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THEORY FOR THE SEPARATION OF VERY LARGE DNA MOLECULES BY RADIAL MIGRATION

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The separation of very large biological macromolecules is not presently possible with conventional techniques such as sedimentation and gel electrophoresis. For molecules larger than about 5×10^8 daltons, such as chromosomal DNA, it is necessary to develop new separation methods. Herein we describe the principle for a new device which shows promise for separating molecules in this size range, as a function of molecular weight. It is based on the deformability of random coil molecules, and the normal stresses which they generate in a certain class of rheological flows. In particular, when a solution of large DNA molecules (we have used the intact chromosome from phage T2) is contained between two concentric cones, one of which rotates relative to the other, there will be a "radial migration" of the DNA toward the center of the cones. The velocity with which the macromolecules migrate is highly dependent on the molecular weight, and therefore the potential exists for separating these large molecules.

1. Introduction

In parallel with the evolution of molecular biology has been the evolution of physical techniques for separating biological molecules on the basis of their size, shape, charge, and so forth. There has been increasing interest in the last few years in separating larger and larger molecules, and the methods of choice have been sedimentation, sieve gels, and gel electrophoresis. The recent discovery that very large, single DNA molecules may constitute whole chromosomes [1] has led to many attempts to extend separation techniques even further to DNA molecules of chromosomal size, larger than 10^8 daltons [2,3].

There are many problems, however. It has been shown theoretically and experimentally [4,5] that at very high molecular weights, random coil DNAs have anomalous sedimentation coefficients, making separations impractical in the centrifuge. Gel electrophoresis is limited by the inhomogeneity of the gel structure at the very low gel concentrations required. To our knowledge, the largest molecules presently separable on gels have a molecular weight around 5×10^8 daltons, and in this range the resolution is not very good [2]. "Chromosome sorting" by a computer controlled flow microfluorimeter [3] shows great promise for the analytic separation of chromosomes, but not for DNA. Its costs and slow one-at-a-time sorting procedure do not make it practical as a preparative lab technique.

In this work, we describe a new preparative device which shows promise for separating large DNA molecules by molecular weight. It is based on a novel separation principle, that of viscoelasticity. We present experimental results elsewhere [6]. Here we present the theory and discuss the design considerations for a simple and inexpensive apparatus.

2. A radial migration separator

Dilute solutions of DNA exhibit viscoelasticity. It has long been known that viscoelastic solutions have some unusual properties when compared to ordinary viscous (Newtonian) solutions. One such property is the "Weissenberg effect". When a viscous liquid is

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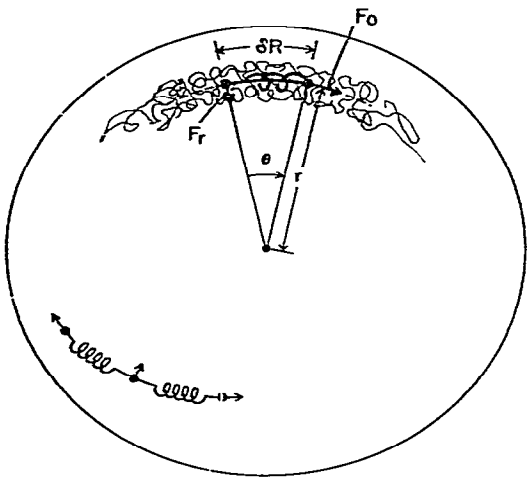

Fig. 1. Radial migration and the Weissenberg effect. The circular shearing flow stretches the springs (increases the elastic free energy), which causes a small component of the force to act radially inward. This produces an inward radial (Weissenberg) pressure, and the molecules migrate toward the center.

sheared in the gap between two concentric cylinders, the pressure is highest at the outside cylinder because liquid is thrown to the outside by inertia. But when a viscoelastic liquid is similarly sheared, the liquid creeps up the inside cylinder, and in this case the pressure is highest at the inside. This is known as the Weissenberg effect [7]. Recently, Shafer and others [8–10] have given a molecular explanation for this phenomenon. In simplest terms, each molecule acts like an elastic band moving along the circular flow lines between the cylinders. The relative motion of the cylinders applies a shear force in the solution which stretches the molecules. Although the applied shear is parallel to the flow lines, and perpendicular to the radius, a small component of the tension in the molecule acts to produce a normal force radially inward on the molecule (see fig. 1). Summing these radial forces from all the polymer molecules in solution gives the Weissenberg pressure. But this same force is the source of an inward radial migration of the individual molecules. The velocity with which this migration occurs is highly dependent on the molecular weight of the macromolecule – the larger the molecule, the more of the curvature is "seen" by the molecule, so the greater will be the velocity of migration radially inward. This variation of the radial migration velocity with molecular weight is the principle on which we base the separation of large DNA molecules. From the theory of Shafer et al. [8–10] we can infer that this radial migration effect would occur in any circular shearing flow. For the practical purpose of separating molecules, we use concentric cones. Therefore we first present a much simplified approach to the molecular theory, in which we generalize the earlier results to other shearing geometries such as concentric cones. Also we can get more physical insight from this than from the more formal approach. The result is valid to within a multiplicative constant, which we then get from the more detailed concentric cylinder theory [9,10].

3. The theory of radial migration

When large randomly-coiled polymer molecules are dissolved in a viscous solvent, even to concentrations of only a few parts per million, the solution will be viscoelastic. That is, in addition to increasing the viscosity, or capacity for energy dissipation, the polymer also adds to the solution the capacity for energy storage (elasticity). The viscosity increment is just a result of the volume of the polymer, which is so large as to traverse neighboring planes of shear, increasing the frictional coupling between them. But the elasticity is due to the deformability of the coil. The recoverable free energy is a result of the entropy due to the large number of configurations available to the molecule. Using a mean-field argument, Flory [11] – and more recently deGennes [12] – have shown that this average free energy of a chain follows a square law. That is, if the excluded volume effects due to intrachain repulsive interactions are small,

$$\Delta G_{\text{elastic}} \sim kT(\delta R)^2/R_0^2, \tag{1}$$

where kT is the Boltzmann constant times temperature and

$$\delta R = R - R_0, \tag{2}$$

is the rms deviation from the unperturbed radius, R_0, of an ideal gaussian coil. Thus the free energy is a minimum when $R = R_0$. (Note that we will leave out constants of order one throughout this section, and will just indicate dependence on the variables by "$\sim$". We will use "$\simeq$" to mean "approximately equal to".) We

can see that deforming the molecule to a radius larger than R_0 increases the free energy. Brownian thermal motion acting on parts of the chain relative to other parts will cause the molecule to "relax" to its equilibrium configuration, a lower free energy state. This entropic tendency to equilibrium can be described by a force,

$$F_{\text{spring}} \sim -kT\,\delta R/R_0^2. \tag{3}$$

This force is linear in δR, thus it acts like a Hooke's law spring. This is the source of the linear elasticity of viscoelastic solutions. The molecular model commonly used to describe the dynamics of viscoelastic solutions is the Rouse-Zimm beads-springs theory, in which the viscosity increment due to the polymer is assumed to occur at frictional beads along the chain, and the entropic elastic force is assumed to occur in springs connecting the beads.

We assume a laminar circular shearing flow in steady state. Then the force applied to the molecule by the shearing flow acts along the circular flow lines. There is a small component of this force which acts radially inward, and tends to cause the molecule to migrate toward the center of the flow.

From fig. 1, we can see that the component of force acting radially on the molecule is

$$F_r = F_0 \sin\theta. \tag{4}$$

Since the molecule is small relative to the dimensions of the device, then

$$F_r = F_0 \delta R/r. \tag{5}$$

The velocity of radial migration is

$$v_r = F_r/f = F_0 \delta R/fr, \tag{6}$$

where f is the translational friction coefficient, which is

$$f \sim \eta R_0. \tag{7}$$

It remains for us to determine δR and F_0. To get δR, we assume a steady state in which there is a balance of the entropic spring force with the applied shear force, therefore:

$$-F_{\text{spring}} = kT\delta R/R_0^2 = F_0, \quad \text{so that } \delta R = F_0 R_0^2/kT. \tag{8}$$

Finally, to calculate the applied shear force, F_0, we assume the solvent to be a newtonian viscous liquid, which means that:

$$\sigma_0 = \eta_s K(r) \tag{9}$$

where σ_0 is the applied shear stress, η_s is the solution viscosity, and $K(r)$ is the shear rate, which depends on the flow geometry and the angular velocity of the solution. We assume an infinitely dilute solution, so that the viscosity increment is small, $\eta_{sp} \ll 1$, and the solution viscosity

$$\eta_s = \eta(1 + \eta_{sp}) \simeq \eta \tag{10}$$

reduces to the solvent viscosity. The applied shear stress is the force applied per unit area of the polymer molecule, which scales as:

$$\sigma_0 \sim F_0/R_0^2 \quad \text{so that } F_0 \sim \eta R_0^2 K(r). \tag{11}$$

So finally, from eqs. (6), (7), (8), (11), the velocity of radial migration is:

$$v_r \sim \frac{\eta K^2(r)}{r\,kT} R_0^5. \tag{12}$$

From the random walk theory of chain statistics, we can in turn calculate the dependence of this velocity on molecular weight, M. Since

$$R_0 = (A_0 M)^{1/2}, \tag{13}$$

where A_0 is a constant then

$$v_r = \text{const}\,\frac{\eta K^2(r)}{r\,kT} M^{5/2}, \tag{14}$$

which is the form of the radial migration velocity expression given by Shafer et al. [8,9], but generalized to include other circular shearing flows. Note the high sensitivity of this radial migration velocity to molecular weight.

We now calculate the constant in eq. (14). Standard solvents for DNA are not θ-solvents. To account for this non-gaussian coil expansion, Dill and Shafer [10] have computed the constant for linear and covalently-closed circular chains under a variety of excluded volume conditions, using the Peterlin-Tschoegl expansion parameter, ϵ [14,15]. So for example, for most reasonable solvents, the ionic strength dependence of radial migration can be calculated. For linear chains, the radial migration velocity according to eq. (23) of Dill and Shafer [10] is:

$$v_r = B(\epsilon)\frac{\eta K^2(r)}{r\,kT} R_0^4 R_\epsilon, \tag{15}$$

where $B(\epsilon)$ is a constant which they have tabulated for a completely non-draining coil, R_ϵ is the rms radius of the expanded coil, and R_0 is the rms radius of the molecule as it would be in a θ-solvent, as we have used it above.

We get R_ϵ from the Flory relation:

$$R_\epsilon = (M[\eta]/6^{3/2}\Phi(\epsilon))^{1/3}, \quad (16)$$

where $\Phi(\epsilon)$ is tabulated by Bloomfield and Zimm [16], or is given approximately by Tschoegl [15] as:

$$\Phi(\epsilon) = 2.843 \times 10^{23}\,(1 - 2.531\,\epsilon + 2.273\,\epsilon^2). \quad (17)$$

This expression is valid for non-draining coils, or DNA larger than about 10^9 daltons. For T2 DNA, where hydrodynamic interaction does not completely dominate [17], the use of the above expression leads to an error of about 5%. To calculate $B(\epsilon)$ and $\Phi(\epsilon)$, we need to know ϵ, which depends on the counterions in the solvent. If the salt is primarily Na^+, then [14]:

$$\epsilon = 0.05 - 0.063 \log [Na^+]. \quad (18)$$

Combining eqs. (13), (15), and (16), we get:

$$v_r = \frac{A_0^2 B(\epsilon)}{6^{1/2}\Phi(\epsilon)^{1/3}} \frac{\eta K^2(r)}{rkT} [\eta]^{1/3} M^{7/3}. \quad (19)$$

Since it is difficult to find a θ-solvent for DNA, the greatest uncertainty in eq. (19) is A_0. We take this from the light-scattering data quoted by Hays et al. [18] on T7 DNA. T7 DNA has a molecular weight of 25×10^6, and they show the radius to be approximately 5000 Å. From their second-virial-coefficient excluded-volume correction, we calculate:

$$R_0 \simeq 4500 \text{ Å}.$$

Using eq. (13), we get:

$$A_0 = 8.10 \times 10^{-17} \text{ cm}^2 \text{ mole g}^{-1} \quad (20)$$

and this should not depend either on molecular weight of the DNA or on the solvent. We note that a radius of 5000 Å corresponds to a wormlike chain model of about 600–700 Å persistence length. Their data is also consistent with a radius of about 6000 Å, corresponding to a persistence length of about 900 Å. Finally, for a θ-solvent, we get:

$$v_r = \frac{\eta K^2(r)}{rkT} B(0) A_0^{5/2} M^{5/2} = 4.423 \times 10^{-41} \frac{\eta K^2(r)}{rkT} M^{5/2}, \quad (21)$$

which is just eq. (19), evaluated with $\epsilon = 0$. In particular, for $\epsilon = 0$, we have used the equivalence of eqs. (13) and (16):

$$[\eta]_{\epsilon=0} = 6^{3/2}\,\Phi(0)\,A_0^{3/2} M^{1/2}. \quad (22)$$

4. Concentration profile

A diagram of the concentric cone device is shown in fig. 2. We fill the gap between the cones with a DNA solution which has the same concentration everywhere, c_0, at time $t = 0$. The top cone is then spun with a steady angular velocity, Ω. Under these conditions we expect that the DNA will migrate to the center of the device, increasing the DNA concentration there. At larger radii, the concentration will be smaller than c_0. At the end of the experiment, we drip fractions slowly enough so as not to disturb the concentration profile in the gap. In this way we recover the distribution of DNA, its concentration as a function of radius and time, $c(r, t)$. We can theoretically predict the shape of this function, and calculate the time required for all the DNA to have migrated to the center. To do so, we take the calculation for the radial migration velocity of a single molecule, eqs. (19) or (21), and sum up over

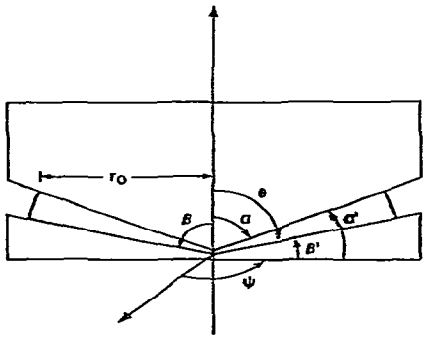

Fig. 2. Coaxial cone device for radial migration. The top cone spins, the bottom cone is fixed. The DNA solution is between them. $\alpha' = 13.7°$, $\beta' = 10.0°$, $r_0 = 2.77$ cm.

all the molecules using the conservation of mass equation:

$$\partial c(r, t)/\partial t = -\nabla \cdot J(r, t), \tag{23}$$

where $J(r, t)$ is the current, which is:

$$J(r, t) = c(r, t)v(r). \tag{24}$$

In the next section, we show how to calculate the shear rate, K, and we show that it is practically constant, independent of r and θ. Therefore, eq. (19) can be rewritten as:

$$v(r) = k_0/r, \tag{25}$$

where

$$k_0 = (2.679 \times 10^{-33}) \frac{B(\epsilon)}{\Phi(\epsilon)^{1/3}} \frac{\eta K^2}{kT} [\eta]^{1/3} M^{7/3}. \tag{26}$$

In spherical coordinates:

$$\nabla \cdot J(r, t) = \frac{1}{r^2} \frac{\partial}{\partial r} r^2 J(r, t), \tag{27}$$

where we get $J(r, t)$ from eqs. (24) and (25). Substituting into eq. (23), we get the following differential equation to solve for $c(r, t)$:

$$\frac{\partial c(r, t)}{\partial t} - \frac{k_0}{r} \frac{\partial c(r, t)}{\partial r} = \frac{k_0 c(r, t)}{r^2}, \tag{28}$$

subject to the initial condition that at time $t = 0$,

$$c(r, t) = c_0. \tag{29}$$

By the "method of characteristics", we find the solution to be:

$$c(r, t) = c_0(r^2 + 2k_0 t)^{1/2}/r. \tag{30}$$

Finally, in a manner analogous to boundary sedimentation, we assume that outside a particular radius, r^*, the concentration is zero. The motion of that outside boundary must move along the characteristic curve:

$$r^* = (r_0^2 - 2k_0 t)^{1/2}, \tag{31}$$

where r_0 is the outside radius of the DNA at time $t = 0$. Therefore, we can also calculate the time required for all the DNA to have migrated to the center of the device. At that time, t^*, $r^* = 0$, where

$$t^* = r_0^2/2k_0. \tag{32}$$

5. Shear rate

For the angles α and β defined in fig. 2, Oka [19] has shown that a coaxial cone device has only one nonzero shear stress component, $\tau_{\theta\phi}$. Therefore, for a newtonian solvent, we can calculate the shear rate, K to be:

$$K(\theta) = \tau_{\theta\phi}/\eta = 2\Omega/\sin^2\theta \left[\frac{\cos\alpha}{\sin^2\alpha} - \frac{\cos\beta}{\sin^2\beta} + \ln \frac{\tan\beta/2}{\tan\alpha/2} \right], \tag{33}$$

where Ω is the angular velocity of the top cone. Note that when $\beta' = 0$, and $\alpha' \simeq 0$, the geometry becomes that of a cone-plate, and the shear rate must reduce to

$$K \simeq \Omega/\alpha'. \tag{34}$$

To check that, note that since

$$\frac{\cos\beta}{\sin^2\beta} = 0, \quad \sin^2\theta \simeq 1, \quad \text{and } \sin^2\theta \frac{\cos\alpha}{\sin^2\alpha} \simeq \pi/2 - \alpha,$$

then

$$K \simeq 2\Omega/(\pi/2 - \alpha - \ln \tan \alpha/2) \tag{35}$$

We let $\alpha/2 = \pi/4 + \delta$, where δ is small so

$$-\ln \tan(\pi/4 + \delta) \quad \text{becomes} \quad -\ln\left(\frac{1 + \tan\delta}{1 - \tan\delta}\right), \tag{36}$$

which can be expanded as

$$-2(\tan\delta + \tfrac{1}{3}\tan^3\delta + \ldots) \simeq -2\delta = \pi/2 - \alpha, \tag{37}$$

so $K \simeq \Omega/(\pi/2 - \alpha) = \Omega/\alpha'$ which indeed checks.

For the concentric cones shown in fig. 2, we get

$$K = \Omega/0.06566, \qquad K\ (\mathrm{s}^{-1}) \qquad \Omega\ (\mathrm{rad\ s}^{-1}) \tag{38}$$

which is only about 2% different from the cone-plate shear rate. The variation of $\tau_{\theta\phi}$, and therefore of K, with θ, is less than about 4%, which justifies the assumption that it is approximately constant. For a given volume of solution between the cones, it is not easy to measure accurately the outside radius, r_0, of eq. (31). However, we can calculate it from the known angles α' and β'. We can see through the clear plexiglas apparatus that the meniscus is approximately vertical at all rota-

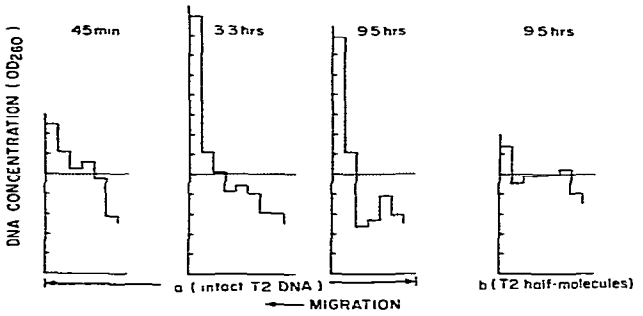

Fig. 3. Experimental radial migration profiles. The first fraction collected from each experimental run (nearest the center of the cones) is at the left of each profile. The dotted line shows the initial DNA concentration everywhere in the chamber. In (a), the DNA was whole T2; in (b) the DNA was T2 half molecules. Shown above each profile is the time during which migration occurred.

tion speeds. That agrees with our intuition that the meniscus will adopt a shape which minimizes the surface area. We can calculate then that the volume of solution, V, is related to r_0 by:

$$V = (2\pi/3)\, r_0^3 [\tan \alpha' - \tan \beta'] . \tag{39}$$

Therefore for a volume of 3 ml of solution, $r_0 = 2.77$ cm.

6. Results

Complete experimental results are presented elsewhere [6]. Fig. 3 is a summary of the data. Fig. 3a shows the concentration profile for the case when T2 DNA alone migrates in the absence of other DNA. Indeed there is a concentration enhancement at the center of the device, and this higher concentration comes off in the first fraction. Under these same conditions, calf thymus DNA (molecular weight $10–20 \times 10^6$) does not migrate measurably — the concentration of all the fractions is the same, and is equal to that of the reference fraction. As a test of the molecular weight sensitivity, fig. 3b shows the radial migration of T2 molecules which had been previously sheared to half-sized molecules by the method of Adam and Zimm [20], and as measured by viscoelastic retardation times). The conditions were identical to those of fig. 3a, and it is evident there is much less radial migration, as expected. We have also shown that in the presence

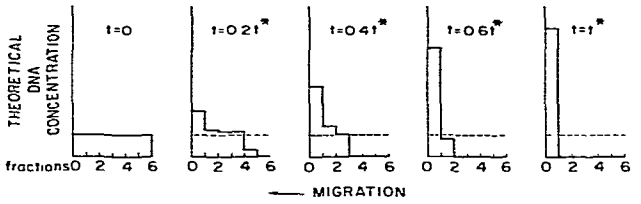

Fig. 4. Theoretical radial migration concentration profiles. The center of the cones corresponds to the first fraction on the left. From left to right are increasing amounts of radial migration time. The time required for complete migration is defined as t^*. Calculated using eq. (30) in conjunction with eqs. (40)–(44). We have assumed $r_0 = 2k_0 = 1$.

of calf thymus DNA, the T2 whole DNA migrates while the calf thymus DNA does not. These results show that selective radial migration occurs in this device, and that there is indeed a high sensitivity to molecular weight, as predicted by the theory.

Fig. 4 shows the theoretically predicted concentration profile, which we get from eq. (30). To make a meaningful comparison with the experimental results in fig. 3a, in which fractions are of fixed volume, we plot against r^3. Since the number of fractions is small, we integrate the theoretical profile to get the average relative concentration in each:

$$\bar{c}(r_{n-1} \to r_n, t) = \frac{1}{v_n} \int_{r_{n-1}}^{r_n} \int_{\alpha}^{\beta} \int_{0}^{2\pi} c(r, t)\, r^2 \sin\theta \, d\phi \, d\theta \, dr, \tag{40}$$

where v_n is the volume of the nth sample collected, and $\bar{c}(r_{n-1} \to r_n, t)$ is the average (measured) concentration in that sample from radius r_{n-1} to r_n. We normalize to $c(r, 0)$:

$$\bar{c}(r_{n-1} \to r_n, t)/c_0 = v_T \int_{r_{n-1}}^{r_n} c(r, t) r^2 dr \Big/ v_n \int_0^{r^*} c(r, 0)\, r^2 dr \tag{41}$$

$$= \frac{v_T}{v_n r_0^3} (r^2 + 2k_0 t)^{3/2} \Big|_{r_{n-1}}^{r_n}, \tag{42}$$

where v_T is the total initial volume. For the first frac-

tion, $\bar{c}(r_0 \to r_1, t)/c_0$ is computed according to eq. (42) above, but summed with an additional term due to conservation of material:

$$\frac{v_T}{v_n}\left(1 - \frac{1}{c_0 r_0^3}\int_0^{r^*} c(r,t)\, r^2 \mathrm{d}r\right). \tag{43}$$

Altogether, for the first fraction we get:

$$\frac{\bar{c}(r_0 \to r_1, t)}{c_0} = \frac{v_T}{v_n r_0^3}(r_1^2 + 2k_0 t)^{3/2}. \tag{44}$$

Experimental conditions were:

$\Omega = 2.62$ rad s^{-1}, $K = 39.9\ s^{-1}$, $\eta = 2.9$ poise,

$kT = 4.14 \times 10^{-14}$ erg, $[Na^+] = 0.05$ M,

$M = 1.25 \times 10^8$ g $mole^{-1}$ (ref. [15]),

$[\eta] = 3.51 \times 10^4$ cm^3 g^{-1} (ref. [15]),

$\epsilon = 0.132$, $B(\epsilon) = 0.555$, $\Phi(\epsilon) = 2.01 \times 10^{23}$,

$r_0 = 2.77$ cm, $k_0 = 7.24 \times 10^{-4}$ cm^2 s^{-1}.

Using eqs. (26) and (32), we calculate that complete radial migration should have occurred in a time $t^* = 1.5$ h, and in reasonable agreement with this calculation our experiments show that no further change in the concentration profile occurs after about two hours.

7. Conclusions

Separation of very large macromolecules by sedimentation is limited by the deformability of those molecules. Large DNA molecules are quite easily deformed, and this causes anomalously slow sedimentation velocities. Here, we use deformability to our advantage. We have described the theory for radial migration — a new method which may be able to separate very large DNAs, in which there is a motion of the DNA toward the center of a laminar, circular, shearing flow. With a coaxial cone device [6], which is simple and inexpensive to build, we have shown that radial migration occurs. The velocity with which a macromolecule migrates to the center of such a device depends highly on molecular weight — large molecules migrate much faster than small ones. The calculations and experiments presented herein pertain to intact phage T2 DNA, of molecular weight 1.25×10^8 daltons. We have preliminary evidence, including the original observations which motivated this work, that radial migration also occurs with molecules of larger size, 10^9–10^{10} daltons. Consequently, we believe this principle shows promise as a separation method for very large DNA molecules.

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