

[CONTRIBUTION FROM THE RESEARCH AND DEVELOPMENT DIVISION, AMERICAN VISCOSE CORPORATION]

Concentration Dependence of Flow Birefringence of Polymer Solutions¹

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By using Peterlin's concept of effective viscosity, both extinction angle χ and birefringence increment $\Delta n/c$ of polymer solutions at constant rate of shear D can be related, as a first approximation, to the corresponding reduced viscosity, η_{sp}/c , by the expression: $\cot 2\chi/\cot 2\chi_0 = (\Delta n/c)/(\Delta n/c)_0 = (\eta_{sp}/c)/[\eta]$, where the subscript zero refers to the intrinsic value at infinite dilution. By replacing $(\eta_{sp}/c)/[\eta]$ with Huggins' or Martin's viscosity-concentration equation one obtains: $\cot 2\chi/\cot 2\chi_0 = (\Delta n/c)/(\Delta n/c)_0 = 1 + k'[\eta]c$, in dilute solutions, or, if Martin's equation is applicable: $\log (\cot 2\chi/\cot 2\chi_0) = \log [(\Delta n/c)/(\Delta n/c)_0] = k[\eta]c$. With these relations it is possible to eliminate the concentration dependence through linear extrapolation. For polyelectrolytes without added salts both $\cot 2\chi$ and $\Delta n/c$ at constant D increase rather than decrease with dilution in the same manner as the η_{sp}/c behavior of a polyion. By analogy the proposed equations can be extended to rigid particles. The linearity of the particle length or the reciprocal of the rotary diffusion constant with respect to concentration can be explained with the same concept of effective viscosity. Improved graphical determination of the initial slopes, $-(d\chi/dD)$ and $[d(\Delta n/c)/dD]_0 = \frac{1}{2}k$ can be achieved by plotting $(1/2)(\cot 2\chi/D) \frac{1}{c} = \frac{1}{2}k$ and $(\Delta n/c)/D$ against D and reading off the intercepts on the ordinate. For rigid particles the upper limit of the particle length can be estimated with less uncertainty.

Introduction

As is true for most theoretical treatments, the theory of flow birefringence is applicable only to an infinitely dilute solution of macromolecules. Experimentally, however, one is forced to interpret the measurements of flow birefringence at finite concentrations. The common practice is to carry out these experiments at sufficiently low concentrations where the concentration effect appears negligible. At present no satisfactory method of extrapolation to zero concentration has yet been accepted among the workers in this field.

Since both flow birefringence and viscosity of a macromolecular solution arise as a result of the shearing stress to which the system is subjected in a hydrodynamic field, one is tempted to look for a common relation describing the concentration dependence of these two types of measurements. It is a well-known fact that at constant rate of shear D the apparent orientation of the solute particles increases and thereby the corresponding extinction angle χ usually decreases with increasing concentration as a result of solute interaction. Thus the conventional $\chi-D$ or $\chi-D\eta_0$ plot (η_0 = solvent viscosity) exhibits a downward shift as the concentration increases. Attempts to plot χ as a function of shearing stress, τ ($= D\eta_{soln}$) rather than D or τ_0 ($= D\eta_0$) above, resulted in an overcorrection, as reflected by the apparently larger χ at higher concentration. This led Peterlin² to develop the concept of effective viscosity η^* by supposing that the solute particles move in a medium of intermediate viscosity between solution and solvent

$$\eta_s = \eta_0 + c[\eta]\eta^* \quad (1a)$$

or

$$\eta^* = (\eta_s - \eta_0)/c[\eta] \quad (1b)$$

where c is the concentration of the solute. This formal approach has proved highly successful in the flow birefringence study of polystyrene, nitrocellulose and rubber solutions.² By plotting χ against $D\eta^*$ or Δn against $D\eta^*c$ it was found that all the

experimental data fell on a composite curve over a wide range of concentrations, even at concentrations having a relative viscosity as high as 2500. Peterlin, however, has emphatically stated that there are only a few measurements of flow birefringence and viscosity at different concentrations and rates of shear and the precision of the measurements of the whole is rather low. Thus the general validity of his proposed theory still awaits many more precise measurements of the birefringence and viscosity of various polymer solutions over a wide range of concentrations and rates of shear. In particular, anomalies might arise at higher concentrations due to polymer association and entanglement of polymer coils.

The theories of flow birefringence for polymer coils are still in a rapidly developing stage. Peterlin² has found that only the product of $[\eta]\eta_0$ (when $c \rightarrow 0$) or $[\eta]\eta^*$ (in finite concentrations) enters the expression for the extinction angle χ and birefringence increment $\Delta n/c$ and so very likely this product $[\eta]\eta^*$ only has a physical meaning in concentrated solutions. Actually, in concentrated solutions one can simply substitute the reduced viscosity, η_{sp}/c , for $[\eta]$ in the product $[\eta]\eta_0$ without the aid of eq. 1, noting that $[\eta]\eta^* = (\eta_{sp}/c)\eta_0$. If both $\cot 2\chi$ (in the whole range of D) and $\Delta n/c$ (at low D) were proportional to $[\eta]\eta^*$ at any constant D one can easily derive the expressions

$$\cot 2\chi/\cot 2\chi_0 = (\eta_{sp}/c)/[\eta] \quad (2a)$$

and

$$(\Delta n/c)/(\Delta n/c)_0 = (\eta_{sp}/c)/[\eta] \quad (2b)$$

where the subscript zero refers to the value at zero concentration. χ_0 can be considered as the intrinsic extinction angle similar to the intrinsic viscosity. Similarly, $(\Delta n/c)_0$ may be tentatively named as intrinsic birefringence increment, although confusion might arise from the fact that the term intrinsic birefringence frequently has been used to designate the part of birefringence which is different from the form birefringence.

Very recently Cerf³ has presented a more adequate theory of flow birefringence by taking into account the internal viscosity of the polymer coils. The final expression for birefringence predicts a di-

(1) Presented at the 133rd American Chemical Society Meeting, San Francisco, April, 1958.

(2) A. Peterlin and R. Signer, *Helv. Chim. Acta*, **36**, 1575 (1953); A. Peterlin, *Proc. 2nd. Intern. Congr. Rheol.*, Oxford, 1953, p. 343; *J. Polymer Sci.*, **12**, 45 (1954); A. Peterlin in F. R. Eirich, Ed., "Rheology—Theory and Applications," Vol. I, Academic Press, New York, N. Y., 1956, p. 615.

(3) R. Cerf, *Compt. rend.*, **248**, 1875 (1956); *J. Polymer Sci.*, **23**, 125 (1957); **25**, 247 (1957).

rect proportionality between $\Delta n/c$ and $[\eta]\eta_0$ ($c \rightarrow 0$). Thus in finite concentrations it again yields eq. 2b. On the other hand, the extinction angle equation involves an internal viscosity term and can be written as

$$\tan \alpha \equiv - \left(\frac{d\chi_0}{dD} \right)_{D=0} = \frac{1}{2} \left(\frac{d \cot 2\chi_0}{dD} \right)_{D=0} = \frac{a M[\eta]\eta_0}{RT} + B \quad (3)$$

Here a is a constant characteristic of the chain stiffness and B is the internal viscosity term of the polymer coils. Upon integration of eq. 3 one finds that $\cot 2\chi_0$ is still a function of $[\eta]\eta_0$ but eq. 2a is no longer valid unless B is extremely small as compared with the first term on the right side of eq. 3.

Another complication arises from the form anisotropy of polymer solutions which appears with non-vanishing difference in refractive indices between the polymer and the solvent. According to Čopič,⁴ this form anisotropy accounts for the parabolic dependence of Δn on the refractive index of the solvent, as has most recently been observed by Tsvetkov.⁵ The theory of Čopič, however, indicates that for a given polymer at low D , $\Delta n/c$ is proportional to $[\eta]\eta_0$ ($c \rightarrow 0$), thus making it possible to apply equation 2b to moderately concentrated solutions.

By combining equation 2 with the viscosity-concentration function, the birefringence-concentration equations easily may be deduced. Thus in dilute solutions where Huggins' equation

$$\eta_{sp}/c = [\eta](1 + k'[\eta]c) \quad (4)$$

can be employed we have

$$\cot 2\chi = \cot 2\chi_0 (1 + k'[\eta]c) \quad (5a)$$

and

$$\Delta n/c = (\Delta n/c)_0 (1 + k'[\eta]c) \quad (5b)$$

In moderately concentrated solutions where Martin's equation⁶

$$\log (\eta_{sp}/c) = \log [\eta] + k[\eta]c \quad (6)$$

may be applicable, eq. 2a and 2b become

$$\log \cot 2\chi = \log \cot 2\chi_0 + k[\eta]c \quad (7a)^7$$

and

$$\log (\Delta n/c) = \log (\Delta n/c)_0 + k[\eta]c \quad (7b)^7$$

Even with Cerf's treatment the uncertainty about the proportionality between extinction angle and viscosity in eq. 2a does not invalidate eqs. 5 and 7, since one can always expand the ratio $\cot 2\chi/\cot 2\chi_0$ into a power series of concentration through the use of Huggins' or Martin's viscosity equations, the first two terms of which will be still of the same form as those in eq. 5 and 7. Needless to say the coefficient of c will no longer be identical with $k'[\eta]$, or $k[\eta]$. It is the purpose of this paper to test the pro-

(4) M. Čopič, *J. Poly. Sci.*, **20**, 593 (1956); *J. Chem. Phys.*, **26**, 1382 (1957).

(5) V. N. Tsvetkov, *Chem. Listy*, **49**, 1419 (1955); *J. Polymer Sci.*, **23**, 151 (1957).

(6) A. F. Martin, Am. Chem. Soc. Meeting, Memphis, April, 1942; H. M. Spurlin, A. F. Martin and H. G. Tennent, *J. Polymer Sci.*, **1**, 63 (1946).

(7) In practice equations 7a and 7b obviously would fail when χ approaches 45° or Δn approaches zero, since in both cases the term on the left side becomes $-\infty$.

posed flow birefringence equations and thereby develop methods of extrapolation to infinite dilution. For this reason the literature data on polystyrene (flexible chains) and poly-4-vinyl-*n*-butylpyridinium bromide (polyelectrolyte) were re-interpreted in the light of the proposed equations. The same concept was further extended to rigid particles, using poly- γ -benzyl-L-glutamate as an example. The literature data on rabbit actomyosin and bovine albumin were employed as additional illustrations for the applicability of the proposed equations.

Results and Discussion

Part I. Random Coils

Polystyrene in Toluene.—Schmidli's study⁸ of the birefringence and viscosity of polystyrene solutions in toluene over a concentration range of from 0.5 to 6% has been quoted by Peterlin² in a series of papers in support of his concept of effective viscosity. It is therefore only necessary to re-interpret these data according to our proposed equations 2, 5 and 7. In Figs. 1 and 2 $\cot 2\chi$ and $\Delta n/c$ are plotted against η_{sp}/c ⁹ at constant rates of shear D on a log-log scale. The lines are drawn with a slope of unity. For the birefringence increment, $\Delta n/c$, the agreement between experimental data and the proposed equation was as good as could be expected, especially in view of the over 100-fold variation in the solution viscosity. In the case of extinction angle χ there appeared to be a persistent inverse S-shape deviation from the straight line, indicating that eq. 2a was not obeyed exactly. Very probably this was the result of the polydispersity of the polymer which influences the concentration behavior of extinction angle and viscosity in a rather different way. In fact, Peterlin¹¹ has found that the extinction angle curves in many cases cannot be reduced to the same composite curve due to the fact that the polymers studied were highly polydisperse.

The portions of dotted lines in the figures should be considered with some reservations. Due to the wide variation in solution viscosity the ranges of rates of shear covered in the flow birefringence measurements varied from one concentration to another. For example, for the particular polystyrene sample at 0.5% no extinction angle could be measured at D less than 2590 sec.^{-1} , whereas at 6% the upper limit of D as reported by Schmidli was only

(8) B. Schmidli, thesis, Zurich, 1952.

(9) In his original paper, Schmidli has used the average rate of shear \bar{D}

$$\bar{D} = \frac{8Q}{3\pi R^3}$$

Here R is the radius of the capillary tube and Q the volume flow rate in ml. per sec. We have converted it into the maximum rate of shear at the wall, D_m

$$D_m = \frac{4Q}{\pi R^3}$$

for reasons mentioned previously.¹⁰ Another correction was applied to D_m in the non-Newtonian region by the expression

$$D_{cor} = \frac{(n+3)}{4} D_m$$

where n is the slope of the $\log D$ - $\log \tau$ plot (τ being the shearing stress).¹⁰

(10) J. T. Yang, *THIS JOURNAL*, **80**, 1783 (1958).

(11) A. Peterlin, private communication.

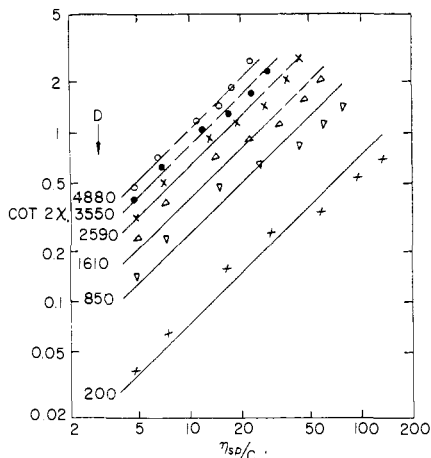


Fig. 1.—Extinction angles *versus* reduced viscosities of polystyrene in toluene at constant rates of shear. Data were taken from ref. 8. Concentrations at each rate of shear (from left to right): 0.5, 1, 2, 3, 4, 5 and 6%.

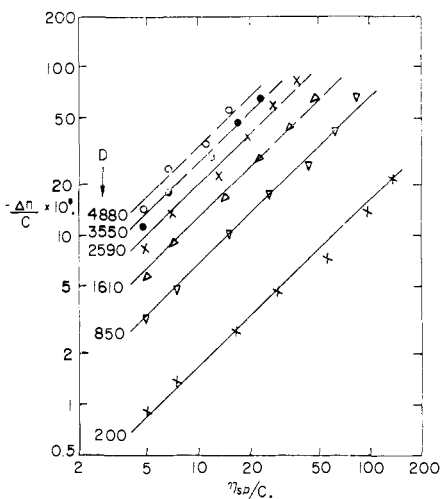


Fig. 2.—Birefringence increments *versus* reduced viscosities of polystyrene in toluene at constant rates of shear. Data were taken from ref. 8. Concentrations, same as those in Fig. 1.

850 sec.⁻¹. Consequently the χ - D curves had to be extrapolated to the same range of rates of shear. Fortunately, because of similarity in the shape of the χ -curves, the errors thus introduced in the extrapolation were reasonably small. Even so the errors would be magnified when χ was converted to $\cot 2\chi$. It is clear beyond any doubt, however, that a close parallelism between flow birefringence and viscosity behavior of polymer solutions with respect to concentration does exist. As more experimental data accumulate it will be possible to determine the nature of the inverse-S extinction angle curves. Eventually, we may reach an ultimate understanding of flow birefringence in concentrated solutions.

Viscosity-Concentration Functions.—It is a well-known fact that any viscosity-concentration equation is valid only over a more or less limited range of concentration. For dilute solutions it is common practice to employ Huggins' equation. In

moderately concentrated solutions caution should be taken to determine the applicability of the chosen equation. Martin's equation, for example, has been applied successfully to many polymer solutions, even up to a concentration of 20%.¹² This representation, however, is only satisfactory for systems in "poor" solvents.¹³ In "good" solvents, the Martin plot gives a straight line only above a certain critical concentration, and below this experimental points usually deviate downward from the straight line. Thus, the values obtained from such a plot by straight line extrapolation may only be mathematical fiction and not represent the true intrinsic value, $[\eta]$ being overestimated and k underestimated. As a result of equation 2, the same argument is equally applicable to the flow birefringence measurements. In Fig. 3 is shown a

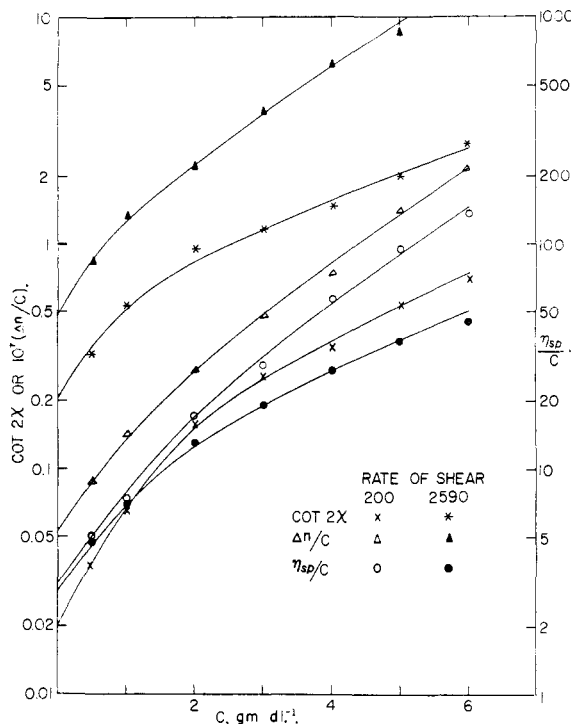


Fig. 3.—Comparison of $\cot 2\chi$, $\Delta n/c$ and η_{sp}/c of polystyrene in toluene as a function of concentrations.

semi-log plot of $\cot 2\chi$, $\Delta n/c$ and η_{sp}/c *versus* the concentration at constant rate of shear. All the experimental points at $c > 2\%$ obeyed Martin's equation very satisfactorily but bent downward below that concentration. Thus, precisely these apparently perfect straight lines would give false intercepts for both $\cot 2\chi_0$ and $(\Delta n/c)_0$ had there been no measurement at 0.5 and 1%. It is therefore of uppermost importance to test the limitation of any chosen concentration function, especially when moderately concentrated solutions are employed. Since the viscosity of a dilute solution is easily measured while the flow birefringence of the same dilute solution might be unmeasurable, it is suggested that reliable information concerning the

(12) See, for example, R. S. Spencer and J. L. Williams, *J. Colloid Sci.*, **2**, 117 (1947).

(13) S. G. Weissberg, R. Simha and S. Rothman, *J. Research Natl. Bur. Standards*, **47**, 298 (1951).

latter can only be obtained with the aid of viscosity data, and it is not necessary, although desirable, to investigate the whole flow curve of the solution.

The similarity in the shape of the curves in Fig. 3 is self-explanatory and is a natural consequence of the simple relationship in equation 2. A more logical comparison can be made by plotting $\cot 2\chi/\cot 2\chi_0$ and $(\Delta n/c)/(\Delta n/c)_0$ against c and comparing them with the $(n_{sp}/c)/[\eta]$ versus c plot. This procedure was not employed here mainly because the intrinsic values in Fig. 3 were not very accurate due to lack of sufficient measurements in dilute solutions.

If one accepts our proposed equations two additional features soon become apparent in the determination of intrinsic values, χ_0 and $(\Delta n/c)_0$. First, it is not necessary to determine the non-Newtonian viscosity η_s of the polymer solution throughout the same whole range of rates of shear as that for the flow birefringence, which was required with Peterlin's equations. Secondly, the extent of concentration dependence of flow birefringence can be predicted from both the interaction constant k' or k and the intrinsic viscosity of the solution. In a poor solvent where k' or k is large, $\cot 2\chi$ will rise more rapidly with concentration than in a good solvent. Likewise, a polymer having a high $[\eta]$ will exhibit a strong concentration effect. In other words the concentration dependence becomes most significant when $k'[\eta]c$ or $k[\eta]c$ is much greater than unity.

The Initial Slopes of χ and $\Delta n/c$ versus D Curves.—For theoretical interpretation of flow birefringence it is of great significance to determine the values of $-d\chi/dD$ and $d(\Delta n/c)/dD$ at $c = 0$ and $D = 0$ with high precision. Yet at low concentrations and also at low rates of shear the measurements of χ and Δn are most difficult to perform. As a consequence appreciable errors can easily be introduced into the experimental data. Such difficulties can be greatly minimized by performing the experiments in moderately concentrated solutions followed by proper extrapolation to zero concentration. For example, for the polystyrene solution at 0.5% no reliable measurements could be made at $D < 2000 \text{ sec.}^{-1}$, whereas at $c > 2\%$ it was not difficult to measure the extinction angle even at $D = 200 \text{ sec.}^{-1}$. By using moderately concentrated solutions it will be possible to collect more experimental points at lower rates of shear, thus yielding a more accurate χ_0 - D curve (at zero concentration).

With the complication due to concentration dependence eliminated one should in principle be able to determine the initial slopes with confidence. Additional improvement in the graphic method is, however, necessary and seems worth mentioning here. In Fig. 4A is plotted the χ_0 - D curve of the polystyrene sample, the χ_0 values of which were obtained through extrapolation of a $\cot 2\chi$ - c plot (not shown). It is not difficult to see that the tangent drawn through the first few experimental points could involve appreciable uncertainty. In many cases, a plot of $\cot 2\chi$ versus D would yield a straight line over a wide range of rates of shear. This is illustrated in Fig. 4b. An additional improvement can be made by means of a

simple transformation method, the advantage of which already has been discussed elsewhere.¹⁴ Since the curve in Fig. 4B can always be expressed as

$$\cot 2\chi = AD - BD^2 + \dots \quad (8a)$$

one can rearrange the equation into

$$(\cot 2\chi)/D = A - BD + \dots \quad (8b)$$

Thus by plotting $(\cot 2\chi)/D$ against D one can determine the slope, $d(\cot 2\chi)/dD$, from the intercept A rather than from the slope in Fig. 4B. This rearrangement appears highly satisfactory for the determination of the initial slope, $-d\chi/dD$, as can be seen in Fig. 4C.¹⁵ In fact, it can even magnify inaccurate experimental points as indicated by the question mark in the figures. The same method can equally well be applied to the determination of $d(\Delta n/c)/dD$.

From theoretical considerations it would be possible to determine the molecular weight of the polymer from the initial slope $-d\chi/dD$, and conversely test the validity of various theories with a polymer of known molecular weight. No such calculations, however, are attempted in this paper mainly because the degree of polydispersity unavoidably complicates any theoretical interpretation. To date it is still not known what kind of average value the flow birefringence measurements yield.

Polyelectrolytes.—It is now a well-known fact that the reduced viscosity ($= n_{sp}/c$) of polyelectrolytes in the absence of added electrolyte rises upon dilution in a striking manner due to the expansion of the coiled polyions. A similar behavior in the flow birefringence of polyions would be expected to exist according to our earlier deductions (eq. 2). Very recently Fuoss and Signer¹⁶ have published an interesting note on the flow birefringence of salt-free poly-4-vinyl-*p*-butylpyridinium bromide solutions. This polyion exhibited unusual behavior of both χ and Δn . The χ decreased rather than increased upon dilution, whereas the magnitude of Δn went through a maximum as a function of concentration. These were in marked contrast to uncharged flexible coils which exhibit a normal increase of χ and decrease of Δn on dilution. In Fig. 5 Fuoss and Signer's data are replotted in the light of the proposed equations. It is interesting to note that both the $\cot 2\chi$ and $\Delta n/c$ curves closely resemble the n_{sp}/c versus c plot of a typical polyelectrolyte. Furthermore, the maximum (between $c = 0.1$ and 0.2%) in the Δn - c curves completely disappears when one plots $\Delta n/c$ rather than Δn alone. Clearly here is another indication that a close relationship exists between flow birefringence and viscosity of polymer solutions.

At concentrations lower than those of Fig. 5 the published data were not sufficient to warrant any reliable extrapolation. However, it appeared that the χ curve bent toward 45° ($\cot 2\chi = 0$), whereas $\Delta n/c$ dropped sharply toward the origin. All these were in perfect agreement with the viscosity behavior of polyions. To pursue this point further one

(14) J. T. Yang, *J. Polymer Sci.*, **26**, 305 (1957).

(15) In a recent paper, J. Leray (*Compt. rend.*, **246**, 107 (1958)) has devised a different graphic method by plotting τ_0/Φ against τ_0 . Here $\tau_0 = D\eta_0$ and $\Phi = \pi/4 - \chi$. In this way the intercept on the ordinate gives $\eta_0/\tan \alpha$. Here $\tan \alpha$ is simply $-(d\chi/dD)_{D=0, c=0}$.

(16) R. M. Fuoss and R. Signer, *THIS JOURNAL*, **73**, 5872 (1951).

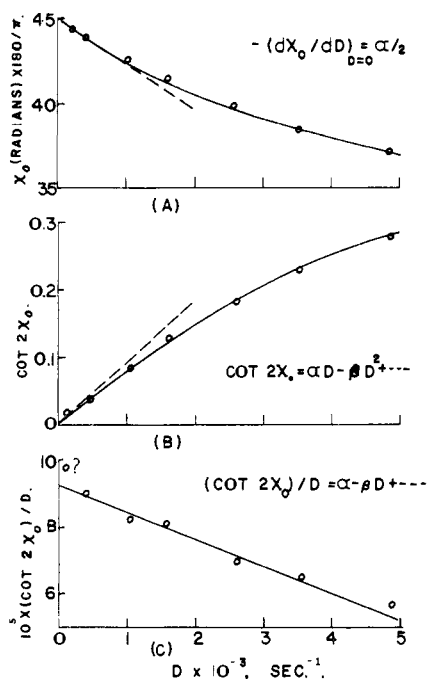


Fig. 4.—Graphic determination of the initial slope of an α - D curve.

would expect that these expressions exist

$$\frac{1}{\cot 2\chi} = \tan 2\chi = A + B\sqrt{C} \quad (9a)$$

and

$$\frac{C}{\Delta n} = A' + B'\sqrt{C} \quad (9b)$$

just as Fuoss¹⁷ had deduced for the viscosity of polyelectrolytes. (Here A , B , A' and B' are numerical constants.) In the case of extinction angle such a relation does indeed exist. For the birefringence, however, the $c/\Delta n$ versus c plot appeared to reveal a slight concave-upward curvature (figure not shown). Whether this might be attributed to experimental errors cannot be answered without further investigations.

Similar results on the same polyion also have been observed by Rosen, Kamath and Eirich.¹⁸ In addition they have found a normal flow birefringence behavior when dilutions were carried out in the same manner as the viscosity behavior of a polyion. They have also calculated the apparent rotary diffusion constants, using the theory of rigid ellipsoids. This interpretation is, however, questionable in view of our present understanding of polyion configurations.

Part II. Rigid Particles

Experimental. a. Materials.—The poly- γ -benzyl-L-glutamate sample (Lot. no. 416) which had been used previously for flow curve investigations,¹⁰ was a gift of Dr. E. R. Blout and Professor P. M. Doty.

The reagent grade *m*-cresol (Fisher Scientific Company) was redistilled under vacuum before use.

b. Flow Birefringence Measurements.—All the experi-

mental measurements were carried out in a Rao Flow Birefringence Viscometer, Model 4-B. The basic design of this instrument has been described elsewhere.¹⁹ This new model has a water jacket around the outer cylinder for temperature control. It also has a magnet attachment which together with a Hewlett Packard model 500B frequency meter, makes it possible to adjust the desirable speed of rotation with ease. The extinction angle and birefringence at any specified r.p.m. were measured in both forward and backward directions (by reversing the rotation of the motor). In this way the errors involved in the "zeroing" of the polarizer can be eliminated.

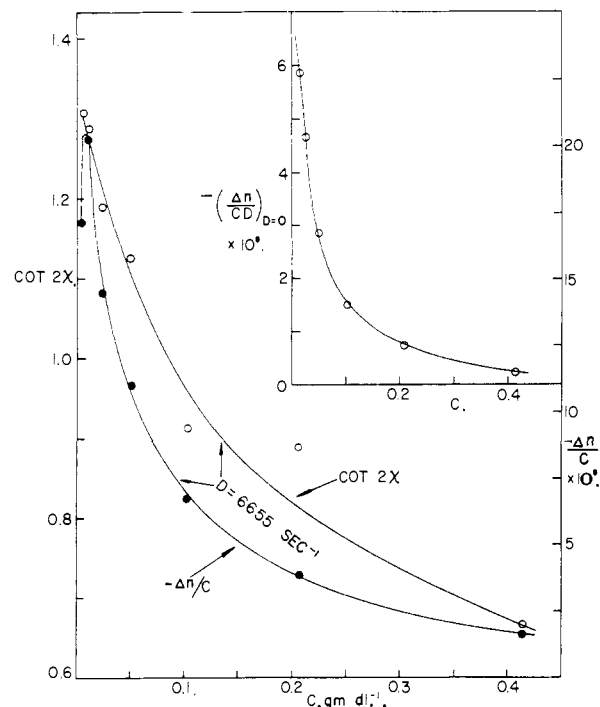


Fig. 5.—Extinction angles and birefringence increments of poly-4-vinyl-*n*-butylpyridinium bromide (in the absence of added electrolyte) as a function of concentrations at constant rate of shear. Insert: birefringence increments extrapolated to zero rate of shear. Data were taken from ref. 16.

Poly- γ -benzyl-L-glutamate in *m*-Cresol.—The concept of effective viscosity can be equally well applied to rigid particles. It is of interest to find out whether by analogy the same proposed equations can be extended to rigid systems. For this purpose we have carried out a detailed study of a poly- γ -benzyl-L-glutamate (PBLG) sample which exists as rod-like α -helices in several poor solvents such as *m*-cresol and ethylene dichloride.²⁰ In Figs. 6 and 7 are shown the extinction angle and birefringence curves as functions of rate of shear over a 60-fold variation in concentrations. In Fig. 8 is plotted $\log \cot 2\chi$ against concentration according to eq. 7. Previous viscosity study¹⁰ of the same polymer had indicated that Martin's equation ($\log \eta_{sp}/c$ versus c) was applicable within this range of concentrations. It is interesting to note that the $\log \cot 2\chi$ plot does indeed yield a straight line just as did the $\log \eta_{sp}/c$ plot. It is also not difficult to see that a $\cot 2\chi$ vs. c plot would result in a concave upward curve in the same manner as the Huggins viscosity equation for this polymer at concentration greater than 0.5%.

In Table I are listed the values of the slopes as calculated from Fig. 8 at several rates of shear. Also included

(17) R. M. Fuoss and U. P. Strauss, *Ann. N. Y. Acad. Sci.*, **51**, 836 (1949).

(18) B. Rosen, P. Kamath and F. Eirich, *Disc. Faraday Soc.*, **11**, 135 (1951); P. Kamath and F. Eirich, *Phys. Rev.*, **86**, 657 (1952).

(19) J. T. Edsall, A. Rich and M. Goldstein, *Rev. Sci. Instr.*, **23**, 695 (1952).

(20) P. Doty, A. M. Holtzer, J. H. Bradbury and E. R. Blout, *This Journal*, **76**, 4493 (1954); P. Doty, J. H. Bradbury and A. M. Holtzer, *ibid.*, **78**, 947 (1956).

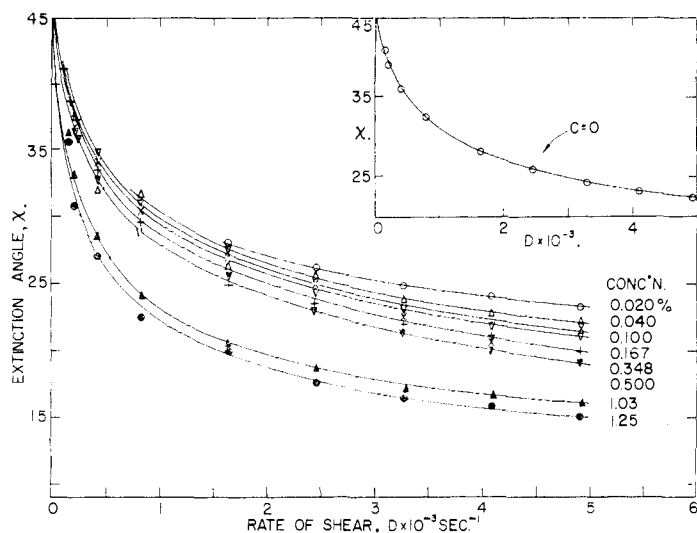


Fig. 6.—Extinction angles of poly- γ -benzyl-L-glutamate No. 416 in *m*-cresol at various concentrations. Insert, intrinsic extinction angle curve.

are the values of $k[\eta]$ (k = Martin constant) from previous flow curve study.¹⁰ The agreement between flow birefringence and viscosity slopes was indeed as good as could be expected. Thus at least as a first approximation the relationship in equation 2 appears also to hold for rigid systems.

In Fig. 8 and Table I the values of the slopes appear gradually to decrease with increasing rate of shear. This drop is mostly the result of the non-Newtonian viscosity which caused a marked drop in intrinsic viscosity when the shearing stress rises over a critical point.¹⁰ Of particular interest is the finding that the $\cot 2\chi$ versus c curves at high concentrations (Fig. 7) approach one another due mainly to the difference in the slopes at various rates of shear. Thus the χ versus D curves become flatter with increasing concentration which agreed with all the literature data.

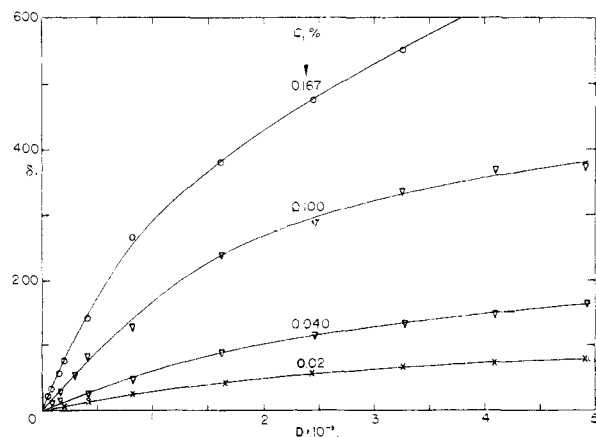


Fig. 7.—Birefringence (represented by twice the phase differences) of poly- γ -benzyl-L-glutamate No. 416 in *m*-cresol as a function of concentrations: $\Delta n = 1.39 \times 10^{-8} \delta$.

Figure 8 also points out two interesting features. At very low concentrations, *e.g.*, less than 0.1%, the concentration dependence is indeed insignificant due to the fact that the product of $k[\eta]c$ in this case becomes much less than one. It is conceivable, however, that the experimental data under these conditions would also involve greater errors. The usefulness of our proposed equation lies in the fact that it can smooth out the scattering of points, thus yielding a more reliable intrinsic value. The second feature is that at high concentrations or, for that matter, where $k[\eta]c$ is much

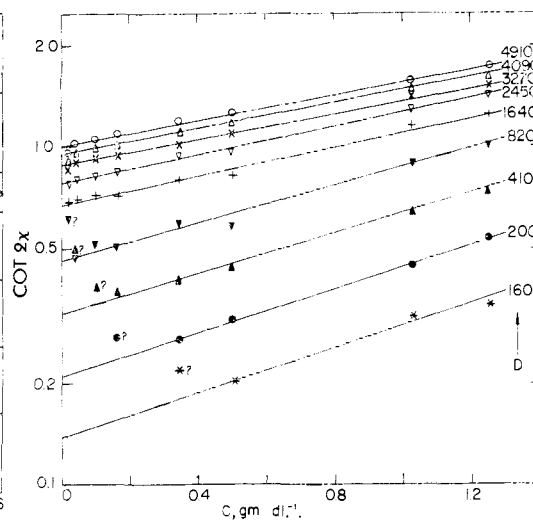


Fig. 8.—Extinction angles of PBLG No. 416 as a function of concentrations. Data were taken from Fig. 6.

TABLE I
COMPARISON OF THE CONCENTRATION DEPENDENCE OF FLOW BIREFRINGENCE AND VISCOSITY OF PBLG No. 416 IN *m*-CRESOL

Rate of shear D , sec. ⁻¹	Flow birefringence ^a $\cot 2\chi$ eq.	Slope, $k[\eta]$	
		($1/\theta$) eq.	Viscosity ^b
160	0.33	..	0.43
200	.32	0.36	.41
410	.30	.36	.36
820	.28	.33	.29
1640	.21	.28	.22
2450	.21	.28	.17
3270	.19	.28	.16
4090	.19	.27	.15
4910	.19	.28	.15

^a Calculated from Figs. 8 and 10. ^b Calculated from the data in ref. 10.

greater than unity, the results of any simple experiment would most certainly yield values that are far different from the intrinsic ones. Not infrequently one would also find that the concentration effect predominates even at such dilutions that the extinction angle becomes difficult to measure. In these cases our proposed equations will be most useful in the determination of intrinsic extinction angle.

Our results on the birefringence Δn were much less than satisfactory. The inherent imperfection in the optical arrangement of the instrument made it extremely difficult to obtain very precise measurements. All we can say is that the $\Delta n/c$ at any specified rate of shear did gradually increase with increasing concentration. Unfortunately the present apparatus could not detect this subtle difference with high precision. Thus, this problem awaits further investigation with an improved apparatus.

Poly- γ -benzyl-L-glutamate in Ethylene Dichloride.—Previous study of PBLG No. 397 ($M_w = 334,000$) in ethylene dichloride²¹ is another sample for illustrating eq. 5. Since the rates of shear in that study varied slightly from one concentration to another a bilinear plot similar to that of a light scattering envelope was designed by plotting $\cot 2\chi$ against $D\eta_0/T + Kc$ (K being an arbitrary constant). The results are shown in Fig. 9. Clearly all the constant-concentration curves (solid lines) fell on the same $\cot 2\chi = 0$ line since χ approaches 45° at $D = 0$. At such low concentrations (0.01 to 0.30%) all the constant $-D\eta_0/T$ curves (broken lines) were found to be straight lines according to eq. 5. The slopes, $k'[\eta] \cot 2\chi_0$ gradually rose with in-

(21) P. Doty and J. T. Yang, manuscript in preparation.

creasing rate of shear due to the fact that increase in $\cot 2\chi$ at higher rate of shear more than compensated the decrease in $k'[\eta]$. The data at the lowest concentration used (0.015%) were definitely in error, as was evidenced from their crossing over the zero-concentration curve (heavy line).

According to Peterlin and Stuart²² the initial slope of the extinction angle curve is related to the rotary diffusion constant Θ of the polymer by the expression

$$(d \cot 2\chi/dD)_{c=0, D=0} = -2(dx/dD)_{c=0, D=0} = 1/6\Theta \quad (10)$$

The initial slope in Fig. 9 was equal to $T/6\eta_0\Theta$, the choice of which was based on the fact that $\eta_0\Theta/T$ rather than Θ alone is characteristic of a polymer system. As can be seen in the figure appreciable error could be introduced into the drawing of the tangent line of the zero-concentration curve at $D = 0$. In the insert of Fig. 9 there is plotted $(\cot 2\chi)_{c=0}/(D\eta_0/T)$ against $D\eta_0/T$, the intercept of which on the ordinate equals $T/6\eta_0\Theta$ (see eq. 8b). Thus the length L_0 of the polypeptide was found to be 3200 Å. according to Perrin's well known equations for ellipsoids of revolution.²³ As D approaches zero, only extremely asymmetric particles will be oriented by the shearing force. Thus L_0 would be expected to approach closely the upper limit of the length of a polydisperse system. For the sake of comparison it can be mentioned that the weight-average length of PBLG No. 397 as calculated from the molecular weight was 2300 Å.²⁰ and the ratio of M_w to M_n was 1.4.²¹ The magnitude of these two values of particle length appeared to be fairly reasonable.

Other Flow Birefringence-Concentration Equations.—It has been found empirically that for desoxyribonucleic acid of squid testes the reciprocal of the apparent rotary diffusion constant $1/\Theta$ is a linear function of concentration,²⁴ whereas in other cases such as bovine fibrinogen and detergent micelles the apparent length varies linearly with concentration.²⁵ If the concept of effective viscosity is accepted such relationships would indeed be expected to exist. Since $\eta_0\Theta_0/T$ is a constant characteristic of each macromolecular system, one can write at constant temperature

$$\eta_0\Theta_0 = \eta^*\Theta \quad (11)$$

where η_0 is the solvent viscosity and Θ_0 the rotary diffusion constant at zero concentration. By combining equation 11 with equations 1 and 4 we have

$$\frac{1}{\Theta} = \frac{1}{\Theta_0} \frac{(\eta_{sp}/c)_D}{[\eta]_D} \quad (12a)$$

or

$$\frac{1}{\Theta} = \frac{1}{\Theta_0} (1 + k'[\eta]_D c) \quad (12b)$$

In other words

$$\alpha = \alpha_0 (\eta_{sp}/c)_D / [\eta]_D \quad (13a)$$

or

$$\alpha = \alpha_0 (1 + k'[\eta]_D c), (\alpha = D/\Theta) \quad (13b)$$

at any chosen rate of shear. As a first approximation the rotary diffusion constant of a prolate ellipsoid varies inversely as the cube of the length L . Eq. 12b then becomes

$$L = L_0 (1 + k'[\eta]_D c)^{1/3} \quad (14a)$$

or

$$L = L_0 \left(1 + \frac{k'}{3} [\eta]_D c + \dots \right) \quad (14b)$$

Thus there is nothing contradictory between eq. 12b and 14b, the latter being simply a further modification of the former.

In Fig. 10 is shown a plot of $\log 1/\Theta$ versus the concentration for PBLG No. 416 in *m*-cresol (Fig. 6). The corresponding $k'[\eta]$ values are included in Table I. Clearly the concentration dependence of the extinction angle and thereby the rotary diffusion constant can be equally well represented by eq. 5 or 12. In fact, for rigid particles eq. 5

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(23) F. Perrin, *J. phys. radium* [7], **7**, 1 (1936).

(24) J. T. Edsall, H. A. Scheraga and A. Rich, *Absts. 119th ACS Meeting, April, 1951*, p. 5J; A. Rich, p. 35c.

(25) J. K. Backus and H. A. Scheraga, *J. Colloid Sci.*, **6**, 508 (1951); H. A. Scheraga and J. K. Backus, *This Journal*, **74**, 979 (1952).

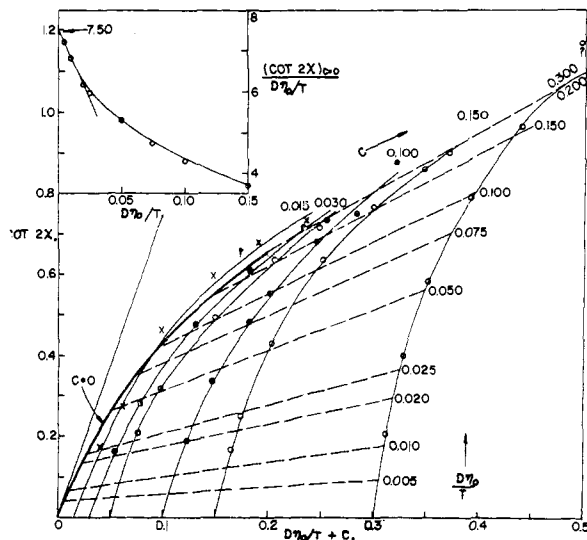


Fig. 9.—A double plot of the extinction angles of PBLG No. 397 in ethylene dichloride. Data were taken from ref. 21.

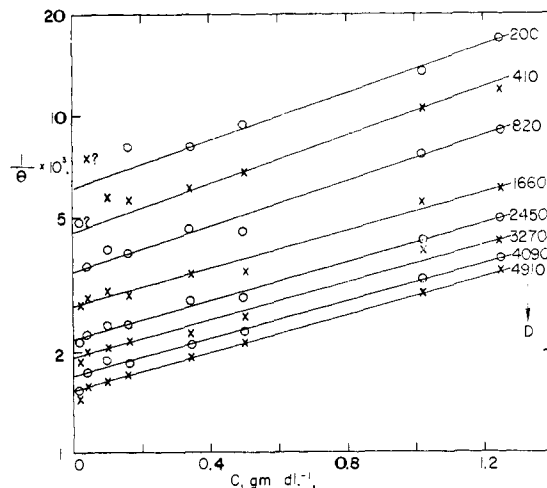


Fig. 10.—Intrinsic rotary diffusion constants of PBLG No. 416 in *m*-cresol at various rates of shear.

can also be deduced from the concept of effective viscosity. Since χ is a function of D/Θ , one can likewise write

$$\cot 2\chi = F(D/\Theta) \quad (15)$$

By substituting eq. 12b into eq. 15 and expanding the function F into a power series it becomes at constant rate of shear D

$$\cot 2\chi = \cot 2\chi_0 (1 + \text{constant } C + \dots) \quad (5a')$$

The advantage of using eq. 5a' lies in the fact that it can be applied to any polymer solution. On the other hand, one has to be sure that the system is rigid so that eq. 12 can be applied.

If the system were monodisperse, all the straight lines in Fig. 10 would have the same intercept, since Θ_0 in this case would be independent of the rate of shear. It is noted that this type of plot is extremely sensitive to the degree of polydispersity due to the fact that Θ_0 varies inversely with the cube of the particle length or, for that matter, the molecular weight. For PBLG No. 416 the $1/\Theta_0$ at $D = 0$ was found to be 0.007–0.009 through extrapolations, which in turn gave a L_0 of about 2200–2400 Å. as compared with the weight-average length of 1430 Å. (M_w/M_n being 1.3).²¹

Test of Protein Solutions.—In recent years flow birefringence has become one of the powerful tools for the study of biological systems, in particular the proteins which exhibit

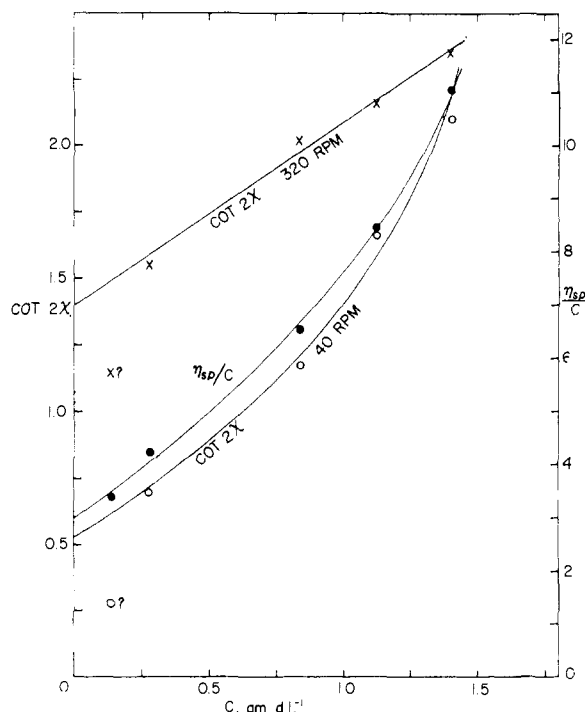


Fig. 11.—Comparison of $\cot 2\chi$ - C and (η_{sp}/C) - C plots of rabbit actomyosin solution. Data were taken from ref. 26.

a high degree of rigidity. It is therefore of interest to test the applicability of the proposed equations to these macromolecular systems. As an example we will choose von Murralt and Edsall's pioneer work on actomyosin.²⁶ In Fig. 11 are plotted $\cot 2\chi$ against the concentration (assuming the protein contains 16% N) at two rates of shear (the lowest and the highest measurable RPM). Also included in the figure is the (η_{sp}/c) - c curve, the data of which were measured

(26) A. von Murralt and J. T. Edsall, *J. Biol. Chem.*, **89**, 375, 351 (1930); J. T. Edsall, *Trans. Faraday Soc.*, **26**, 837 (1930).

in an Ostwald viscometer presumably at very low rate of shear. The close similarity in the shape of both flow birefringence (at low r.p.m.) and viscosity is self-explanatory. According to the original paper, the lower R.P.M. corresponded to about $D = 10 \text{ sec.}^{-1}$ and $\alpha (= D/\theta)$ was close to 1. Although the actual rate of shear in the viscometer was not mentioned, the listed values seemed also to lie close to the Newtonian region. Thus the two curves were comparable at about the same range of rates of shear. The apparently straight line obtained at a high r.p.m. was mostly certainly due to the sharp drop in $k'[\eta]$ in eq. 5, since this protein exhibits extremely strong non-Newtonian viscosity at higher rates of shear.

Bovine plasma albumin is another extreme case for illustration. Due to its low asymmetry this protein can only be oriented in a highly viscous medium and at a very high concentration. Edsall and Foster²⁷ had reported an estimated length of 190–200 Å. for a 4.48% (w./v.) solution in 88.45% (w./w.) glycerol. At this concentration the ratio of $(\eta_{sp}/c)/[\eta]$ was estimated to be about 1.4,²⁸ using the viscosity data in aqueous solutions. Using eq. 12 one finds a calculated value of $\eta_0\theta_0/T$ of 42 rather than the published value of 30. Consequently the estimated length of the ellipsoid should be close to 170 Å., which is in better agreement with the currently accepted value of 150 Å. It seems highly desirable to reinvestigate this protein in several more concentrated solutions so that any scattering in experimental points can be smoothed out. In fact, with our proposed equations it will be possible to extend the lower limit of flow birefringence technique, thus enabling us to study many proteins of very low asymmetry. Likewise, for those having very high asymmetry it is of pertinent importance to determine the extent of concentration dependence. These studies would certainly prove or disprove the general applicability of our proposed equations.

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

Kinetics of the Reversible Michaelis–Menten Mechanism and the Applicability of the Steady-state Approximation¹

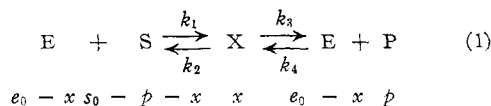
BY WILMER G. MILLER² AND ROBERT A. ALBERTY

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The exact analytical solutions to the rate equations for the reversible Michaelis–Menten mechanism are derived for the case that $k_1 = k_4$. The steady-state approximation is shown to be a good approximation if $s_0 \gg e_0$ or if $(e_0 + s_0) \ll (k_2 + k_3)/k_1$. A perturbation solution is developed for the case that $k_1 \neq k_4$ and the applicability of the steady-state approximation for this case is discussed.

Introduction

Enzyme kinetic data frequently can be represented by the reversible Michaelis–Menten mechanism



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(2) Socony-Mobil Fellow (1957–1958).

where E represents enzyme,³ S and P represent substrates, and X is the intermediate. The total molar concentration of enzyme is represented by e_0 and the initial concentration of substrate by s_0 . The concentrations of the intermediate and product

(3) The symbol E actually represents the enzymatic site rather than the enzyme but generally the number of sites per molecule is unknown. Letting E represent the enzyme rather than the enzymatic site so that e_0 represents the total molar concentration of the enzyme increases the rate constants by a factor equal to the number of sites per molecule, assuming no site–site interaction. The mechanism is not restricted to enzyme catalysis but might be applied to heterogeneous catalysis, with E representing the sites on the surface of the solid.