biological macromolecules with a large axis ratio, such as DNA. For such macromolecules the intrinsic viscosity $[\eta]$ and the Simha coefficient ν are large, and $[\eta]c$ is large even for low concentrations. Then a good approximation for Eq. (5) is

$$S = \frac{S_0}{1 + k_2 c}$$

where k_2 is close to $[\eta]$ ($[\eta]/\nu \ll [\eta]$). As an experimental verification, we give the data for tobacco mosaic virus. Lauffer experimentally shows in Ref. *10* that $S = S_0/(1 + 27.8c)$. For tobacco mosaic virus (*3*, *11*), $[\eta] = 28$ mL/g, $\nu = 37$, and $\bar{v}_2 = 0.73$ mL/g. By using our model, we obtain from Eq. (5)

$$S = S_0\frac{1 - (0.73 + 28/37)c}{1 + 28c} = S_0\frac{1 - 1.49c}{1 + 28c} = \frac{S_0}{1 + 29.5c}$$

for a relatively small concentration c, which is in good agreement with Lauffer's results.

The second model, $S = S_0(1 - k_1 c)$, fits well the data for globular proteins and spherical viruses. For such particles, ν is near to 2.5 and $[\eta]$ is somewhat less than 4 mL/g. Then $[\eta]c \ll 1$, even for high concentrations, and Eq. (5) becomes

$$S = S_0\left(1 - \left(\bar{v}_2 + [\eta] + \frac{[\eta]}{\nu}\right)c\right) \tag{8}$$

As an example we give the data for bovine serum albumin. Taylor (*12*) found that $S = S_0(1 - 5.4c)$, where $S_0 = 4.31S$. From Ref. *4* we have $[\eta] = 3.9$ mL/g, $\nu = 5.25$, and $\bar{v}_2 = 0.73$, and from Eq. (8) we obtain $S = 4.31\ (1 - 5.37c)$.

Table 1 gives a comparison of K_s published data from Ref. *4* and the corresponding values from our model.

It should be noted, however, that in some cases the values of K_s predicted by our model and the data contained in the above-mentioned table are essentially different; for instance, for myosin rabbit, myosin rabbit guanidine-denatured, and α_2-macroglobulin.

The general model of Eqs. (1.1)–(1.3) with S from Eq. (5) (via Eq. 2) is a nonlinear initial boundary value problem for the concentration of the sedimenting particles. For a nonlinear problem of this type there exists a global solution for any initial concentrations $c_0(r) \geq 0$ (*15*).

An interactive system for a numerical solution of the above initial boundary value problem was created for IBM PC/XT/AT and compatibles. By means of this system it is possible to simulate sedimentation and separation processes in a centrifuge. The algorithm for solving the nonlinear boundary value problem uses the finite elements method presented for this problem by Claverie, Dreux, and Cohen (*5*, *6*).

TABLE 1

Substance	K_s published (mL/g)	K_s from Eq. (8) (mL/g)
Polystyrene latex spheres	38.6	33.6
Ovalbumin	6.2	5.8
Bovine serum albumin	5.4	5.4
Met-haemerythrin	7.0	5.3
Lactate dehydrogenase, rat liver	5.2	5.4
Albolase	6.0	5.5
Aldolase, urea-dissociated	15.0	14.5
Fetuin, calf, oxidized	22.2	22.4
Fibrinogen, bovine, urea-denatured	42.0	43.4
Zn-α_2-glycoprotein, human	6.4	6.5
γ-Globulin, human	7.8	7.5
γ-Globulin, horse	7.3	7.9
"Pathological macroglobulin"	8.7	8.2
Bushy stunt virus	7.1	5.4
Myosin, rabbit	73.0	218.5
Gelatin, parent	59.0	56.0
Myosin, rabbit, guanidine-denatured	162.0	94.0
α_2-Macroglobulin, human	5.5	8.3

The input contains information about the centrifuge parameters and the experimental conditions. The initial particle concentration and the gradient of the solution can be represented by using the graphical editor. If the user knows the parameters of the sedimenting particles, he can specify them. Otherwise he can use a special module of the system to obtain them. This module solves the inverse problem, i.e., it determines the unknown particle parameters from the experimental data. Further information about this module will be given below.

At every moment t the system displays the graph of the function $c(r,t)$—the particle concentration versus radius r. At any moment of the calculations the user can obtain some global characteristics of the solution such as the mass center, the maximum concentration, and the zone width (which is the size of the domain where the particle concentration is greater than a given value).

The simulated experiments are in good agreement with the real experimental data. Table 2 shows the accuracy of the calculations of the global solution characteristics, such as the radius of the maximum concentration.

Next, some details about the inverse problem module are given. The nonlinear least-squares method is used (*7*, *14*). The Gauss–Newton method is well adapted for the minimization of the functional

$$F(P) = \sum_{i=1}^{m} w_i(Y_i - Z_i(P))^2$$

TABLE 2[a]

	Time					
	16 h		22 h		29 h	
c_0 (mg/mL)	r_{sim} (cm)	r_{exp} (cm)	r_{sim} (cm)	r_{exp} (cm)	r_{sim} (cm)	r_{exp} (cm)
1.015	8.42	8.38	8.96	8.87	9.65	9.55
2.09	8.42	8.38	8.96	8.87	9.66	9.55
11.36	8.38	8.32	8.92	—	9.59	9.50
21.15	8.34	8.25	8.88	8.81	9.56	—
46	8.24	8.19	8.76	8.75	9.42	9.28

[a]Experimental conditions: Rotor Sw40Ti, R_{min} = 6.84 cm, R_{max} = 15.87 cm, ω = 35,000 rpm; sucrose gradient: 7.5–20.5% w/w; sample: 0.4 mL BSA with initial concentration c_0, $S_{20,w}$ = 4.31*S*, $D_{20,w}$ = 6.1 × 10^{-7} cm^2/s, k_1 = 5.8 mL/g, $\bar{v}_2$ = 0.734 mg/mL; time = 16 h 15 min, 22 h, and 29 h. r_{sim} and r_{exp} are the radius of the maximum concentration from simulation and experiment, respectively.

TABLE 3[a]

S (mg/mL)	S_0 (error %)	k_1 (error %)
0.002	0.2	0.17
0.015	2.25	0.3
0.027	2.5	3.1

[a]Calculations are made for $S_{20,w} = 40S$, $D_{20,w} = 60 \times 10^{-7}$ cm²/s, $k_1 = 0.3$ mL/mg, and all other experimental conditions are as in Table 2.

where $Y_i (i = 1...m)$ are the experimental data, P is the vector of the unknown parameters, $Z_i(P)$ are the simulated data corresponding to Y_i, and w_i are appropriately chosen weights. During the calculations the Jacobian is determined with the finite difference approximation.

The test of the inverse problem module is done in the following way. The solution of the system with known parameters is disturbed randomly in the corridor of a given error. These disturbed data are used as input experimental data for the inverse problem module.

Table 3 shows the accuracy of the estimate of the parameters S_0 and k_1 for random error with different SD (SD = $\sqrt{F(P)/w(m - p)}$, where $w = \Sigma w_i/m$ and p is the number of the unknown parameters).

Acknowledgment

This work is partially supported by Committee of Science under Grant 59/1987.

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Received by editor December 3, 1990