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# ANOMALIES IN SEDIMENTATION.

## V. Chains at high fields, practical consequences

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The theory of a preceding paper [B.H. Zimm, Biophys. Chem. 1 (1974) 279] is used to calculate a numerical table for the change of sedimentation coefficient with centrifugal field for chain molecules. A simple formula is found to fit the results within 1.3% up to the centrifugal field at which $S/S^0 = 0.377$; this formula is

$$S/S^0 = (1+0.1155y^2)^{-1/4} ,$$

where $y$ is proportional to $M^2/S^0$ times the centripetal acceleration, $M$ being the molecular weight and $S^0$ the sedimentation coefficient at zero acceleration. Applying this formula to DNA, we conclude that at a given centrifuge speed the sedimentation coefficient must reach a maximum at a particular molecular weight and be smaller at higher molecular weights. The value of the maximum depends on the conditions, but can come at less than 150 S for DNA under typical conditions. When a maximum is present, the profile of a sedimenting non-homogeneous band is also severely distorted.

## 1. Introduction

In the preceding paper of this series [1] [hereafter called (IV)] we obtained a formula, applying to chain molecules, for the change in sedimentation coefficient induced by high sedimenting forces. In this paper we consider how the coefficient change affects practical matters involving sedimentation of large chain molecules. As we noted in IV, the effect in question, which results from a distortion of the coiling chain by non-uniform frictional forces, is important only for very large molecules, in effect, only for DNA molecules of molecular weight greater than $10^8$ daltons; on the other hand, it can become very important for such molecules, as we show below.

Note, however, that our basic formula, eq. (3) below, is still only an approximation to reality in both the mathematical and the physical senses. Only one term of a normal-mode expansion has been retained, and the molecule has been approximated by a chain of Hooke's-law springs with perfectly flexible joints. Nevertheless, eq. (3) is the only formula that we have at this point, and it seems worthwhile to evaluate it numerically and to examine its consequences.

In IV we showed that the dependence of the sedimentation coefficient, $S$, on centrifuge speed could be represented by the formula

$$S/S^0 = Y(y) , \tag{1}$$

where $S^0$ is the limiting sedimentation coefficient as the speed approaches zero and $Y(y)$ is a function only of the dimensionless parameter $y$ given by

$$y = 8.374 \times 10^{-24} M^2 (1-\bar{v}\rho)^2 (\text{rpm})^2 x / T\eta S^0 , \tag{2}$$

where $M$ is the molecular weight and $\bar{v}$ the partial specific volume of the solute, $\rho$ is the density of the solvent, rpm is the speed of the rotor in revolutions per minute and $x$ is the radius of the rotor, $T$ = the absolute temperature, and $\eta$ is the viscosity of the solvent. We obtained representations of the functions $Y(y)$ either as a power series in $y$ or as a triple integral with $y$ as a parameter. The integral seems more useful when

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Table 1
Calculated values of the function $Y(y)$

| $y$ | $Y(y)$ | $Y_1(y)$ from eq. (4) |
|---|---|---|
| 0.0 | 1.0000 | 1.0000 |
| 0.5 | 0.9929 | 0.9929 |
| 1.0 | 0.9730 | 0.9730 |
| 1.5 | 0.9439 | 0.9439 |
| 2.0 | 0.9097 | 0.9094 |
| 2.5 | 0.8737 | 0.8730 |
| 3.0 | 0.8383 | 0.8368 |
| 3.5 | 0.8047 | 0.8022 |
| 4.0 | 0.7734 | 0.7698 |
| 4.5 | 0.7444 | 0.7398 |
| 5 | 0.7177 | 0.7122 |
| 6 | 0.6705 | 0.6636 |
| 7 | 0.6301 | 0.6225 |
| 8 | 0.5953 | 0.5876 |
| 9 | 0.5648 | 0.5575 |
| 10 | 0.5379 | 0.5313 |
| 12 | 0.4925 | 0.4880 |
| 14 | 0.4556 | 0.4535 |
| 16 | 0.4249 | 0.4253 |
| 18 | 0.3990 | 0.4017 |
| 20 | 0.3769 | 0.3815 |
| 25 | 0.3325 | 0.3419 |
| 30 | 0.2992 | 0.3124 |
| 40 | 0.2517 | 0.2709 |
| 50 | 0.2192 | 0.2424 |
| 60 | 0.1953 | 0.2213 |
| 80 | 0.1622 | 0.1917 |
| 100 | 0.1402 | 0.1715 |
| 200 | 0.0876 | 0.1213 |
| 300 | 0.0653 | 0.0990 |

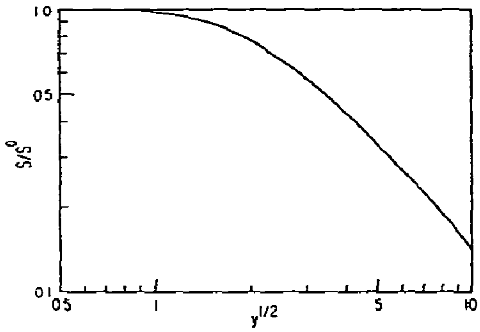

Fig. 1. Change of sedimentation coefficient with the parameter $y^{1/2}$, which is proportional to centrifuge speed. The upper part of this curve, plotted on a different scale, is shown enlarged in fig. 1 of ref. [1].

the domain of $y$ covers a wide range of values; it is

$$Y(y) = \frac{9}{4y^2}\int_0^1\int_0^{1-q} \frac{q^{1/2}\exp(-y^2PQ/4q)}{PQ}$$

$$\times \int_0^\infty \exp(-q\omega^2/y^2PQ) \tag{3}$$

$$\times \left(\sinh\omega - \frac{\cosh\omega}{\omega} + \frac{\sinh\omega}{\omega^2}\right) d\omega\, dp\, dq\ ,$$

where

$$PQ = \sin^2\pi p\ \sin^2\pi q\ . \tag{3a}$$

The evaluation of this integral by numerical quadratures is described in the Appendix.

Results are presented in table 1. The entries at $y$ equals 2, 4, and 6 were checked by summing 50 terms of the equivalent power series, eq. (44) of IV, using numerical integration by Simpson's rule with 99 points to evaluate the coefficients. The results were 0.909657, 0.773465 and 0.67046 from the power series compared to 0.909665, 0.773354 and 0.67047 from numerical quadrature. While the series is convenient at small values of $y$, 50 terms were barely enough to secure convergence to five decimal places at $y = 6$.

These results are plotted in various ways in figs. 1, 2 and 3. Fig. 1, which is similar to fig. 8 of Levin and Hutchison [2], is essentially a log–log plot of $S/S^0$ against centrifuge speed, since $y^{1/2}$ is proportional

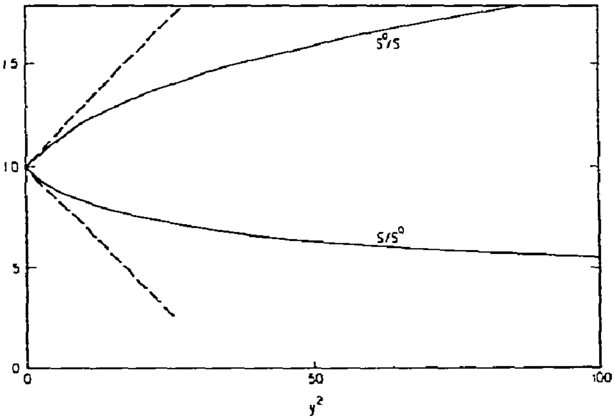

Fig. 2. Anamorphoses of the curve of fig. 1. The dashed lines are the limiting tangents.

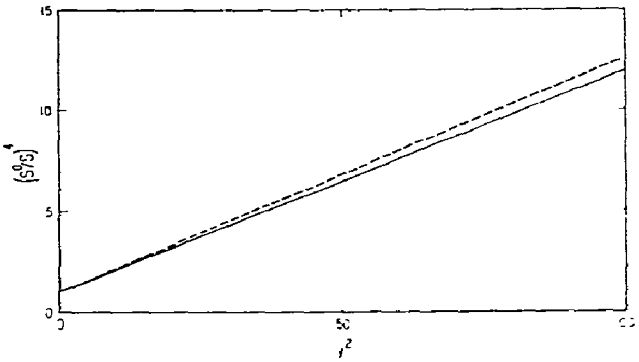

Fig. 3. A further anamorphosis of the curve of fig. 1. The dashed line is again the limiting tangent.

to rpm. Fig. 2 is an attempt to find a way to straighten out the plot. Since the leading term in the power series for $Y(y)$ involves $y^2$, a plot of $S/S^0$ against $y^2$ [which is effectively a plot against $(\text{rpm})^4$] does straighten out the initial decrease of $S$, but the plot as a whole is strongly curved. $S^0/S$ is somewhat better, but still not attractive as a means of plotting data for the purpose of extrapolating to $S^0$. On the other hand, a plot of $(S^0/S)^4$ against $y^2$, fig. 3, is strikingly effective. For finding $S^0$ by extrapolation of data at finite centrifuge speeds, then, we can recommend that $1/S^4$ be plotted against $(\text{rpm})^4$; an approximately straight line should result.

The dashed line in fig. 3, which is the limiting tangent to the true curve, is represented by the simple formula

$$Y_1(y) = (1+0.1155y^2)^{-1/4} , \tag{4}$$

or

$$S/S^0 = [1+8.099\times10^{-48}M^4(1-\bar{v}\rho)^4 \times (\text{rpm})^4 x^2/(T\eta S^0)^2]^{-1/4} . \tag{4a}$$

This formula fits the values of $S/S^0$ calculated by quadrature to within 0.0077 up to $y=20$, $S/S^0 = 0.377$, in addition to approaching perfect agreement at small $y$. It should be useful as an approximate interpolating formula. (The formula has already been published in a previous note [3].) If a more accurate interpolating formula is needed, the following is accurate to within two percent up to $y = 300$:

$$Y_2(y) = (1+0.1155y^2+0.00175y^3\,e^{-20/y})^{-1/4} . \tag{5}$$

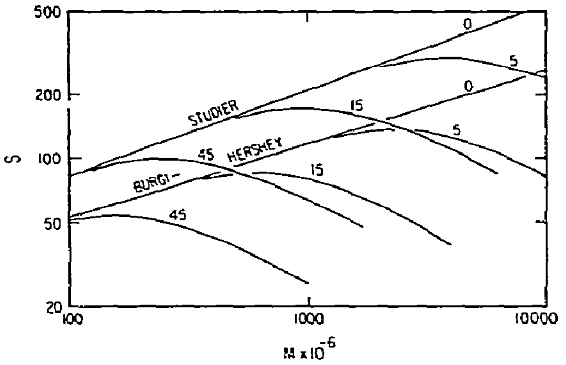

Fig. 4. Sedimentation coefficient of single- and double-standard DNA as a function of molecular weight at various centrifuge speeds according to eq. (7). Rotor radius, etc. as in fig. 1 of ref. [1]. The numbers on the curves are the centrifuge speed in thousands of revolutions per minute. The Studier [4] and Burgi–Hershey [5] values of the parameters $K$ and $a$ were used for single-stranded and double-stranded DNA respectively. At high speeds eq. (5) instead of eq. (4) was used in eq. (7).

## 2. The curve of $S$ versus $M$ has a maximum

An unexpected result of these calculations was pointed out by Levin and Hutchinson [2] and by Chia and Schumacher [3]. This is the prediction that the sedimentation coefficient of a chain molecule should not increase indefinitely with increase of molecular weight at a given centrifuge speed, but should reach a maximum value and then decline. For example, assume that $S^0$ can be represented as a function of $M$ by the familiar relation,

$$S^0 = KM^a , \tag{6}$$

while $Y(y)$ is given by eq. (4). Then from eq. (1),

$$S = KM^a(1+0.1155y^2)^{-1/4} , \tag{7}$$

with $y$ given by (2). This curve as a function of $M$ has a maximum, which comes when

$$y^2 = 2a/0.1155(2-3a) , \tag{8}$$

$$\frac{S}{S^0} = \left(\frac{2-3a}{2-a}\right)^{1/4} , \tag{9}$$

that is, when $y = 2.94$ and $S/S^0 = 2^{-1/4} = 0.841$ if $a = 0.4$. This value for the exponent $a$ occurs in the widely

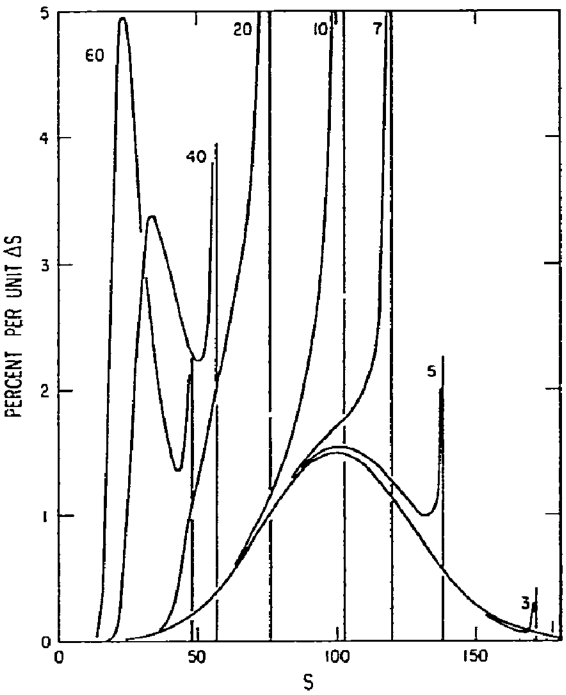

Fig. 5. Distortion of weight distribution curves as a function of speed, according to eq. (12), for a randomly degraded double-stranded DNA with a peak value of 100 for $S$. These curves also represent the profile of an initially sharp, non-diffusing band of this DNA in a band sedimentation experiment. The ordinate is the weight percent of material per unit range of $S$. The numbers on the curves are the speeds in thousands of revolutions per minute, with the other parameters the same as in fig. 4.

used Studier [4] relation for single-stranded DNA. If $a$ is taken to be 0.35, as in the Burgi–Hershey [5] relation for native DNA, then the maximum occurs at $y = 2.53$ and $S/S^0 = 0.871$, not significantly different.

Fig. 4 shows several log–log plots of $S$ versus $M$ corresponding to typical experimental conditions. The numbers on the curves state the rotor speed in thousands of revolutions per minute; a rotor radius of 5.2 cm and $T$ of 293 K was assumed. Note that the onset of the speed dependence is rather sudden as a function of molecular weight, and that the difference between $S$ and $S^0$ at the maxima of the curves is not large, although the functional dependence of $S$ is very different from that of $S^0$ on $M$. Obviously, it is not possible to determine $M$ unambiguously from a measurement of $S$ at one centrifuge speed with molecules in the indicated size range, since there are now two possible values of $M$ for a given $S$, one much larger than the other.

The presence of a maximum in $S$ as a function of $M$ could, in the right combination of circumstances, have a dramatic effect on the shape of a sedimenting band. Fig. 5 shows an example. Here a heterogeneous material is assumed, corresponding to randomly broken linear chains, with the distribution of $M$ given by the limiting form of the Montroll–Simha function [6] for an extensively broken chain:

$$w(M)\,\mathrm{d}M = M\mu^{-2}\exp(-M/\mu)\,\mathrm{d}M\,, \qquad (10)$$

where $w(M)\,\mathrm{d}M$ is the weight fraction of material of molecular weight $M$ in the range $\mathrm{d}M$, and $\mu$ is the number-average of $M$. The profile of a sedimenting band is ideally a plot of weight of material against $S$, in other words, $W(S)$, which is related to $w(M)$ by

$$W(S)\,\mathrm{d}S = w(M)\,\mathrm{d}M\,, \qquad (11)$$

or

$$W(S) = w(M)(\mathrm{d}M/\mathrm{d}S) = \frac{w(M)(\mathrm{d}M/\mathrm{d}S^0)}{\mathrm{d}S/\mathrm{d}S^0}\,. \qquad (12)$$

In fig. 5 $\mu$ was taken to be $3.76 \times 10^8$, corresponding to a typical degraded bacterial DNA of initial molecular weight over $10^9$; the maximum in the corresponding sedimentation distribution, using the Burgi–Hershey relation, comes at $S^0 = 100$.

The derivatives in eq. (12) were evaluated by the use of eqs. (2), (4), and (6). Since there is a maximum in the curve $S$ versus $S^0$, $\mathrm{d}S/\mathrm{d}S^0$ vanishes there, and an infinity appears in $W(S)$ as a result of the speed effect. The infinity moves to lower values of $S$ as the speed is increased, until the shape of the sedimenting band, which is effectively $W(S)$, is greatly distorted. When the infinity is near the maximum in the molecular-weight distribution, at 10 000 rpm in this example, the band is artificially sharpened. When the infinity moves to lower $S$ values, at higher speeds, the size of the infinity diminishes, and the band mostly recovers its original bell-shaped form, but the maximum in the bell now comes at values of $S$ much lower than 100, the original low-speed maximum. Furthermore, the distribution is now inverted, that is, the material of highest $M$ is now sedimenting most slowly, except near the infinity, where a wide range of molecular weights sediments all together.

Chia and Schumaker [3] have demonstrated experimentally the crossing over the sedimentation coefficients of high- and low-molecular-weight DNA at high speeds, and the distortion of the shape of sedimenting bands has been observed by many workers, for example, by Levin and Hutchinson [2], Rubenstein and Leighton [7], and Ormerod and Lehman [8].

## 3. Conclusions

The potential of this speed effect for producing artifacts and misleading sedimentation profiles is obviously high. One does not normally expect to find high-molecular-weight material sedimenting slower than homologous material of lower molecular weight. To what extent this effect may have defeated early attempts to obtain and to characterize very large DNA molecules is unknown, but it is probably great. For a discussion, see Levin and Hutchinson [2] or Ormerod [9]. Once the existence of the speed effect was recognized, a number of workers at once were able to identify with certainty DNA larger than $10^8$ daltons. For example, double-stranded DNA near $10^9$ daltons in size has been described by Kavenoff [11], Levin and Hutchinson [2], Rubenstein and Leighton [7,10], and Chia and Schumaker [3], while a speed effect with very large single-stranded DNA has been reported by McBurney et al. [12], Elkind [13], and Ormerod and Lehman [8].

It seems unlikely that any of the results described in figs. 4 and 5 would be changed qualitatively by improvements in our calculations. The maximum in the $S$ against $M$ curve comes at relatively small values of the molecular distortion parameter, $y$, in fact, at values of $y$ less than three. Here the inaccuracy of neglecting the higher terms in the normal-mode expansion should not be great, and the deviations of a real DNA molecule from the ideal beads-and-springs model should also be small. On the other hand, we can confidently expect that more exact calculations for large values of $y$ will differ quantitatively from those presented here. However, the existence of a maximum in the curve of $S$ against $M$, and even the approximate position of that maximum, as well as the resulting distortion of the sedimenting band and boundary profiles, seem indisputable.

## Appendix: Evaluation of the function $Y(y)$ by numerical quadratures (Carl B. Zimm)

The integral, eq. (3), was put into the form:

$$Y(y) = \frac{9}{2}\int_0^1\int_0^{1-w^2} A(p,w)\int_0^\infty H(\omega)\,\mathrm{d}\omega\,\mathrm{d}p\,\mathrm{d}w\ , \qquad \text{(A-1)}$$

where $w^2$ has replaced $q$, eliminating a singularity at the origin,

$$A(p,w) = w^2/y^2(\sin \pi p)^2(\sin \pi w^2)^2\ , \qquad \text{(A-2)}$$

and

$$H(\omega) = \frac{1}{2}\left\{\left(1-\frac{1}{\omega}+\frac{1}{\omega^2}\right)\exp\left(\omega - A\omega^2 - 1/4A\right) - \left(1+\frac{1}{\omega}+\frac{1}{\omega^2}\right)\exp\left(-\omega - A\omega^2 - 1/4A\right)\right\}\ , \qquad \text{(A-3)}$$

with $A$ given by eq. (A-2).

The integration over $\omega$ was effected by Gauss–Hermite or Gauss–Laguerre quadrature [14] with 8 points scaled to cover the significant portions of the integrand. Gauss–Hermite quadrature was used when $A \leqslant 0.03$; here the integrand is everywhere negligible except for a narrow, nearly symmetrycal peak centered at $\omega = 1/2A$. Gauss–Laguerre quadrature was used when $A > 0.03$; here the integrand rises nearly linearly from the origin to a broad asymmetrical peak. The power-series expansion of $H(\omega)$ was used when $\omega$ was less than 0.1 to avoid possible division by zero.

Integrations over $p$ and $w$ employed Gauss–Legendre quadrature [14]. Eight points were used for each integration when the parameter $y$ was less than ten; 16 points otherwise. Use of 16 points instead of eight near $y = 10$ produced a negligible change in $Y(y)$: for $y = 10$, change 0.00027; for $y = 16$, change 0.00020.

Actual numerical work was carried out by means of an ALGOL program on an automatic digital computer (Burroughs 6700).

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