is a dimensionless quantity of the order of unity. The entropy change ΔS_X is given accordingly as $k\ln[\Omega_\kappa(\Delta\tau)^{\nu/2}]$, or

$$\Delta S_x = k\nu[(1/2)lnC - 9/4 + (3/4)\ln(\nu/N_s)] \quad (66)$$

This result probably is to be preferred over a previous estimate[42] which, in the present notation and with neglect of the term corresponding to $\ln C$, is

$$\Delta S_x = k\nu[(9/8)ln(\nu/N_s) - 3.4]$$

Schellman[18] recently treated the effect of intramolecular cross linkages in stabilizing the configuration of an individual (folded) polypeptide chain, with a result not unlike that given in eq. 66.

According to eq. 59 with neglect of ΔS_{el}^{i}, and eq. 66

$$(1/T^{\circ}_m - 1/T^{i}_m) = (R\nu/N_s\Delta h')(A - (3/4)ln(\nu/N_s)) \quad (67)$$

where $A = 9/4 - (1/2)\ln C$. For moderate or low degrees of cross-linking, such that the fraction N_s/ν of segments cross-linked is small, the logarithmic term in eq. 67 should exceed A. We thus conclude that cross-linking of oriented fibers should result in an increase in melting temperature T^{i}_m. This is indeed true of collagen fibers cross-linked with various tanning agents. Ordinary vulcanization of rubber, on the other hand, depresses its melting point somewhat. This again is in accord with theoretical prediction, as set forth above.

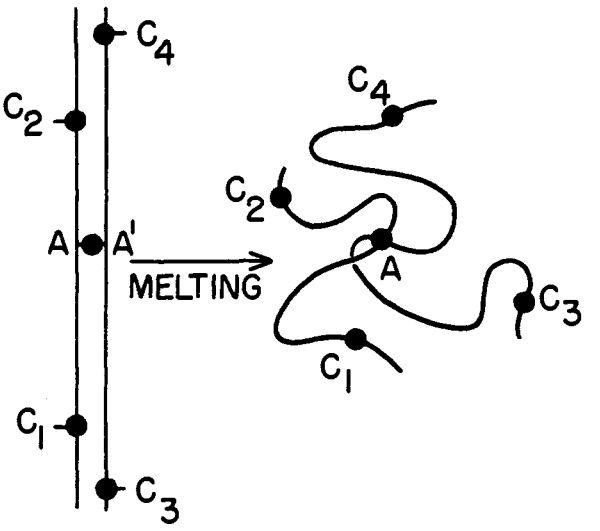

Fig. 3.

ITHACA, NEW YORK

[CONTRIBUTION FROM THE LABORATORIES OF THE SLOAN-KETTERING DIVISION OF CORNELL UNIVERSITY MEDICAL COLLEGE]

Flow Dichroism and its Application to the Study of Deoxyribonucleic Acid Structure[1]

BY LIEBE F. CAVALIERI, BARBARA H. ROSENBERG AND MORTON ROSOFF

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An apparatus has been constructed which enables dichroism measurements to be made on flowing solutions. Viscosities can also be determined using a manometer in the system. An analytical treatment has been developed which permits the calculation of apparent rotary diffusion constants and dichroism values at complete orientation. The method has been applied to solutions of deoxyribonucleic acid and the results are discussed in terms of its structure.

Introduction

The dichroism of flowing solutions has been measured in a number of cases[2,3] but the technique has not been fully developed as an analytical tool. In the course of an investigation dealing with the effects of salt and pH changes on the macrostructure of DNA, we have found dichroism measurements, using plane-polarized ultraviolet light, to be extremely sensitive to changes in structure. In general, the sensitivity to changes in shape is approximately equal to that of viscosity measurements. Furthermore, although the method is analogous in some respects to flow birefringence, it is unique in that it yields information about specific parts of the molecule. In this paper we present an analytical treatment which leads to apparent rotary diffusion constants and dichroism values for complete orientation, thus providing information concerning both the shape and the "internal" structure of the DNA molecule.

Experimental

Description of the Flow Dichroism Apparatus. The Cell.—The cell[4] was constructed of clear fused quartz according to the following specifications: it was rectangular with inner dimensions of 0.238 × 12 × 120 mm., the optical path being 0.238 mm. The thickness of the quartz walls was 2 mm. Quartz tubing (outer diameter 6 mm.) was fused at either end of this section, providing a facile inlet and outlet. The tubes were gently tapered at the junction of the rectangular section to minimize pressure losses in the flowing solutions.

The housing for the cell was constructed in the Sloan-Kettering Institute Machine Shop. Appropriate guides were inserted to ensure that the rectangular faces were parallel to the Beckman spectrophotometer housing block. Provision was also made for sliding the cell away from the light path in order to balance the spectrophotometer.

The Driving Mechanism.—The solutions were placed in a 25-ml. medical syringe driven by a worm gear actuated by a synchronous Bodine motor (output = 6 r.p.m.). A set of gears between the motor and the worm gear provided about 15 speeds. At the end of the path of the syringe the worm gear was reversed automatically by means of a microswitch. The rate of discharge from the rectangular section was easily calculated from the volume discharged from the calibrated syringe.

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, Public Health Service (Grant No. C-471) and from the Atomic Energy Commission (Contract No. AT(30-1)-910).

(2) A. Butenandt, H. Freidrich-Frickson, G. Hartung and G. Scheibe, *Z. physiol. Chem.*, **274**, 276 (1942).

(3) (a) W. E. Seeds and M. H. F. Wilkins, *Disc. Faraday Soc.*, **9**, 417 (1950); (b) D. Zucker, J. F. Foster and G. H. Miller, *J. Phys. Chem.*, **56**, 170 (1952).

(4) Made by the Amersil Company, Hillside, New Jersey.

The Optical System.—Measurements were made in a Model DU Beckman Spectrophotometer. A modified Glan-Foucault calcite prism[5] was used as a polarizer. This was inserted in the removable block adjacent to the exit slits of the spectrophotometer. A worm gear attached to the housing of the prism provided a simple means of rotating the prism and hence the plane of the light, and a dial was mounted on the worm gear to indicate the position of the prism. The slit image was checked at various distances from the prism and found not to diverge to any significant extent. The photo tube was found to be only slightly sensitive to the plane of polarized light; however, this introduced no error since it was shown that the plane of light remained unchanged on passing through the solution.

Method of Measurement.—The quartz cell was removed from the light path and the dark current balanced; the "check" knob of the spectrophotometer was then put in the 0.1 position, and with the transmission dial at 0% the galvanometer was balanced with the sensitivity knob. Next, the cell containing the DNA solution was placed in the light path and a reading at 260 mμ was taken with the solution at rest. Flow was begun and after approximately one second a "flow" reading was taken, equilibrium being established in this time. Edge and end effects were minimal or nonexistent since only a small area (*ca.* 9 mm.^{2}) of the quartz cell was used. This area was in the center of the cell with respect to both the long and short sides.

Precision.—Optical density readings were reproducible to within 0.001. This corresponds to an error of around 2% in dichroism for most values, although at the lowest concentration (concentration range, 0.023–0.107%) and shear rates the error is higher.

Calculation of Shear Rates and Viscosities.—The average linear velocity of the solution in the flow cell is given by $\bar{v} = Q/bd$, where Q is the volume discharged per second, b is the thickness of the optical path, and d is its width. $\bar{v}$ is also given by

$$\bar{v} = \frac{2}{3} \times \frac{b^2}{8\eta} \times \frac{P_1 - P_2}{L} \qquad (1)$$

where η is the viscosity, $P_1 - P_2$, the pressure drop, and L the length of the rectangular section of the cell. Thus, by placing a manometer across the cell, it can be calibrated for pressure drops at various values of $\bar{v}$, using water as the flowing liquid. Once the apparatus is calibrated, it is possible, using eq. 1, to determine the viscosities of solutions under the conditions of the dichroism measurements. For example, a 0.125% solution of DNA was found to have a relative viscosity of 1.8 in various salt concentrations between 10^{-3} and 0.1 M, at shear rates between 1760 and 9480 sec.$^{-1}$. Therefore, rotary diffusion constants and dichroism values at different salt concentrations for a finite DNA concentration are comparable at these high shear rates. As the shear rate was decreased (< 1760 sec.$^{-1}$) the relative viscosities increased.

Using the expression $\bar{v}bp/\eta$ for Reynold's number (where $\bar{v}$ is the average linear velocity, b the optical path, p the density and η the viscosity), a value of 72 was calculated from the highest average linear velocity used in the experiments. This value is well below that for the onset of turbulence between parallel plates, namely, 890.[6]

The maximum shear rate, β, is given by

$$\beta = \frac{b}{2} \times \frac{P_1 - P_2}{\eta L} \qquad (2a)$$

The shear rate is maximal at the walls and decreases linearly to zero at the center of the cell. The average shear rate, $\bar{\beta}$, is given by

$$\bar{\beta} = \frac{b}{3} \times \frac{P_1 - P_2}{\eta L} \qquad (2b)$$

Materials.—Sample S-II was prepared according to the procedure of Schwander and Signer.[7] D-III was prepared according to Kay, Simmons and Dounce,[8] with the exception that it was never dissolved in water as prescribed[8] but rather in 0.005 M salt. Complete analytical and physical data for these samples are given in the following paper.

(5) Made by Bausch and Lomb Company, Rochester, New York.

(6) G. Barr, "A Monograph of Viscosity," Oxford University Press, London, 1931, p. 145.

(7) H. Schwander and R. Signer, *Helv. Chim. Acta*, **33**, 1521 (1950).

(8) E. R. M. Kay, N. Simmons and A. L. Dounce, THIS JOURNAL, **74**, 1724 (1952).

Theory

Dichroism, D, is conveniently defined as the ratio $(R - A_{\parallel})/R$, where R is the optical density reading of the solution at rest, and $A_{\parallel}$ the reading when the electric vector of the incident plane-polarized light is parallel to the flow lines of the solution. Defined in this manner, D is an intensive property. Two parameters are of interest: the apparent rotary diffusion constant, θ', and the dichroism at infinite shear, which will be called D'_∞. The latter, D'_∞, will be discussed in terms of the intrinsic dichroism, D_i, in the next section.

D'_∞ may be evaluated empirically by determining the intercept of a D *versus* $\bar{\beta}^{-1/3}$ plot, which is linear at high $\bar{\beta}$. Theoretically, the dichroism, D, at any shear rate is related to D'_∞ by an orientation function which depends on the dimensions of the molecule and the shear rate β. The following relation should hold

$$D = D'_\infty \bar{f}'(\bar{\beta}/\theta') \qquad (3)$$

where $\bar{f}'(\bar{\beta}/\theta')$ is an average orientation factor.

The orientation factors calculated by Scheraga, Edsall and Gadd[9] for the Peterlin–Stuart[10] distribution cannot be used throughout the entire shear rate region since in our system the beam of entering light is parallel to a plane containing the flow lines and the velocity gradient. The factors computed by Scheraga, Edsall and Gadd[9] apply to a conventional flow birefringence apparatus where the light enters in a direction perpendicular to the velocity gradient. The orientation factors for our system, $f'(\bar{\beta}/\theta')$, can be shown to be given by

$$2f'(\bar{\beta}/\theta') = f(\bar{\beta}/\theta') - \overline{\sin^2\phi} + \overline{2\cos^2\phi} \qquad (4)$$

where ϕ is the angle between the y-axis and the major axis of the molecule as defined by Peterlin and Stuart,[10] and $f(\bar{\beta}/\theta')$ is the orientation factor tabulated by Scheraga, Edsall and Gadd.[9] At low values of $\bar{\beta}/\theta'$, eq. 4 reduces to

$$f'(\bar{\beta}/\theta') = f(\bar{\beta}/\theta')/2 \qquad (5)[11]$$

This can be used to determine an apparent rotary diffusion constant, θ', in the following manner. A series of $\bar{f}'$ values[12] for various shear rates may be calculated from eq. 3 and from the extrapolated value D'_∞. θ' is related to $\bar{f}$ and $\bar{\beta}$ by[9]

$$\bar{f}(\bar{\beta}/\theta', \mathrm{R}) = \frac{\bar{\beta}R}{15\theta}\left[1 - \frac{\bar{\beta}^2}{72(\theta')^2}\left(1 + 6\,\frac{R^2}{35}\right) + \ldots\right] \qquad (6a)$$

(9) H. Scheraga, J. T. Edsall and J. Gadd, *J. Chem. Phys.*, **19**, 1101 (1951).

(10) A. Peterlin and H. Stuart, *Z. Physik.*, **112**, 129 (1939).

(11) The averages $\sin^2\phi$ and $\cos^2\phi$ may be evaluated by multiplying each function by F, the distribution function, and integrating. One obtains

$$\overline{\cos^2\phi} = {}^1/_3 + {}^4/_{15}\pi\, R \sum_{j=1}^{\infty} R^{J-1} a_{10J}$$

and

$$\overline{\sin^2\phi} = {}^2/_3 - {}^4/_{15}\pi\, R \sum_{j=1}^{\infty} R^{J-1} a_{10J}$$

the symbols having the same meaning as in reference 9. It would be useful to compute these coefficients to enable f' values to be calculated for this particular experimental case. When $\bar{\beta}/\theta'$ is small, no term in $\bar{\beta}/\theta'$ appears in the limiting series as given in reference 10.

(12) The orientation factors and shear gradients must be considered as average values, since the shear gradient is not constant across the cell.

where R is related to the axial ratio of the molecule. Differentiating

$$\frac{d\bar{f}(\bar{\beta}/\theta',R)}{d\bar{\beta}} = \frac{R}{15\theta'} - \frac{3\bar{\beta}^2}{72(\theta')^3} + \cdots \quad (6b)$$

From a plot of $\bar{f}'$ *versus* $\bar{\beta}$, the slope at any point may be determined by graphical differentiation. When the slopes are plotted against the corresponding values of $\bar{\beta}^2$, the intercept (*i.e.*, at $\bar{\beta}^2 = 0$) is given by $R/30\theta'$, as obtained from eq. 5 and 6b. If the molecule is assumed to be a long rigid ellipsoid a value of θ' may be computed.

Results and Discussion

The results are contained in the accompanying figures and table. It can be seen from Fig. 1 that the D *versus* $\bar{\beta}$ plot is similar to an $f(\beta/\theta')$ *versus* β/θ' plot based on the values of Scheraga, *et al.*[9] Relative values for the rotary diffusion contants can be evaluated simply by comparing the normalized slopes, $(dD/d\beta)/D_\infty$, at corresponding $\bar{\beta}$ values. The fact that the solutions are Newtonian above 1760 sec.$^{-1}$ (see Experimental) shows that the quantity $(\eta - \eta_0)/c$, where η is the solution viscosity and η_0 is the solvent viscosity, is constant. A plot of dichroism *vs.* $((\eta - \eta_0)/c)\bar{\beta}$ would therefore be equivalent to a plot of dichroism *vs.* $\bar{\beta}$.

A special situation may arise in dichroism measurements which requires comment. If a sample contains ultraviolet-absorbing material which is not oriented by flow or which has no intrinsic dichroism, the dichroism of the solution is, of course, decreased. Further, under these circumstances, the calculated rotary diffusion constant will not reflect all the species present, and in this sense the constant represents an apparent value. It is impossible to say to what extent, if any, such situations occur, but the possibility must be kept in mind.

To evaluate the effect of ionic strength on molecular length, it is necessary to extrapolate θ' and D'_∞ to zero DNA concentration, giving limiting values of θ'_0 and D_∞ (Table I).[13]

TABLE I

APPARENT ROTARY DIFFUSION CONSTANTS AND DICHROISM VALUES

Sample	Salt concn., M	θ_0' (sec.$^{-1}$)[a]	D_∞[a]	Mol. wt. $\times 10^{-6}$ [b]
S-II	0.2	137	0.170	5.5
	10^{-3}	55	.200	
D-III	0.2	54	.175	6.8

[a] Obtained by extrapolation to zero DNA concentration. From light scattering in 0.2 M sodium chloride.

At infinite dilution θ is given by

$$\theta = \frac{3kT}{16\pi\eta a^3}\left(2\ln\frac{2a}{b} - 1\right) \quad (7)$$

(for a prolate ellipsoid), where a and b are the lengths of the semi-major and semi-minor axes, respectively, η is the solvent viscosity, and k is the Boltzmann constant. There appears to be about a 25% decrease in length in 0.2 M salt.

It can be seen that D_∞ is higher in 10^{-3} M than in 0.2 M salt. Since dichroism is actually a measure of the angle(s) made by the chromophores with the flow lines of the solution, we conclude from the results that the chromophores are more nearly perpendicular to the flow lines in the low salt solutions. This may result from a rearrangement of the bases with the molecule with little or no change in molecular shape, or from distention of a kinked molecule with little or no change in the sugar-base angles. The latter appears more probable in view of the smaller θ' in 10^{-3} M salt, although the possibility of some change in the sugar-base angles cannot be ruled out. Viscosity data[14] also support the view that DNA is more extended in low salt. The effect of ionic strength on θ' and D'_∞ at a finite DNA concentration is shown in Fig. 3. The steep portion of the θ' curve is approximate; nevertheless it can be seen that at low ionic strengths, interactions sharply diminish with small increases in salt concentration. That the DNA does not contract to any significant extent is shown by the insensitivity of D'_∞ in this region (<0.01 M salt). Beyond this region, D'_∞ decreases while θ' increases but slightly. This suggests that small decreases in length greatly affect the dichroism.

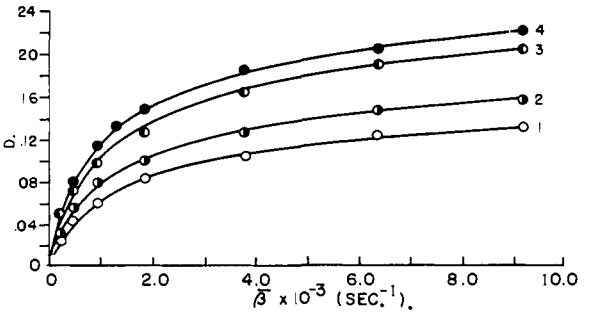

Fig. 1.—Curves 1, 2, 3 and 4 are, respectively, 0.0240, 0.0465, 0.0830 and 0.107% D-III DNA, measured in 0.2 M sodium chloride. The ordinate is dichroism, $D = (R - A_{\|})/R$; along the abscissa are the shear rates, $\bar{\beta}$ (see Experimental).

That dichroism increases with increasing DNA concentration (Fig. 2A) indicates that the greater degree of interaction results in a distention of the molecule. The fact that in 0.2 M salt the slope of the D'_∞ *vs.* c curve for D-III is slightly greater than that for S-II may be associated with its slightly greater length and perhaps greater flexibility. It is of further interest that although S-II has a much higher θ', its D_∞ is very nearly equal to that of D-III. This suggests that the orientable components of each sample have the same intrinsic dichroism.

Intrinsic Dichroism.—It is possible to derive an expression for the dichroism of any given molecular model, based on the extinction coefficients of the various absorption axes. In solution, the absorption of each chromophore may be conveniently resolved into threemutuallyperpendicular axes[15] and if the extinction coefficient of each is known, the angles which they make with an ar-

(13) Since the measurements cannot be carried out at a very low DNA concentration, the extrapolations must be viewed with some caution.

(14) L. F. Cavalieri, M. Rosoff and B. H. Rosenberg, THIS JOURNAL, **78**, 5239 (1956).

(15) R. D. C. Fraser, London, Thesis, 1951 (*cf.* Seeds, *Prog. Biophys. Biophys. Chem.*, **3**, 27 (1953)) has derived an expression for a uniaxial fiber assuming only one absorption axis. Eq. 8 in the text reduces to that of Fraser.

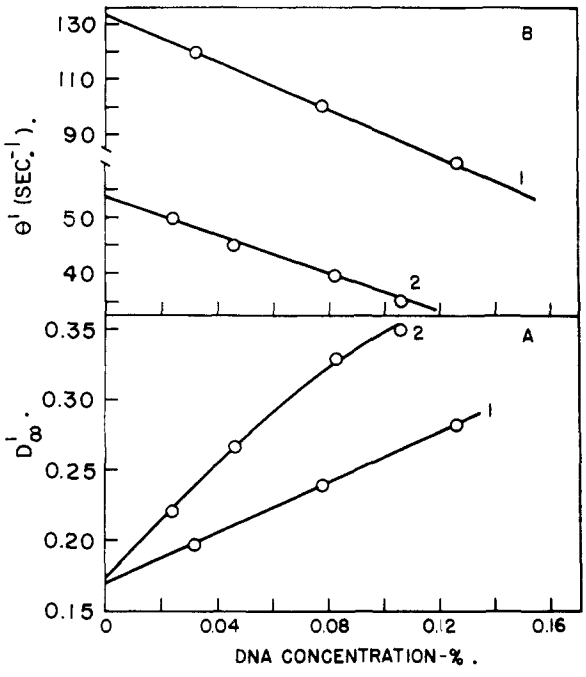

Fig. 2.—All curves determined in 0.2 M salt. Figure 2A contains D'_∞ values plotted as a function of DNA concentration; D'_∞ represents the dichroism at infinite shear. Figure 2B shows the apparent rotary diffusion constants as a function of DNA concentration. Curves 1 are for S-II, curves 2 for D-III DNA.

bitrarily chosen coördinate system may be calculated. For a long, rigid molecule in which one of the absorption axes, a, extends directly from the long axis of the molecule, the following equation for dichroism, as defined in this paper, may be readily derived

$$D_i = \left(\frac{R - A_{||}}{R}\right)_i = 1 - \frac{3}{(u + \delta + 1)} [u \sin^2\psi + \cos^2\psi (\sin^2\lambda[1 - \delta] + \delta)] \quad (8)$$

This expression was derived assuming the long molecular axis to be parallel to the flow lines. In terms of DNA where the chromophores are planar rings, u is the ratio of the average extinction coefficients of the two absorption axes, a and b, in the plane of the ring; δ is the ratio of the average extinction coefficient of the axis perpendicular to the plane of the ring to the average coefficient of b. ψ and λ are the angles made by a and b, respectively, with a plane perpendicular to the flow lines. ψ and λ are thus the average orientation angles of the chromophores. Rotational symmetry, if not a property of each molecule, is always a statistical property of the molecules in solution, and thus of the model.

Since the intrinsic dichroism, Dl, refers to a rigid rod, it can be related to D_∞ only if it is known that the molecules in solution are fully extended (*i.e.*, unkinked) and rigid. In other words, it is possible that the intrinsic dichroism of a chain element of a kinked molecule (I) is the same as that for a rigid rod (II) but D_∞ for the former will be lower if the

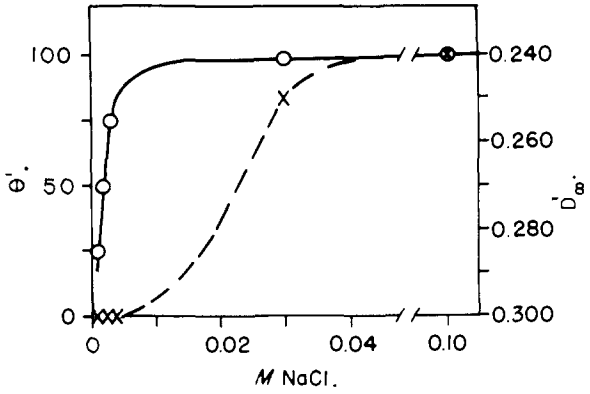

Fig. 3.—Effect of ionic strength on θ' and D'_∞. The DNA concentration was 0.079%. The solid curve refers to the ordinate on the left (θ'); the broken curve refers to D'_∞. Because of the steepness of the curve, the θ' values at low ionic strength were not as reproducible as those at high ionic strength where the precision was good.

molecule remains kinked under the conditions of the experiment; further, such a molecule (I) could not be distinguished from a rod (III) whose intrinsic dichroism is lower. Thus, without a shape function, it is impossible at present to calculate the values of ψ and λ for DNA even if the reasonable assumptions are made[11] that $\delta = 0$, and $u = 1$. Nevertheless, as has been pointed out above, dichroism measurements do provide information about alterations in structure.

An equation similar to (8) can be derived for $(R - A_\perp)/R$, and it can then be shown that

$$R - A_{||} = 2(A_\perp - R) \quad (9)$$

where $A_\perp$ is the optical density when the plane of the polarized light is perpendicular to the flow lines. Equation 9 has been verified experimentally. Therefore, from the experimental values R and $A_{||}$, it is possible to calculate the dichroic ratio, $A_\perp/A_{||}$, without measuring $A_\perp$. Likewise, the optical density increment, $R - A_\phi$, when the incident light is polarized in any other direction, can be calculated from the relation

$$|(R - A\phi)| = (1.5 \sin^2\phi - 1) |(R - A_{||})| \quad (10)$$

where ϕ is the angle between the plane of polarization and the flow lines. No independent information concerning the optical properties of the solution can be obtained by the use of unpolarized incident light, for the optical density increment, $R - A_u$, is calculable from $A_{||}$, as

$$R - A_u = R - \tfrac{1}{2}(A + A_\perp) \quad (11)$$

where $A_{||}$ is the optical density of the flowing solution obtained with unpolarized light.

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