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Viscoelastic properties of entangled DNA solutions

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Abstract – Viscoelastic properties of double-stranded DNA (bacteriophage T2 DNA) were measured in semi-dilute high-salt aqueous solutions in a concentration range from 0.16 to 1.6 mg/cm^3. The viscoelastic properties follow the same universal behaviour as synthetic linear polymers. The reduced variables for the concentration dependence of the viscosity are η/η_{Rouse} and C/C_e, where η_{Rouse} is the viscosity in a Rouse regime and C_e the concentration at which DNA starts to be entangled. The concentration C_e differs from the overlap concentration C^* because n_e blobs are needed for the chains to be entangled. For phage T2 DNA, the concentration C_e is equal to 0.25 mg/cm^3.

Propriétés viscoélastiques de solutions d'ADN enchevêtré

Résumé – Les propriétés viscoélastiques de chaînes d'ADN double brin (ADN de bactériophage T2) ont été mesurées en solutions semi-diluées aqueuses salines dans un intervalle de concentrations allant de 0,16 à 1,6 mg/cm^3. Les propriétés viscoélastiques suivent les mêmes lois universelles que les polymères synthétiques linéaires. Les variables réduites pour le comportement en concentration de la viscosité sont η/η_{Rouse} et C/C_e, où η_{Rouse} est la viscosité dans un régime de Rouse et C_e la concentration à laquelle les chaînes commencent à être enchevêtrées. La concentration C_e est égale à 0,25 mg/cm^3.

Version française abrégée – Les propriétés dynamiques de l'ADN chromosomique à l'intérieur de la cellule posent un problème de physique des polymères. Les échelles de temps biologiques sont très rapides comparées à ce que l'on attend du fait de la concentration et de la longueur des chaînes. Les ADN topoisomérases de type II (topo II) jouent un rôle essentiel dans ce problème. Ces enzymes catalysent le passage, l'un à travers l'autre, des doubles brins d'ADN, effaçant ainsi les contraintes topologiques qui ralentissent la dynamique. Il est prévu théoriquement pour la dynamique des chaînes le passage d'un mode de reptation en l'absence d'enzyme, à un mode de Rouse en sa présence (Sikorav et Jannink, 1993). Pour vérifier expérimentalement cette conjecture il convient au préalable de caractériser les propriétés viscoélastiques des chaînes d'ADN en solution semi-diluées : l'ADN se comporte-t-il comme les polymères synthétiques ? Quelle est la concentration à partir de laquelle les chaînes sont enchevêtrées ? Pour répondre à ces questions, nous avons déterminé la viscosité et le temps de relaxation le plus long pour des solutions semi-diluées aqueuses salines de l'ADN de bactériophage T2 dans un intervalle de concentration de 0,16 à 1,2 mg/cm^3 (tableau).

RAPPELS THÉORIQUES. – Les propriétés statiques des polymères linéaires en solution semi-diluée s'expriment à partir de la taille ξ du blob qui correspond à la longueur d'écrantage des interactions thermodynamiques. Ainsi la pression osmotique π divisée par la concentration C, réduite par sa valeur à C tendant vers zéro, est une fonction universelle du nombre de blobs par chaîne N/g, g étant le nombre de monomères dans un blob. Si C^* est la concentration de recouvrement des chaînes, on a : $\pi/\pi_0 = N/g = (C/C^*)^{1/(3\nu-1)}$ pour $C/C^* \gg 1$. Les propriétés dynamiques font intervenir une seconde longueur A, proportionnelle à ξ, dont le facteur de proportionnalité dépend de la nature chimique du polymère. Dans un régime de concentration tel que la taille du polymère $\xi(N/g)^{1/2}$ soit inférieure à A, les propriétés viscoélastiques correspondent à un régime de Rouse et s'expriment en fonction du nombre N/g de blobs par chaîne. On a pour la viscosité

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$\eta_{\text{Rouse}} = \eta_0 (N/g) = (C/C^*)^{1/(3\nu-1)}$, où η_0 est la viscosité du solvant. Dans un régime de concentration tel que $\xi (N/g)^{1/2}$ soit supérieur à A, c'est-à-dire tel que N/g soit supérieur à un nombre $n_e = (A/\xi)^2$, la chaîne se déplace à l'intérieur d'un tube de diamètre A par reptation. Les propriétés viscoélastiques s'expriment en fonction du nombre d'enchevêtrements par chaîne $(N/g)/n_e = (C/C_e)^{1/(3\nu-1)}$, où C_e est la concentration à laquelle $N/g = n_e$, soit $C_e = C^* n_e^{3\nu-1}$. On a : $\eta/\eta_{\text{Rouse}} = ((N/g)/n_e)^2 = (C/C_e)^{2/(3\nu-1)}$ (Raspaud *et al.*, 1995).

RÉSULTATS EXPÉRIMENTAUX. – Afin de comparer le comportement de l'ADN à celui des polymères synthétiques, la concentration d'enchevêtrement C_e a été calculée selon : $C_e = C^* (K/G)^{3\nu-1} = [3\nu/(3\nu-1)]^{3\nu-1}\ C/(G/G_{\text{Rouse}})^{3\nu-1} = 1,9 \times C/(G/G_{\text{Rouse}})^{3\nu-1}$, où $K = C \times d\pi/dC = [3\nu/(3\nu-1)]\,\pi$ est le module osmotique, $G_{\text{Rouse}} = kTC/M$ la masse molaire M de l'ADN étant connue par ailleurs. La valeur moyenne est $C_e = 0,25 \pm 0,04$ mg/cm^3 (*fig.* 2). La viscosité réduite est obtenue en prenant $\eta_{\text{Rouse}} = \eta_0 ([\eta]\, C)^{1/(3\nu-1)}$, où η_0 est la viscosité de l'eau et $[\eta]$ est la viscosité intrinsèque de l'ADN de phage T2 (Chapman *et al.*, 1969). En utilisant pour variables réduites η/η_{Rouse} et C/C_e les résultats de l'ADN se superposent avec la courbe maîtresse obtenue pour les polymères synthétiques (*fig.* 3). Le régime enchevêtré est décrit par la relation : $\eta/\eta_{\text{Rouse}} = 60 \times (C/C_e)^{3,4}$.

INTRODUCTION. – Dynamical properties of chromosomal DNA within the cell raise a problem of polymer physics. Due to the very high degree of polymerization of the chains and their non-dilute state, the biological time-scales are unexpectedly fast. Type II DNA topoisomerases (topo II) play an essential role in this problem. These enzymes catalyze the passage of double-stranded DNA segments through one another (Liu *et al.*, 1980). Topo II are required for instance during anaphase for the segregation of intertwined sister chromatids pulled apart by the mitotic spindle (Spell and Holm, 1994).

In the entangled regime, the motion of linear synthetic polymers is strongly slowed down by neighbouring chains, which form a tube within which the chain moves. Polymer dynamics in this regime can be described by the reptation model (de Gennes, 1979). It has been conjectured that topo II release the topological constraints leading to reptation (Sikorav and Jannink, 1993). In a solution of entangled DNA, the action of topo II could change the dynamics from a reptation to a Rouse type behaviour. A prerequisite to test this prediction is the characterization of the viscoelastic properties of semi-dilute DNA in the absence of enzyme. Two questions arise: do semi-flexible polymers such as DNA behave as flexible linear synthetic polymers, and what is the concentration at which chains start to be entangled? We study here high-salt aqueous semi-dilute solutions of bacteriophage T2 DNA for concentrations ranging from 0.16 to 1.6 mg/cm^3.

THEORETICAL BACKGROUND. – Static properties of linear polymers in solution depend on a single characteristic concentration C^* proportional to the internal concentration of the polymer coil at the zero concentration limit: $C^* \cong N/R^3$, N being the degree of polymerization and $R \cong N^\nu$ the radius of gyration of the coil. From this concentration, one defines reduced variables allowing universal laws to be written (de Gennes, 1979). For instance, the osmotic pressure π divided by the concentration C and reduced by its zero concentration limit $\pi_0/C = kT/N$, is independent of the degree of polymerization and of the polymer species when plotted as a function of C/C^*. Such universal scaling laws can be established using the notion of "blob", *i.e.* the correlation volume ξ^3 of concentration fluctuations: $\pi = kT/\xi^3$. The length ξ corresponds to the screening length of excluded

volume and thermodynamical interactions, the screening being due to the presence of other polymer chains. The g monomers inside a blob belong to the same polymer chain, leading to $C = g/\xi^3$. For $C/C^* \gg 1$, the reduced osmotic pressure is thus a function of the number N/g of blobs per chain:

$$\frac{\pi}{\pi_0} \cong \frac{N}{g} \cong \left(\frac{C}{C^*}\right)^{1/(3\nu-1)} \tag{1}$$

The dynamics of semi-dilute solutions also depends on the reduced degree of polymerization N/g. While the chains moves, the friction it undergoes is the summation of the N/g friction per blob, leading to a diffusion coefficient $D = D_0/(N/g)$, where $D_0 = kT/(6\pi\eta_0\xi)$ and η_0 is the local viscosity, *i.e.* the solvent viscosity in semi-dilute solutions. The motion of unentangled polymers corresponds to self-diffusion with a relaxation time τ_{Rouse} equal to R^2/D, where $R \cong \xi(N/g)^{1/2}$. This is the Rouse model which considers a chain of N/g hydrodynamically independent particles. The elastic modulus in this situation is proportional to the number of chains per volume unit and the viscosity is obtained from the product $\eta_{\text{Rouse}} = G_{\text{Rouse}}\,\tau_{\text{Rouse}}$. One obtains:

$$(2a,b,c) \qquad \tau_{\text{Rouse}} \cong \tau_{\text{blob}}\left(\frac{N}{g}\right)^2; \qquad G_{\text{Rouse}} = kT\frac{C}{N}; \qquad \eta_{\text{Rouse}} \cong \eta_0\left(\frac{N}{g}\right)$$

where $\tau_{\text{blob}} = \xi^2/D_0 = 6\pi\eta_0\xi^3/kT$.

For high values of the reduced degree of polymerization N/g, polymers reptate in a tube of length L and diameter A. The diffusion coefficient D corresponds to diffusion within the tube. The corresponding relaxation time is equal to L^2/D. The main question concerns the relation between A, L and ξ. In the melt, the tube diameter is larger than the size "a" of monomers: $A = a \times n_e^{1/2}$ and n_e monomers are needed to form an entanglement, the number n_e depending on the polymer species. In semi-dilute solutions the same number n_e of blobs is needed for polymers to be entangled (Raspaud *et al.*, 1995). A chain of blobs being Gaussian, the tube diameter is equal to $A = \xi\, n_e^{1/2}$ and its length to $L = A(N/g)/n_e = \xi(N/g)/n_e^{1/2}$. The elastic modulus is equal to the number of entanglements per volume unit $G = kT/(n_e\,\xi^3)$. One obtains:

$$(3a,b,c) \qquad \tau \cong \tau_{\text{Rouse}}\left(\frac{N/g}{n_e}\right); \qquad G \cong G_{\text{Rouse}}\left(\frac{N/g}{n_e}\right); \qquad \eta \cong \eta_{\text{Rouse}}\left(\frac{N/g}{n_e}\right)^2$$

These equations show that viscoelastic properties in the entangled regime are a function not only of the number of blobs per chain N/g (*i.e.* of C/C^*), but also of the non-universal parameter n_e. The concentration C_e at which polymers begin to be entangled is proportional to C^* and further depends on the polymer species. At C_e, $n_e = N/g = (C_e/C^*)^{1/(3\nu-1)}$, which gives:

$$C_e = C^*\, n_e^{3\nu-1} \tag{4}$$

The relevant reduced concentration for the dynamical properties is thus C/C_e rather than C/C^*. Equation $(3a,b,c)$ can be rewritten as:

$$(5a,b,c) \qquad \begin{cases} \dfrac{\tau}{\tau_{\text{Rouse}}} \cong \left(\dfrac{C}{C_e}\right)^{1/(3\nu-1)}; \\ \dfrac{G}{G_{\text{Rouse}}} \cong \left(\dfrac{C}{C_e}\right)^{1/(3\nu-1)}; \qquad \dfrac{\eta}{\eta_{\text{Rouse}}} \cong \left(\dfrac{C}{C_e}\right)^{2/(3\nu-1)} \end{cases}$$

These relations are verified (Raspaud *et al.*, 1995). Using the above reduced variables it is possible to lay on a single master curve experimental data obtained on different synthetic polymer species.

SAMPLE CHARACTERISTICS AND EXPERIMENTAL CONDITIONS. – Lyophilized T2 DNA was purchased from Sigma. T2 DNA has a molecular weight M of 1.1×10^8 g/mol corresponding to 164,000 base pairs, and a contour length of 56 μm. DNA solutions were prepared using a buffer containing 50 mM-Tris.HCl (pH 7.5), 100 mM KCl, 10 mM $MgCl_2$ and 0.5 mM EDTA. DNA concentrations were determined by optical density measurements assuming that 0.05 mg/cm^3 gives an absorbance of unity. More than 80% of the DNA chains were intact according to the supplier. This integrity was checked by gel electrophoresis on a 0.2% agarose gel. It was necessary to wait for 2 to 3 weeks to obtain homogeneous samples upon dilution from a stock solution. Zero shear viscosities and the longest relaxation times of solutions were measured using a magnetorheometer. This device allows the use of sealed cells which prevent evaporation and contacts of the sample with external surroundings. Measurements were performed at 30°C.

EXPERIMENTAL RESULTS AND DISCUSSION. – At concentrations lower than 0.16 mg/cm^3 the viscosity of the solutions is too small to be measured with the magnetorheometer. At concentrations higher than 1.2 mg/cm^3, the size of the blob is expected to be of the order of the persistence length (about 50 nm or 150 base pairs; Bloomfield *et al.*, 1974). Thus, for these high concentrations local organization of neighbouring chains should lead to a peculiar viscoelastic behaviour. In fact, a zero shear viscosity can be measured at a concentration of 1.2 mg/cm^3, while at 1.6 mg/cm^3 the solution behaves like a gel with a zero shear elastic modulus.

We measured the viscosity and the longest relaxation times between 0.16 and 1.2 mg/cm^3. Both quantities depend strongly on the shear rate γ. In figure 1, the viscosity η is plotted as a function of the reduced shear rate $\gamma\tau$, where τ is the zero shear limit of the measured longest relaxation time. In this work we focus on the zero shear limit of η and τ. Results are listed in the table, where the plateau elastic modulus G is equal to the ratio η/τ.

TABLE

Zero shear limit of the viscosity η and the longest relaxation time τ for the different concentrations studied. G is the plateau modulus η/τ.

Limite à gradient de cisaillement nul de la viscosité η et du temps de relaxation τ le plus long pour les différentes concentrations étudiées. G est le module élastique au plateau η/τ.

C (mg/cm^3)	η (Pa s)	τ (s)	G (Pa)
0.160	0.043 ± 0.002	–	–
0.269	0.55 ± 0.01	31 ± 0.5	0.0180 ± 0.0005
0.295	2.05 ± 0.05	77.5 ± 0.5	0.0264 ± 0.0002
0.328	1.6 ± 0.1	79.5 ± 0.5	0.020 ± 0.001
0.545	23.5 ± 0.5	235 ± 5	0.100 ± 0.004
0.640	31.0 ± 0.2	243 ± 2	0.127 ± 0.002
0.960	520 ± 20	1 170 ± 10	0.44 ± 0.02
1.210	530 ± 10	930 ± 10	0.57 ± 0.02

In order to compare the viscoelastic behaviour of DNA solutions to the one observed with synthetic polymers and to the theoretical predictions, one needs to reduce the concentration by the entanglement concentration. This concentration can be determined

in two ways. First, using equation (5) one obtains $C'_e = C/(G/G_{\text{Rouse}})^{3\nu-1}$, with $\nu = 0.588$, G_{Rouse} (Pa) $= kTC/M = 0.025 \times C$ (C, mg/cm^3, $M = 1.1 \times 10^3$ g/mol). Second, the number n_e may be directly determined from the ratio $K/G = n_e$, where $K = C \times d\pi/dC = [3\nu/(3\nu - 1)]\pi$ is the osmotic modulus. This leads to $C_e = C^*(K/G)^{3\nu-1} = [3\nu/(3\nu - 1)]^{3\nu-1} \times C'_e = 1.9 \times C'_e$ for the entanglement concentration. The experimental results obtained for five different synthetic polymers having different degrees of polymerization and different chemical natures give $C_e/C'_e = 1.90 \pm 0.05$ (Raspaud *et al.*, 1995). For synthetic polymers, the second definition of the entanglement concentration leads to values of n_e equal to the number of monomers determined in the melt. We therefore adopt this definition. In figure 2 the concentration $C_e = 1.9 \times C/(G/G_{\text{Rouse}})^{3\nu-1}$ is plotted as a function of the concentration. One finds for the entanglement concentration:

$$C_e = 0.25 \pm 0.04 \text{ mg/cm}^3 \tag{6}$$

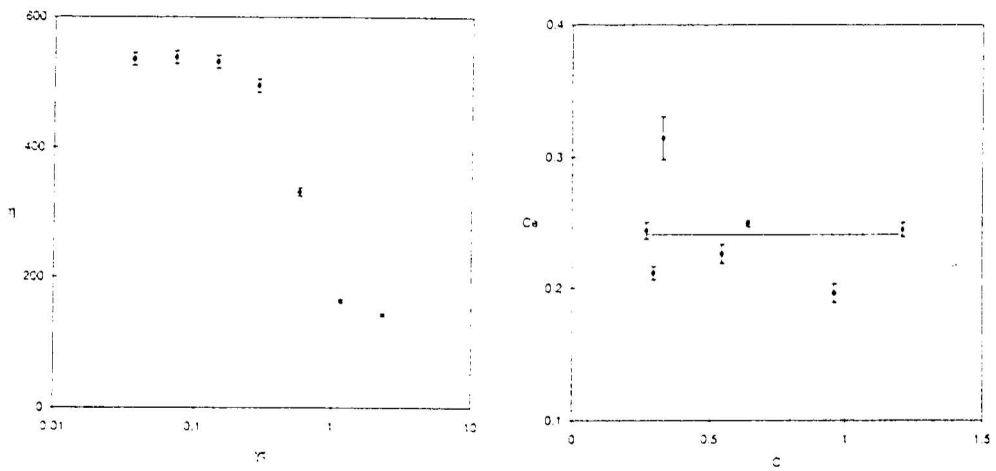

Fig. 1. – Influence of the reduced shear rate $\gamma\tau$ on the measured viscosity η (Pa s) at a concentration $C = 0.960$ mg/cm^3. The shear rate γ is estimated by dividing the flow velocity by the magnetic ball diameter.

Fig. 1. – Influence du gradient de cisaillement réduit $\gamma\tau$ sur la viscosité mesurée η (Pa s) pour une concentration $C = 0.960$ mg/cm^3. Le gradient de cisaillement γ est calculé en divisant la vitesse d'écoulement par le diamètre de la bille magnétique.

Fig. 2. – Entanglement concentration C_e deduced from the plateau elastic modulus G measured at different concentrations (mg/cm^3): $C_e = 1.9 \times C/(G/G_{\text{Rouse}})^{3\nu-1}$, where $\nu = 0.588$ and G_{Rouse} (Pa) $= 0.025 \times C$ (mg/cm^3). The straight line corresponds to the mean value 0.25 mg/cm^3.

Fig. 2. – Concentration d'enchevêtrement C_e déduite du module élastique au plateau G mesuré à différentes concentrations (mg/cm^3): $C_e = 1,9 \times C/(G/G_{\text{Rouse}})^{3\nu-1}$, avec $\nu = 0,588$ et G_{Rouse} (Pa) $= 0,025 \times C$ (mg/cm^3). La droite correspond à la valeur moyenne 0.025 mg/cm^3.

The fact that $C/(G/G_{\text{Rouse}})^{3\nu-1}$ is found to be independent of the concentration, proves that the exponent value for the concentration dependence of the elastic modulus is in fact $1 + 1/(3\nu - 1) = 2.31$.

Equation (5c) shows that the reduced viscosity is η/η_{Rouse}. The problem is to obtain an accurate estimate for η_{Rouse}, which is a function of C/C^*. The concentration C^* of synthetic polymers is usually determined by light scattering, either by measurements of the mass and radius of gyration ($C^* = M/R^3$), or by measurement of the second virial

coefficient ($C^* \cong 1/MA_2$). Due to their size, this cannot be done for DNA molecules. The best way to have access to the internal concentration C^* of DNA chains is by intrinsic viscosity measurements $[\eta] \cong 1/C^*$; for T2 DNA $[\eta] = 30$ cm^3/mg (Chapman *et al.*, 1969). The Rouse viscosity will thus be taken as:

$$\eta_{\text{Rouse}} = \eta_0 ([\eta] C)^{1/(3\nu-1)} \tag{7}$$

where $\eta_0 = 8 \times 10^{-4}$ Pa s is the viscosity of water at 30°C. In figure 3 the reduced viscosity η/η_{Rouse} is plotted as a function of the reduced concentration C/C_e for T2 DNA and compared to the master curve obtained for synthetic polymers. The experimental data here reported lie on this master curve. Note that the partial degradation of the DNA sample leads to an overestimate of the concentration of about 25%. This can account for the slight difference between DNA data and the master curve. The asymptotic behaviour corresponds to the power law:

$$\eta/\eta_{\text{Rouse}} = 60 \times (C/C_e)^{3.4} \tag{8}$$

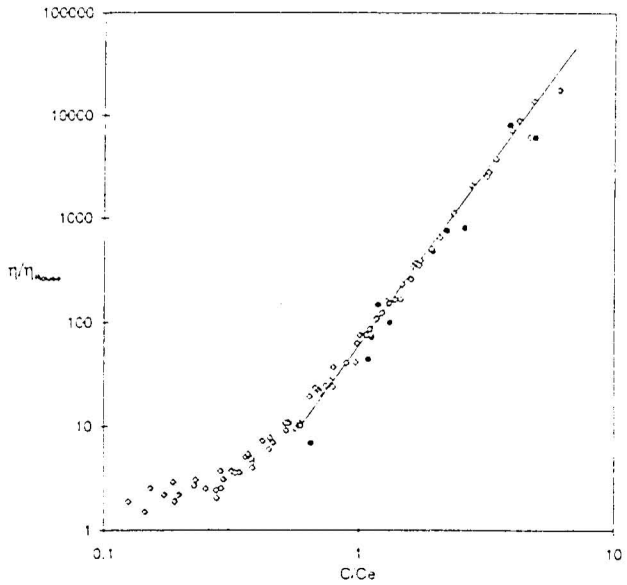

Fig. 3. – Reduced viscosity η/η_{Rouse} as a function of the reduced concentration C/C_e ($\eta_{\text{Rouse}} = \eta_0 ([\eta] C)^{1/(3\nu-1)}$, $\eta_0 = 8 \times 10^{-4}$ Pa s, $[\eta] = 30$ cm^3/g (Chapman *et al.*, 1969), $\nu = 0.588$ and $C_e = 0.25 \pm 0.04$ mg/cm^3). Open symbols correspond to measurements performed on synthetic polymers (Raspaud *et al.*, 1995), while black circles correspond to T2 DNA data. The straight line corresponds to $\eta/\eta_{\text{Rouse}} = 60 \times (C/C_e)^{3.4}$.

Fig. 3. – Viscosité réduite η/η_{Rouse} en fonction de la concentration réduite C/C_e ($\eta_{\text{Rouse}} = \eta_0 ([\eta] C)^{1/(3\nu-1)}$, $\eta_0 = 8 \times 10^{-4}$ Pa s, $[\eta] = 30$ cm^3/g (Chapman et al., *1969), $\nu = 0,588$ et $C_e = 0,25 \pm 0,04$ mg/cm^3). Les symboles clairs correspondent aux mesures effectuées sur des polymères synthétiques, tandis que les symboles noirs correspondent aux valeurs obtenues avec l'ADN T2. La droite correspond à l'équation $\eta/\eta_{\text{Rouse}} = 60 \times (C/C_e)^{3.4}$.*

The exponent in (8) is greather than the value predicted by the reptation model. The same discrepancy is observed for the mass dependence of η in the melt: η varies as $N^{3.4}$ rather than N^3 (Berry and Fox, 1968). The exponent value 3.4 leads to $\eta/\eta_{\text{Rouse}} = (C/C_e)^{3.45}$

[from equation (3c)] rather than $\eta/\eta_{\text{Rouse}} = (C/C_e)^{2/(3\nu-1)} = (C/C_e)^{2.93}$, in agreement with relation (8). In terms of concentration, the prefactor of 60 in (8) corresponds to a factor of $60^{1/3.4} = 3.34$. As for C^*, this factor can clearly be due to the convention used for C_e.

The results obtained here permit analysis of two previous reports dealing with semi-dilute solutions of λ DNA molecules (48.500 base pairs). Using the estimate of C_e obtained above for T2 DNA, it is possible to calculate the entanglement concentration for λ DNA (which scales as $M^{1-3\nu}$). For λ DNA, one finds $C_{e\,(\lambda\,\text{DNA})} = 0.6$ mg/cm^3. Scalettar *et al.* (1989) have studied the self-dilution of λ DNA using fluorescence recovery after photobleaching. The concentration range was 0.017 mg/cm$^3 \leqq C \leqq 0.30$ mg/cm^3. According to our results these solutions are not entangled. This agrees with their observation that the self-diffusion coefficient shows a fairly weak dependence on concentration. Perkins *et al.* (1994) report elegant direct observations of motion of a single DNA molecule (obtained by linking several λ DNA molecules) in a semi-dilute solution of λ DNA ($C = 0.6$ mg/cm^3). The concentration chosen is equal to the entanglement concentration $C_{e\,(\lambda\,\text{DNA})}$. Whether the tube-like motion observed can be described by the reptation model will require quantitative investigations, preferably at higher concentrations.

Concluding remarks. – Viscoelastic properties of entangled DNA solutions have been measured. Results are found to be in agreement with the universal law $\eta/\eta_{\text{Rouse}} = 60 \times (C/C_e)^{3.4}$. The longest measured relaxation time (about 20 min. at 1 mg/cm^3) is equal to the time required for the intracellular development of T-even phages (*see* e.g. Kellenberger, 1961). In contrast with the simplicity of the relaxation process studied here, the phage development cycle is highly complex. The DNA is replicated every 2 min. and at the end of replication the 200 replicated molecules are condensed to yield the mature phages. This condensation process occurs at an average concentration of the order of 50 mg/cm^3. It will be of interest to investigate the role of the phage topo II in the dynamics of these processes.

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