[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF WISCONSIN]

Viscosity and Rigidity of Solutions of Sodium Thymonucleate[1,2]

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Viscosities over a range of small velocity gradients and dynamic rigidities over a range of low audio frequencies have been measured for solutions of sodium desoxyribonucleate (weight-average molecular weight 8×10^6) in 0.2 M sodium chloride at pH 6.2, at concentrations from 0.005 to 0.02 g./ml. and temperatures from 10.5 to 35°. The viscosity is non-Newtonian at velocity gradients as low as 0.05 sec.$^{-1}$, but can be extrapolated to zero gradient from the data obtained. The extrapolated viscosity is an exponential function of the reciprocal absolute temperature, and the apparent heat of activation for viscous flow increases from 6.2 to 7.9 kcal. with increasing concentration. The reduced specific viscosity increases with concentration from 300 to 12,000 (g./dl.)$^{-1}$, as compared with an expected intrinsic viscosity of 62 from the measurements of Pouyet. The dynamic rigidity increases only slightly with increasing frequency or decreasing temperature; these changes are interrelated as predicted by the method of reduced variables. The ratio of rigidity to concentration (at 50 cycles/sec., 25°) is of the order of 10^3 (dynes/cm.^{2})(g./ml.)$^{-1}$, but increases sharply above a concentration of 0.015 g./ml. The Maxwellian relaxation times lie primarily between 0.1 and 1 sec., being much more sharply distributed than in conventional gels or viscoelastic polymer solutions. The unusual mechanical properties of these solutions are due in large part to the presence of a very small rigidity associated with a moderately long relaxation time. The magnitude of the rigidity is similar to that predicted by Kirkwood and Auer for orientation of isolated thin rigid rods, but the relaxation time is much higher than expected for the latter process. The observed mechanical properties are attributed to orientation of rigid elongated particles which are subject to marked hydrodynamic and steric interference but form no interconnecting structure through bonds or localized attractive forces.

Introduction

Solutions of desoxyribonucleic acid (often obtained from thymus, hence thymonucleic acid) and its salts have peculiar mechanical properties, often alluded to by the description "gel-like." This term is not very satisfactory; the properties are certainly markedly different from those of familiar gels[3] such as gelatin or fibrin, and also those of viscoelastic high polymer solutions such as polyisobutylene,[4] for example. Neither qualitative descriptions nor the conventional sort of viscosity measurements can adequately characterize solutions of nucleic acid.

In the present study, two types of mechanical measurements have been applied to sodium desoxyribonucleate in the concentration range from 0.005 to 0.02 g./ml., providing the viscosity at very low rates of shear and the dynamic rigidity at frequencies in the low audio frequency range. These measurements assist in understanding the unusual mechanical properties in terms of well-defined physical constants.

Other experimental methods have given some information about the structure existing in solutions of this material in different ranges of concentration. Riley and Oster[5] concluded from X-ray diffraction that above 0.04 g./ml. there is partial order of a liquid crystalline character, accompanied by extremely high viscosity and, at higher concentrations, spontaneous birefringence. Below 0.04 g./ml., there is no obvious ordering and the viscosity is lower. Nevertheless, from measurements of Kerr effect, Benoit[6] found evidence of strong intermolecular interaction at 0.00005 g./ml., and it was necessary to reduce the concentration below 0.00002 g./ml. to observe orientation with a rotary diffusion constant of the magnitude expected for an isolated nucleic acid molecule. Similarly, the measurements of intrinsic viscosity at low velocity gradients by Pouyet[7] necessitated working in a concentration range below 10^{-4} g./ml. The latter workers used a desoxyribonucleic acid preparation described as VIII by Signer and Schwander,[8] very similar in characteristics to the one used in our present investigation and a previous light scattering study from this Laboratory.[9] (Long-range coulombic effects were reduced by the presence of 1% glycine[6] and 10% sodium chloride,[7] respectively.)

In the concentration range of our present experiments there is evidently substantial interaction (as would indeed be expected for molecules about 7000 Å. in length[9]), but still no quasi-crystalline structure.

Materials and Methods

The sodium desoxyribonucleate (hereafter SDNA), from calf thymus, was the same preparation used in the study of its reaction with mercuric chloride previously reported.[9] Its content of basic protein was less than 0.2% according[9] to the color reaction used by Signer[8]; its weight-average molecular weight determined by light scattering[9] was 8×10^6. Solutions were made up by weight in 0.2 M sodium chloride at pH 6.2. Concentrations are expressed in g. of SDNA per ml. of solution, which differs less than 0.5% from the weight fraction of SDNA.

Viscosities were measured at various velocity gradients by the falling ball method with miniature glass spheres as described by Ehrlich, Shulman and Ferry.[10,11] Instead of a very long glass tube, a 10-cc. measuring cylinder was used to contain the solution; it was placed in an air thermostat with windows, and the rate of fall of the spheres was followed with a cathetometer telescope. A range of velocity gradients was provided for each solution by a series of spheres from 0.1 to 0.5 mm. in diameter. Equations for calculating viscosity and maximum velocity gradient have been given previously.[10]

The velocities of propagation of transverse waves were measured by following their strain birefringence, using

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(1) Presented in part at the 121st Meeting of the American Chemical Society, April 2, 1952.

(2) This paper is part XII of a series on Mechanical Properties of Substances of High Molecular Weight.

(3) J. D. Ferry, *Adv. Protein Chem.*, **4**, 1 (1948).

(4) J. D. Ferry, E. R. Fitzgerald, M. F. Johnson and L. D. Grandine, Jr., *J. Appl. Phys.*, **22**, 717 (1951).

(5) D. P. Riley and G. Oster, *Biochim. Biophys. Acta*, **7**, 526 (1951).

(6) H. M. Benoit, *J. chim. phys.*, **48**, 612 (1951).

(7) J. Pouyet, *ibid.*, **48**, 616 (1951).

(8) R. Signer and H. Schwander, *Helv. Chim. Acta*, **33**, 1522 (1950).

(9) S. Katz, THIS JOURNAL, **74**, 2238 (1952).

(10) P. Ehrlich, S. Shulman and J. D. Ferry, *ibid.*, **74**, 2258 (1952).

(11) We are indebted to the Minnesota Mining and Manuacturing Company for some of these spheres; and also to Dr. V. R. Deitz of the National Bureau of Standards for ten samples of size-graded spheres, selected for sphericity as described by F. G. Carpenter and V. R. Deitz, *J. Research Natl. Bur. Standards*, **47**, 139 (1951).

stroboscopic illumination.[12,13] The damping indices, which were of the order of unity, could not be measured with sufficient accuracy to calculate η', the real part of the dynamic viscosity, but were adequate for calculation of G', the real (or in-phase) part of the dynamic modulus of rigidity, by the equations previously given.[13]

Results

Viscosity.—The apparent viscosity, *i.e.*, that calculated from the Stokes law with the Faxén correction, without taking deviations from Newtonian flow into account, is plotted in Fig. 1 against the maximum velocity gradient at the surface of the sphere, for one SDNA solution at three temperatures. It increases sharply with decreasing velocity gradient, showing non-Newtonian effects which persist down to a gradient of 0.05 sec.$^{-1}$. This result emphasizes the well-known fact that viscosities of such solutions measured in a conventional capillary viscosimeter, where the gradient is of the order of 1000 sec.$^{-1}$, are very far from the values corresponding to zero gradient or random orientation. Only the measurements of Pouyet,[7] made with a specially designed coaxial cylinder apparatus, involve gradients of such small magnitude as those used here.

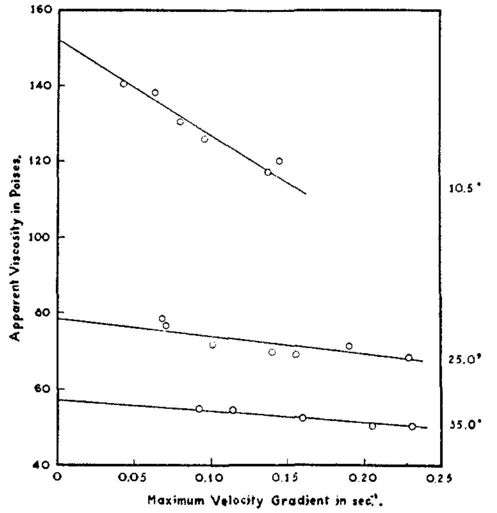

Fig. 1.—Apparent viscosity plotted against maximum velocity gradient for solution of SDNA at concentration of 0.015 g./ml., at three temperatures identified in margin.

The linear dependence of apparent viscosity (η_a) on maximum gradient ($\dot{\gamma}$), shown in Fig. 1 and by similar plots at three other concentrations, permits extrapolation to zero gradient with fair confidence. The values of viscosity thus obtained are given in Table I, together with the slopes of the linear plots, $d\eta_a/d\dot{\gamma}$, and other derived data. Even at a concentration of 0.005 g./ml., the viscosity at 25° is 150 times that of the solvent.

The zero gradient viscosity is plotted logarithmically against the reciprocal absolute temperature for four different concentrations in Fig. 2. These plots show slight upward curvature; the apparent activation energies for viscous flow, Q_η, calculated from their slopes at 25°, are listed in Table I. These values are somewhat higher than that of the solvent (about 4 kcal.), and increase somewhat with increasing concentration.

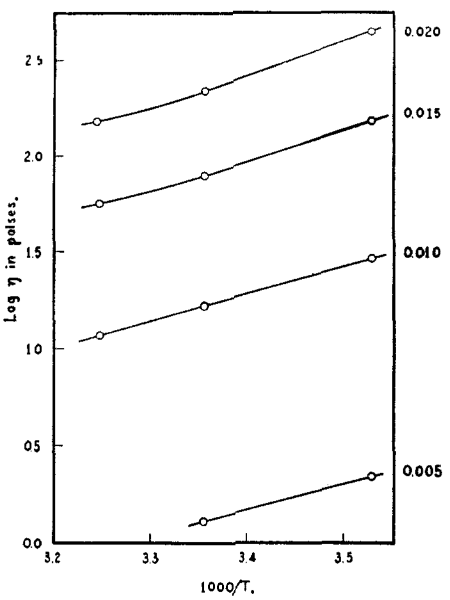

Fig. 2.—Viscosity extrapolated to zero velocity gradient, plotted logarithmically against reciprocal absolute temperature, for SDNA solutions at four concentrations (in g./ml.) identified in margin.

TABLE I

VISCOSITY DATA

Concn., g./ml.	Temp., °C.	η, poises	$d\eta_a/d\dot{\gamma}$, poise-sec.	Q_η, kcal.	$\eta^2/(d\eta_a/d\dot{\gamma})$, dyne/cm.^{2}
0.005	10.5	2.2	0.19		25
	25.0	1.3	0.06	6.2	28
0.010	10.5	29.6	13.3		66
	25.0	16.8	4.2	6.4	67
	35.0	11.8	1.7		82
0.015	10.5	152	237		97
	25.0	79	48	7.0	130
	35.0	57	29		112
0.020	10.5	442	2160		90
	25.0	220	434	7.9	112
	35.2	150	156		144

The reduced specific viscosity, η_{sp}/c (where here c is in g./dl. to provide the conventional units), is given in Table II. The values are enormous, and change so rapidly with concentration that it is clearly impossible to obtain the intrinsic viscosity by extrapolation from this concentration range. The intrinsic viscosity obtained by Pouyet for a

TABLE II

REDUCED SPECIFIC VISCOSITY AT 25°

Concn., g./ml.	η_{sp}/c, (g./dl.)$^{-1}$
0	62[a]
.005	300
.010	1,840
.015	5,700
.020	12,000

[a] From data of Pouyet[7] on preparation VIII of Signer and Schwander[8] in 10% sodium chloride.

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(12) J. D. Ferry, *Rev. Sci. Instruments*, **12**, 79 (1941).

(13) J. N. Ashworth and J. D. Ferry, THIS JOURNAL, **71**, 622 (1949).

similar SDNA preparation (but at a higher ionic strength) is included for comparison.

Rigidity.—The rigidities measured were quite small—from 5 to 200 dynes/cm.^{2}—and are among the smallest ever to be reported for any system.[14] It is very unusual for rigidity mechanisms of this order of magnitude to be associated with relaxation times long enough so that a perceptible dynamic rigidity can be measured in the low audiofrequency range.

The rigidity increased only slightly with increasing frequency from 25 to 160 cycles/sec. or decreasing temperature from 35° to 10.5°. The effects of frequency and temperature could be combined by the method of reduced variables,[17,18] so that at any given concentration a plot of $G'_p = G'T_0/T$ against $\omega_p = \omega\eta T_0/\eta_0 T$ gave a single curve for all temperatures and frequencies. Here η_0 and η are the steady flow viscosities (extrapolated to zero gradient) of the solution at a standard reference temperature, T_0(298°K.), and the temperature of measurement, T; ω is the circular frequency. A density ratio, ρ_0/ρ, which strictly should appear in these expressions, has been omitted because it is negligibly different from unity in this narrow temperature range. The reduced variables G'_p *vs.* ω_p are plotted logarithmically in Fig. 3. The single line obtained at each concentration, within the rather large experimental scatter characteristic of wave propagation measurements, indicates that relaxation times operating in this range have the same temperature dependence.[17,18] These plots represent predictions of the rigidity at 25° over a more extended frequency range.

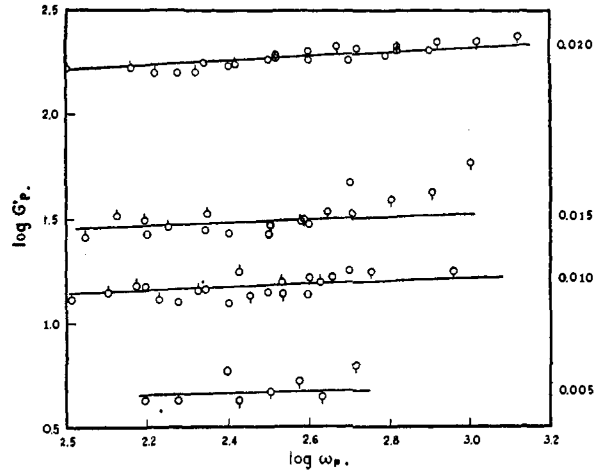

Fig. 3.—Logarithm of reduced dynamic rigidity, $G'T_0/T$, plotted against logarithm of reduced frequency, $\omega\eta T_0/\eta_0 T$, for SDNA solutions at four concentrations identified in margin; reference temperature 25°: downward tags, 10.5°; no tags, 25.0°; upward tags, 35.0°.

It was found that the concentration could not be combined with temperature and frequency in the reduction treatment as usually used for synthetic polymer solutions[17]; either there are elastic mechanisms whose relaxation times depend differently on concentration, or else their magnitudes are not directly proportional to concentration as they are in synthetic polymers.[17] To examine the dependence of rigidity on concentration, therefore, values of G' were compared at constant frequency—50 cycles/sec. at 25°. As shown in Table III, G' increases very rapidly with concentration. Values of G'/c are also given in Table III and are plotted against concentration in Fig. 4 together with η_{sp}/c from Table II. The ratio G'/c experiences a sharp increase above 0.015 g./ml. It may be extrapolated to zero concentration to give an "intrinsic rigidity"[19] of about 400 (dynes/cm.^{2})(g./ml.)$^{-1}$. Such an extrapolation is doubtful, however, in view of the change in structure which apparently takes place[6] below the concentration range covered here, and the fact that the reduced specific viscosity cannot be extrapolated from this range.

TABLE III

REAL PART OF DYNAMIC RIGIDITY AT 25° AND 50 CYCLES/SEC.

c, g./ml.	G', dynes/cm.^{2}	G'/c	η/G', sec.
0.005	4.7	940	0.3
.010	15.2	1520	1.1
.015	31	2100	2.5
.020	182	9100	1.2

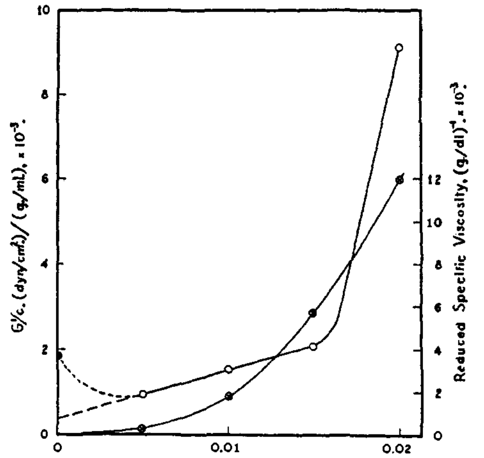

Fig. 4.—G'/c at 50 cycles/sec., 25° (open circles) and η_{sp}/c (crossed circles), plotted against concentration in g./ml. Black point at $c = 0$ denotes theoretical value of G'/c for thin rods at infinite dilution (reference 19).

Discussion

Viscosity.—The enormous values of η_{sp}/c are far greater than those encountered for flexible polymers, polymerized fibrinogen,[10] or even tobacco mosaic virus[20] in a comparable concentration range. They are, however, no greater than would be expected for a molecule with an intrinsic viscosity of 62 on the basis of the Huggins equation, $\eta_{sp}/c = [\eta] + k'[\eta]^2c$. The coefficient k' is predicted theoretically from considerations of hydro-

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[14] Some values in this range were estimated by Van Wazer and Goldberg[15] for solutions of 1.5 to 2% "napalm" in gasoline, and by T. Nakagawa[16] for very dilute aqueous agar gels.

[15] J. R. Van Wazer and H. Goldberg, *J. Applied Phys.*, **18**, 207 (1947).

[16] T. Nakagawa, *J. Chem. Soc. Japan*, **72**, 518 (1951).

[17] J. D. Ferry, THIS JOURNAL, **72**, 3746 (1950).

[18] J. D. Ferry, E. R. Fitzgerald, L. D. Grandine, Jr., and M. L. Williams, *Ind. Eng. Chem.*, **44**, 703 (1952).

[19] J. G. Kirkwood and P. L. Auer, *J. Chem. Phys.*, **19**, 281 (1951).

[20] M. A. Lauffer, THIS JOURNAL, **66**, 1188 (1944).

dynamic interference to be 0.73 for a thin rigid rod[21]; the values for other shapes do not differ greatly, and experimental values for flexible polymers range from 0.2 to 1.0. The value of k' obtained from Pouyet's intrinsic viscosity and η_{sp}/c at our lowest concentration is only 0.12. Thus the concentration dependence of viscosity is, if anything, less than might have been expected. This result, together with the rather low apparent activation energies for viscous flow, suggests that the high viscosities are due primarily to hydrodynamic and steric interference and not to any branching aggregation or "gel structure" such as has been suggested even for dilute solutions of nucleic acid.[22]

No adequate theory exists for interpreting the dependence of viscosity on velocity gradient in such systems, but some interesting parallels can be drawn from treatments of non-Newtonian flow in concentrated synthetic polymer solutions.[23-27] Here shearing stress, $\mathfrak{T}$, rather than velocity gradient, is usually chosen as the independent variable, and the apparent viscosity is expressed in the form $\eta_a = \eta/[1 + f(\mathfrak{T}/G_a)]$; G_a is a constant with the dimensions of a modulus of rigidity, and η is the viscosity extrapolated to zero stress. The function f is variously represented by the ratio $\mathfrak{T}/G_a$ itself or by more complicated expressions, but G_a is generally agreed to be related to molecular orientations, and it has been called an internal modulus of rigidity. In several cases[23,25,26] G_a has been shown to be close in magnitude to G_i, the rigidity calculated from the elastic compliance in steady-state flow or the elastic recovery.

It is of interest, therefore, to estimate G_a for SDNA solutions from the data of Table I. Taking $f(\mathfrak{T}/G_a) = \mathfrak{T}/G_a$, and noting that $\eta_a = \mathfrak{T}/\dot{\gamma}$, it can be shown that $G_a = \eta^2/(\mathrm{d}\eta_a/\mathrm{d}\dot{\gamma})$, where the derivative is taken at vanishing gradient. Values of G_a thus calculated are included in Table I; they are approximately independent of temperature (as in concentrated polymer solutions), and they are actually of the same order of magnitude as the dynamic rigidities in Table III, although they increase less rapidly with concentration.

Relaxation Times.—The fact that the rigidity is almost independent of frequency, as shown in Fig. 3, indicates that the elastic mechanisms operative in the low audiofrequency range are mostly associated with relaxation times considerably longer than the reciprocals of the frequencies involved. On the other hand, the fact that the solutions can be poured and that any lumpiness apparent in the pouring disappears in the order of a second indicates that the relaxation times do not exceed one second. A similar conclusion was reached from qualitative experiments in a coaxial cylinder apparatus,[28,29] where stress relaxation was found to be complete within less than a second. Thus the principal relaxation times are bracketed within 0.1 and 1 sec.—a far narrower range than the spread of many powers of ten which is found both in concentrated solutions of flexible polymers[4,27] and in conventional gels.[28] (The fact that G' does increase slightly with frequency (Fig. 3) shows that there are slight additional elastic contributions with relaxation times in the millisecond range, but they make up a very small proportion of the total rigidity.)

The very sharp distribution of relaxation times suggests that the mechanical properties might be approximately represented by a single relaxation time, as provided by a Maxwell element with a spring and viscous dashpot in series. In this case at frequencies well above the reciprocal of the relaxation time, $G' = G_M$, the rigidity represented by the spring,[30] and the relaxation time is therefore[30] $\tau = \eta/G'$. Values for this ratio are given in Table III and are actually of the order of magnitude of 1 second as deduced above. Moreover, the relaxation time found by Benoit[6] from Kerr effect measurements at a concentration of 0.00005 g./ml. was also about 1 second. A further characteristic of the Maxwell element is that G_i, the reciprocal of the elastic compliance in steady-state flow, equals G_M and therefore G'. It has already been noted that G_a in Table I, which is usually found to be close to G_i, is similar in magnitude to G'. Thus a single Maxwell element may serve to describe roughly the mechanical behavior of SDNA solutions—an unusual circumstance among viscoelastic systems. It is not clear, however, why the relaxation time should change scarcely at all with concentration from 0.00005 g./ml. (Benoit's measurement) to 0.02 g./ml. (our highest value).

Rigidity.—The most unusual feature of the mechanical properties of SNDA solutions is the association of a rigidity of 10–100 dynes/cm.^{2} with a relaxation time of about 1 second. In gelatin gels at a comparable concentration (about 0.01 g./ml.), the rigidity[3] is of the order of 10^3 dynes/cm.^{2} and is associated with relaxation times of the order of minutes[28]; it is attributed to a three-dimensional cross-linked network structure. At lower concentrations, there is no rigidity at all associated with long relaxation times, because the network cannot be formed.[3] In synthetic polymers of very high molecular weight at a comparable concentration, the rigidity[31] is also of the order of 10^3 dynes/cm.^{2} but is associated with a relaxation time of the order of 10^{-3} sec.; it cannot be detected by measurements in the low audiofrequency region. At lower concentrations, rigidities of the order of 100 dynes/cm.^{2} have been measured,[31] but associated, again, with very short relaxation times. Such rigidity[32] is attributed primarily to configurational changes within the highly flexible molecules. In SDNA solutions, the relaxation times are far

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[21] J. Riseman and R. Ullman, *J. Chem. Phys.*, **19**, 585 (1951).

[22] J. A. V. Butler, B. E. Conway and D. W. F. James, *J. chim. phys.*, **48**, 609 (1951).

[23] J. D. Ferry, THIS JOURNAL, **64**, 1330 (1942).

[24] R. S. Spencer, *J. Polymer Sci.*, **5**, 591 (1950).

[25] F. R. Eirich, personal communication.

[26] H. Leaderman and R. G. Smith, *Phys. Rev.*, **81**, 303 (1951).

[27] F. W. Schremp, J. D. Ferry and W. W. Evans, *J. Applied Phys.*, **22**, 711 (1950).

[28] M. Miller, J. D. Ferry, F. W. Schremp and J. E. Eldridge, *J. Phys. Colloid Chem.*, **55**, 1387 (1951).

[29] These experiments were made with the help of Dr. George E. Heckler.

[30] J. D. Ferry, W. M. Sawyer and J. N. Ashworth, *J. Polymer Sci.*, **2**, 593 (1947).

[31] W. O. Baker, W. P. Mason and J. H. Heiss, *ibid.*, 8, 129 (1952).

[32] Other elastic mechanisms with higher rigidities are associated with still shorter relaxation times and are apparent in measurements at extremely high frequencies.[31]

smaller than in gelatin gels and far greater than in synthetic polymer solutions, and the rigidities are smaller than in either. The elastic mechanisms are probably quite different from those of either of the more familiar systems.

Kirkwood and Auer[19] showed that a dilute solution of rigid rod-like molecules should exhibit a dynamic rigidity with Maxwellian dispersion; the limiting high-frequency value for the intrinsic rigidity, G'/c, should be $3RT/5M$ in our units, where M is the molecular weight. This theory can hardly be strictly applicable to SDNA in our concentration range, since it is designed for isolated molecules; in the absence of intermolecular interference, SDNA molecules should orient in water with a relaxation time[33] of about 0.01 sec. instead of 1 sec., and this situation is reached[7] only below 0.00002 g./ml. Nevertheless, the SDNA molecule is apparently rigid and extended,[33–35] and it is of interest therefore to calculate the intrinsic rigidity from the above formula. Taking $M = 8 \times 10^6$, G'/c is found to be 1850 (dynes/cm.^{2}) (g./ml.)$^{-1}$. This value is actually of the correct order of magnitude, and is plotted in Fig. 4 with a dotted line to show how it might be approached at infinite dilution. It is tentatively concluded that not only the viscosities but also the rigidities of SDNA solutions involve primarily the orientations of rigid elongated particles which are crowded together with marked steric hindrance, but form no interconnecting structure through bonds or localized attractive forces.

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(33) H. Schwander and R. Cerf, *Helv. Chim. Acta*, **34**, 436 (1951).

(34) H. Schwander and R. Signer, *ibid.*, **34**, 1344 (1951).

(35) M. E. Reichmann, R. Barin and P. Doty, THIS JOURNAL, **74**, 3203 (1952).

[CONTRIBUTION FROM THE RESEARCH DEPARTMENT, TEXAS DIVISION, MONSANTO CHEMICAL COMPANY]

Physical Properties of *o*-, *m*- and *p*-Methylstyrene

BY H. E. CLEMENTS, K. V. WISE AND S. E. J. JOHNSEN

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High purity *o*-, *m*- and *p*-methylstyrene were prepared from purified samples of the corresponding bromotoluenes. The vapor pressure, index of refraction, density and freezing point were measured for each isomer. Other derived physical properties are reported also.

In connection with their possible uses as monomers, certain physical properties of high purity samples of the methylstyrenes were measured. The methylstyrenes were prepared from the corresponding bromotoluenes by well known methods.[1]

To assure high purity in the final products, the bromotoluenes (starting materials from Eastman Kodak Co.) were each fractionated in a 100-plate column operating at 50:1 reflux ratio. The middle 80% was recovered as product for the preparation of the appropriate tolylmagnesium bromide. Each of the bromotoluenes was assayed for purity before and after fractionation by freezing point methods and equipment described by Taylor and Rossini.[2]

Table I shows the mole per cent. impurity of the bromotoluene samples before and after fractionation, together with our best values of the freezing point for zero impurity (T_{f0},°C.) each of which is the mean of three independent freezing experiments. The impurity concentrations were calculated assuming the cryoscopic constant of all the bromotoluenes to be 0.0242 mole fraction/°C.

All lots of purified methylstyrenes were also assayed by cryoscopic techniques and only those samples assaying 99.4 mole % or better, were used in any measurements reported here. All measured physical properties are given in Table II together with conservative estimates of uncertainties wherever possible. Standard deviations of the vapor pressure measurements are those calculated from the deviations of the measured pressures from the pressures calculated from the Antoine equation fit.

TABLE I

IMPURITY CONCENTRATIONS IN BROMOTOLUENES

	Mole per cent. impurity		
	Original	Purified	T_{f0}, °C.
o-Bromotoluene	0.33	0.02	−26.81 ± 0.04[a,b]
m-Bromotoluene	1.03	.38	−38.14 ± .04
p-Bromotoluene	1.04	.07	+26.58 ± .04

[a] J. Timmermans ("Physico-Chemical Constants of Pure Organic Compounds," Elsevier Publishing Co., Inc., New York, N. Y., 1950, pp. 298–299) gives the freezing point of *o*-bromotoluene as −28.1° and *p*-bromotoluene as +26.7°. [b] All uncertainties of physical data reported in this paper will be in standard deviation units (1 sigma limits, *i.e.*, ±σ).

Experimental

Vapor Pressure Measurements.—The vapor pressures were measured by a static method. All measured point-pairs of temperature and pressure were fitted to the three-constant Antoine equation by the method of least squares described by Rossini, *et al.*[3]

The apparatus used in the vapor pressure measurements consisted of a differential manometer made from two 6 in. long tubes 16 mm. inside diameter. To the bottom bend

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(1) Y. Hirschberg, THIS JOURNAL, **71**, 3241 (1949).

(2) W. J. Taylor and F. D. Rossini, *J. Research Natl. Bur. Standards*, **32**, 197 (1944).

(3) C. B. Willingham, W. J. Taylor, J. M. Pignocco and F. D. Rossini, *ibid.*, **35**, 219 (1945).