characteristic ratio is very insensitive to γ and σ in the vicinity of $\gamma = 10$ and $\sigma = 1.4$: $\partial \ln \langle r^2\rangle_0/\partial \ln \gamma = -0.0449$ and $\partial \ln \langle r^2\rangle_0/\partial \ln \sigma = -0.0786$. The particularly low value of the former quantity is undoubtedly due at least in part to structural features inherent in the chain under consideration. Transitions from states of 0 to ±60° about CH—CH$_2$ bonds are not nearly as effective in changing the dimensions of a polymer chain as are the transitions from 0 to ±120° occurring in polyethylene,[2,3] poly ethers,[5,6,10] vinyl polymers,[8] and other molecules of similar skeletal structure. In any case, the energies determined for both *cis*-1,4-polybutadiene and *cis*-1,4-polyisoprene should be regarded as only rough estimates.

The value $\gamma = 10$ should also be appropriate for *cis*-1,4-polyisoprene; if the value $\langle r^2\rangle_0/nl^2 = 4.7$ is accepted for this polymer (see footnote 34), a value of $\sigma = 2.46$ ($E_\sigma = -0.6$ kcal mole^{-1}) is required. For these values of the parameters, eq 13 predicts $d \ln \langle r^2\rangle_0/dT = 0.56 \times 10^{-3}$ deg^{-1}, in excellent agreement with experiment. It is anticipated, however, that a more reliable estimate of E_σ for 1,4-polyisoprene chains may be obtained from interpretation of $\langle r^2\rangle_0/nl^2$ and $d \ln \langle r^2\rangle_0/dT$ for the *trans* form of this polymer.

Discussion

Comparison of experimental and calculated values of the characteristic ratio and its temperature coefficient for *cis*-1,4-polybutadiene and *cis*-1,4-polyisoprene provides the following information: (i) the energy of a pair of 60° states about bond pairs CH—C$_2$H$_2$—CH relative to conformations ±60°,0° and 0°,±60° is of the order of -1 kcal mole^{-1}, and (ii) the energy of a *gauche* state about a CH$_2$—CH$_2$ bond is either the same as that of a *trans* state or is possibly a few hundred cal mole^{-1} lower.

The basis for the positive temperature coefficient of $\langle r^2\rangle_0$ for these polymers can be seen by examination of the two terms in brackets in eq 13. The increase in dimensions due to transitions to more extended conformations about CH—C$_2$H$_2$—CH bond pairs (*e.g.*, ±60°,±60° → 0°,±60°) as the temperature is increased accounts for approximately 70% of the magnitude of the observed temperature coefficient, the remainder being due to *gauche* to *trans* transitions about CH$_2$—CH$_2$ bonds.

According to the present study, repeat unit $\mathrm{+C{-}C{=}C{-}C+}$ conformations (±60°,180°,±60°,0°) and (±60°,180°,∓60°,0°) should be of approximately the same intramolecular energy as (±60°,180°,±60°, ±120°) and (±60°,180°,∓60°,±120°). X-Ray diffraction studies of the crystal structures of *cis*-1,4-polybutadiene[11,12] and *cis*-1,4-polyisoprene[11,13,14] indicate a conformational sequence $[(\pm60°,180°,\pm60°,0°); (\mp60°,180°,\mp60°,0°)]_{x/2}$ for the chains in the crystalline state. This final choice is apparently made on the basis of the relative efficiencies with which these conformations may be incorporated into a crystalline lattice.

Acknowledgments. The author wishes to thank Professor P. J. Flory for his helpful criticisms of the manuscript, R. Morrill for his assistance in programming the calculations, and the Computation Center of The Polytechnic Institute of Brooklyn for the use of their facilities.

A Kerr-Effect Study of the Low pH Configuration Changes of Bovine Plasma Albumin in Aqueous Solutions

C. L. Riddiford and B. R. Jennings

Contribution from the Department of Physics, University of Southampton,
Southampton, England. *Received April 5, 1966*

Abstract: Electric birefringence measurements have been made on dilute aqueous solutions of bovine plasma albumin (BPA) in order to observe its acid configuration transitions. As the pH was reduced from the isoelectric value of 5.1, the relaxation times of both samples studied fell abruptly at pH 4.1 and at pH 3.6. These decreases were accompanied by increased contributions from the induced dipolar effects and are considered as confirmation of the two-stage expansion of the quadruple-unit molecule proposed by Foster. When studied near the isoelectric point, the two samples displayed different relaxation times, each of which could be substantiated by results recorded in the literature. The difference probably arose from the presence of larger particles in the solutions. Both samples demonstrated the ready formation of nonreproducible quantities of associates of BPA when the pH was lowered below 3.2. The method appears to display molecular configuration changes admirably.

It is now well known that if a solution of randomly arrayed optically anisotropic macromolecules, which are either dipolar or electrically anisotropic, is placed in an electric field, the molecules orientate to a position of minimum potential energy. The ordering of the particles so obtained renders the solution birefringent. This Kerr electrooptical effect is completely reversible in that Brownian motion disorientates the molecules upon removal of the applied field. However, as the molecules require a finite time to respond to changes of the applied field, a relaxation of the birefringence, characterized by a relaxation time of τ sec is observed. τ is a function of the size and shape of the molecules and of the viscosity of the particular solvent. Thus, for applied pulsed fields, observations on both the steady maximum and the transient values of the bire-

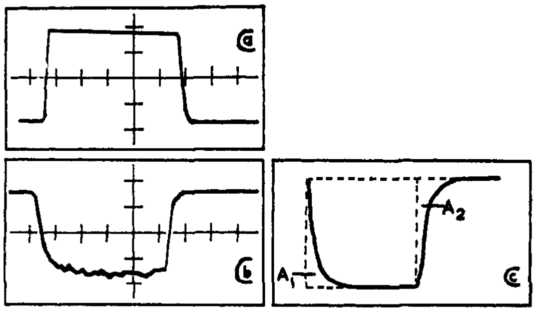

Figure 1. Typical experimental traces: (a) applied pulse, 8 kv for 2.5 μsec; (b) photon-multiplier output signal for solution of sample B at pH 3.1 and concentration 0.7%; (c) frame b redrawn for a linear dependence of birefringence on the pulse height.

fringence of the solutions lead respectively to information about the electrical and geometrical parameters of the molecules.

Recently Krause and O'Konski[1] employed this technique to study aqueous solutions of bovine plasma albumin (BPA). They observed different values for the relaxation times, 210 and 160 nsec for solutions at pH 5.1 and 2.58, respectively. The results from a number of experiments on BPA solutions, which were undertaken by various authors using a variety of techniques,[2] have indicated that molecular configuration changes occur within this pH range. Ingram and Jerrard[3] have also used the Kerr effect to investigate an aqueous BPA solution at pH 5.1 and obtained a relaxation time of only 122 nsec. However, for another sample at the same pH, Ingram[4] quoted a value of 194 nsec in closer agreement with that given by Krause and O'Konski. More recently, Moser, *et al.*,[5] have reported a study in which they have isolated pure BPA, free from any impurities or associated material, and found the much smaller value of 76 nsec after correcting for concentration effects.

The indications that the BPA molecule undergoes an expansion as the pH is lowered, coupled with consideration of the accompanying chemical behavior of the molecule, prompted Foster[2] to postulate a model in which four subunit globules are packed one upon another and linked by the continuity of the constituent flexible polypeptide chain. This most compact arrangement is termed the N state. These subunits are held in pairs as a result of hydrophobic bonding between the two members of a pair, and the two pairs so obtained are bonded electrostatically into a complete four-member globule. As the pH is lowered from the isoelectric point, it is suggested that initially the electrostatic forces are overcome and the molecule divides into its two globular pairs, owing to the flexibility of the peptide links between them, so forming the intermediate F′ form. With further lowering of the pH the electrostatic repulsions increase until the hydrophobic bonds are also ruptured and the molecule exists as four individual but conjoint units. This is called the F state.

The model has been supported by the work of Weber and Young,[6,7] who have sectioned the BPA into three discrete fragments by the action of pepsin on the interlinking polypeptide chain at pH 3. Similarly, Adkins and Foster[8] have split BPA into two corresponding fragments using subtilisin in alkaline solution. It is not unreasonable to expect that in acid solution the individual subunits rotate singly in the applied electric field should Foster's model be correct. We have therefore undertaken the following study of the variation of the relaxation times of the molecular units at different solution acidity with the intention of following and hence manifesting these configuration changes throughout the N to F transitions without permanently severing the interstituent peptide linkages.

Experimental Section

Apparatus. A conventional Kerr-effect optical system was used. Light from a high-pressure mercury arc (GEC 250-w ME/D compact source) was collimated by two cylindrical lenses and a series of diaphragms. It then passed through the cell (C), which contained the solution under investigation, and was brought to a focus on a Mullard 56 AVP photon-multiplier. The cell was placed between crossed polarizer (P) and analyzer (A) with a quarter wave plate (Q) for wavelength 5461 A inserted between C and A at zero azimuth relative to the vibration direction of P. The direction of the field was 45° to the vibration directions of P and A. The fact that no optical filter was used in order to realize the greatest possible intensity of light can be shown to introduce negligibly small errors into the system.[9] Care was taken to position the optical components so as to obtain the maximum light transmission for optimum signal-to-noise ratio.

The cell accommodated 5 ml of solution and was manufactured from stainless steel and lined with polytetrafluoroethylene (PTFE). Two parallel, stainless steel electrodes of 5-cm length were centrally placed 0.32 cm apart. The effect of the steel on the protein solution was not investigated, but was not believed to be important. All joints of the cell were pressure sealed to enable the solution to be continuously pumped by a Watson-Marlow flow inducer (Type MHRK) through a pH cell where the acidity was monitored and controlled by a Radiometer titrator (Type TTTlc with G222B and K4112 electrodes). This titrator governed the behavior of a Radiometer syringe buret unit which supplied the reagent used to adjust the pH of the solution. Simultaneously the quantity of reagent added was recorded on a Honeywell-Brown recorder using the circuit suggested by Josefsson, *et al.*[10]

A hydrogen thyratron and delay line pulse generator system, similar to that of Krause and O'Konski,[1] was used except that the cable was increased in length to give a pulse of up to 8 kv amplitude and of 2.5-μsec duration. Furthermore, improvement in the arrangement and shielding of the system resulted in much cleaner pulses which exhibited negligible "ringing" effects (Figure 1a). The photon-multiplier output was displayed on a Tektronix oscilloscope (Type 585) and photographed using Polaroid 10,000 ASA film.

Materials. Two samples of very pure crystalline BPA were purchased from Messrs. Kock-Light Ltd of Colnbrook, Buckinghamshire, England. These were taken from Batch No. 5856 and 14,164 and hereafter are referred to as samples A and B, respectively. The Sephadex fractionation procedure of Moser, *et al.*,[5] was not followed. However, in order to allow direct comparison with the results of Krause and O'Konski, who have been the only other authors to report Kerr effect results on BPA solutions at more than one pH, solutions of 0.7% concentration were made in freshly distilled, deionized water and the experiments were then undertaken within a few hours to avoid the possibility of the solution aggregating upon standing. The reagent used to lower the pH of the solutions was dilute HCl. This was added to the approximate 25 ml of solution which was circulating through the pH and

---

[1] S. Krause and C. T. O'Konski, *J. Am. Chem. Soc.*, **81**, 5082 (1959).

[2] J. F. Foster, "The Plasma Proteins," Vol. 1, F. W. Putnam, Ed., Academic Press Inc., New York, N. Y., 1960, Chapter 6.

[3] P. Ingram and H. G. Jerrard, *Nature*, **196**, 57 (1962).

[4] P. Ingram, Ph.D. Thesis, University of Southampton, 1963.

[5] P. Moser, P. G. Squire, and C. T. O'Konski, *J. Phys. Chem.*, 70, 744 (1966).

[6] G. Weber and L. B. Young, *J. Biol. Chem.*, **239**, 1415 (1964).

[7] G. Weber and L. B. Young, *ibid.*, **239**, 1424 (1964).

[8] B. J. Adkins and J. F. Foster, *Biochemistry*, **4**, 634 (1965).

[9] H. G. Jerrard, *J. Opt. Soc. Am.*, **44**, 281 (1954).

[10] L. Josefsson, C. Ryberg, and R. Svensson, *Anal. Chem.*, **34**, 173 (1962).

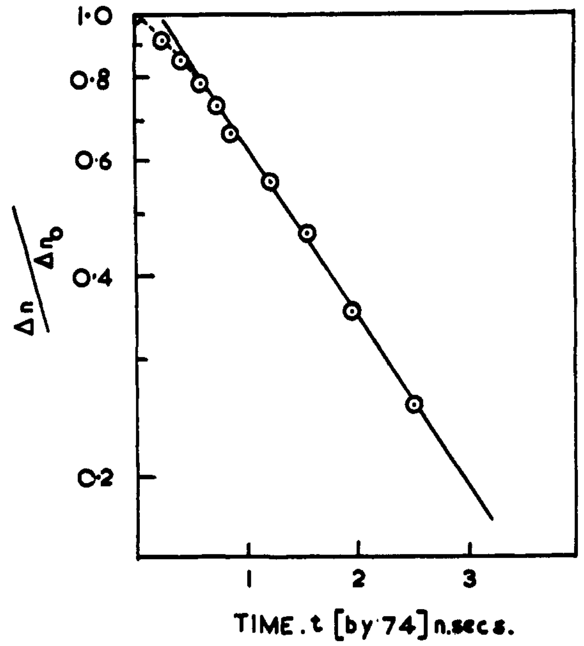

Figure 2. Typical time-dependent variation of the birefringence decay, sample B, at pH 4.0.

Kerr cells. The circulation was stopped prior to the application of the pulse in order to prevent flow birefringent effects and to allow any air bubbles to clear. The Kerr cell was thermostated at 20 ± 1°. No electrolyte was added beyond the HCl required to adjust the pH. The ionic strength of the solutions was thus kept to a minimum as it had been shown[1] that the Kerr constant (and hence the detector signal) decreases with increasing ionic strength.

Results and Treatment of Data

If the electric field is applied in the form of a pulse of such duration that the particles have ample time to align, the birefringence will build up to a steady maximum value Δn_0. This parameter is related to the specific Kerr constant K_{sp} and the electric field strength E applied to a solution of concentration c and refractive index n by the equation[11]

$$K_{sp} = \Delta n_0(cnE^2)^{-1} = \frac{2\pi}{15}(g_1 - g_2)\frac{1}{n^2}\left[\frac{\mu^2}{k^2T^2} + \frac{(\alpha_1 - \alpha_2)}{kT}\right] \quad (1)$$

Here $(g_1 - g_2)$ and $(\alpha_1 - \alpha_2)$ represent the optical and electrical anisotropies, respectively, of a particle which carriers a permanent moment μ along its major axis. The subscripts 1 and 2 refer to the major and minor axes of the particle, respectively. k is Boltzmann's constant and T the absolute temperature. For a given solution and applied field, therefore, eq 1 shows that changes in Δn_0 are related to changes in the electrical parameters of the molecule, provided $(g_1 - g_2)$ remains constant. With the present system the amplitude (κ^2) of the oscilloscope trace is proportional to $(\Delta n_0)^2$ (see Figure 1b) for relatively small values of the birefringence.

The birefringence decay for a dilute monodisperse system obeys the equation[11]

$$\Delta n = \Delta n_0 e^{-t/\tau} \quad (2)$$

where Δn is the birefringence at any time t after the commencement of the decay. The relaxation time (τ) has been obtained by taking data from along the decay curve (Figure 1b) and analyzing the slopes of a log graph of $(\Delta n/\Delta n_0)$ against time (Figure 2).

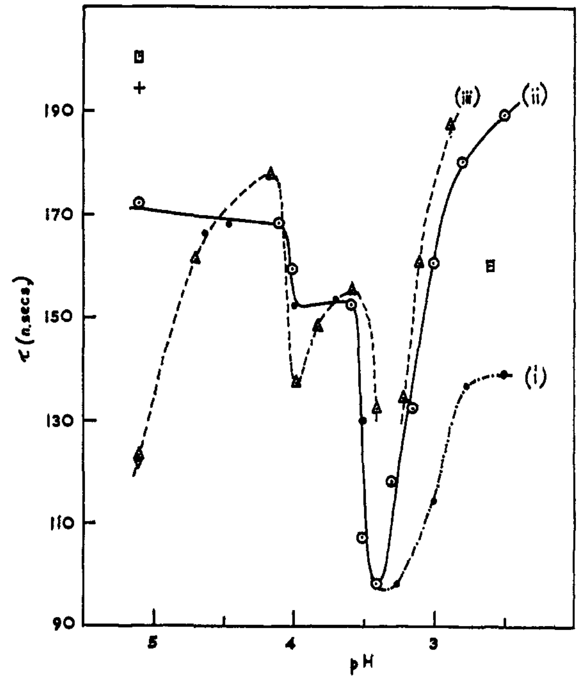

Figure 3. Variation of relaxation times (τ) with pH: runs i and ii for similar fresh preparations of sample A, and iii for sample B; squares, inverted triangle, and cross represent Kerr-effect relaxation times reported in ref 1, 3, and 4, respectively.

Yoshioka and Watanabe[12] have shown that a comparative analysis of the rise and fall of the birefringence leads to information of the relative importance of the permanent and induced orienting mechanisms. They demonstrated that the areas A_1 and A_2 of Figure 1c are related to a parameter r by the expression

$$\frac{A_1}{A_2} = \frac{\int_0^\infty (\Delta n_0 - \Delta n)\mathrm{d}t}{\int_0^\infty \Delta n \mathrm{d}t} = \frac{4r + 1}{r + 1} \quad (3)$$

where

$$r = \frac{\mu^2}{(\alpha_1 - \alpha_2)kT} \quad (4)$$

Photographs of the oscilloscope trace for the birefringence have been taken (Figure 1b) and redrawn so as to convert them from a quadratic to a linear scale of birefringence (Figure 1c). The parameter r has then been computed from the relevant areas. As was originally pointed out by Benoît,[11] a birefringence decay which is symmetrical with the birefringence buildup indicates the absence of permanent dipole moments; *i.e.*, A_1 and A_2 are of equal value and so $r = 0$ as shown above.

Results

The variations of τ throughout the range $2.5 < \text{pH} < 5.5$ are shown for three experimental runs in Figure 3. The data of curves i and ii were obtained on two separately prepared solutions of sample A and are

---

[11] H. Benoît, *Ann. Phys.* (Paris), **6**, 561 (1951).

[12] K. Yoshioka and H. Watanabe, *Nippon Kagaku Zasshi*, **84**, 626 (1963).

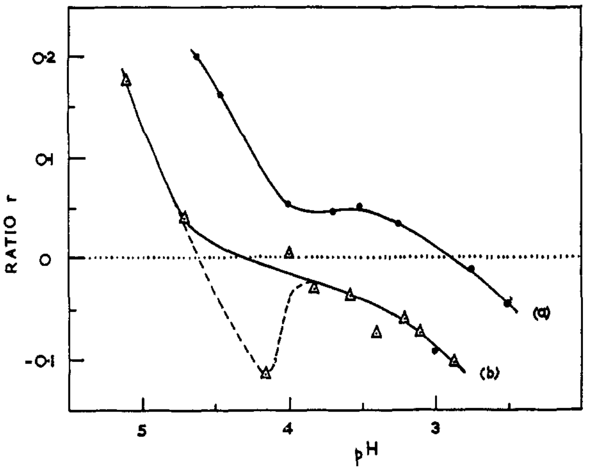

Figure 4. Variation of the ratio r with pH for (a) sample A, run i; and (b) sample B, run iii. Broken line is an improbable alternative to the full line. The error in r is high (see text).

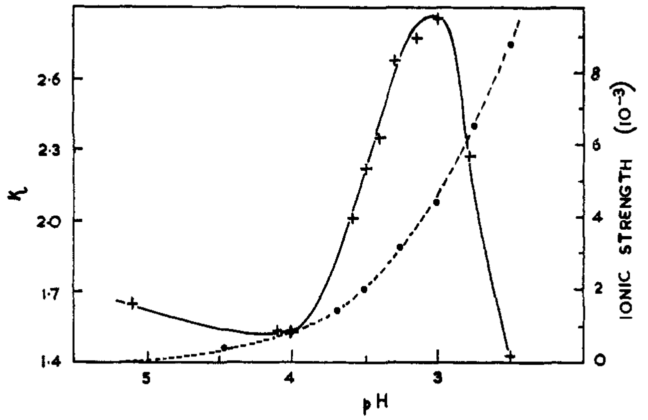

Figure 5. Variation of the ionic strength (broken line) and the square root of the maximum signal amplitude κ (proportional to K_{sp}) with pH: data for run ii of Figure 3. All experiments gave similar curves.

seen to be reproducible for pH greater than 3.4. For these solutions we obtained a value of 172 nsec at pH 5.1, in reasonable but not excellent agreement with the higher values cited by Krause and O'Konski[1] and Ingram.[4] However, our result is practically identical with the value (170 nsec) calculated from the diffusion constant quoted by Edsall and Foster.[13] This was obtained from their streaming birefringence experiments. Reduction of the pH from its value near the isoelectric point was accompanied by little change in the relaxation time, until pH 4.1 where there was a very sharp fall in τ to a steady value of 153 nsec. We believe this to be a manifestation of the N to F′ transition where the complete globular molecule unfolds into its two interlinked pairs, each of which is now able to orientate in the electric field as if it were an individual particle. Further reduction in pH again resulted in little change in τ until pH 3.6 when there occurred another very sudden drop in the relaxation time to a value of 97 nsec. Here again a diminution in the value of τ suggested a configuration change to rotating particles of smaller dimensions. This was probably the F′ to F transition after which each of the four ultimate subunits were able to respond independently to the applied electric field. When the acidity was further increased (pH below 3.4) the results were no longer reproducible, although in all cases there was a very sharp increase in τ.

When a solution of sample B was investigated under the same experimental conditions, curve iii of Figure 3 was obtained. It was interesting to note that in this case the relaxation time at pH 5.1 was 123 nsec which, although different from the value given above, was in excellent agreement with that quoted by Ingram and Jerrard.[3] Curve iii shows decreases in τ similar to those of curves i and ii near the pH values 4.1 and 3.6. It also demonstrates the very large increase in the relaxation time for pH below 3.4. A noticeable difference in the behavior of sample B was observed in the interim pH regions prior to these sharp transitions. Here sample A maintained a steady value for the relaxation time, but for sample B this parameter gradually increased with the acidity of the solution until the onset of the transitions. It thus appears that both samples undergo the same configuration changes at pH 4.1 and 3.6 but differ slightly in their behavior between these changes.

The variable r has been estimated from measured values of A_1 and A_2 using eq 3. A_1 was easily obtained with the aid of a planimeter. In most cases A_2 was difficult to measure accurately owing to the uncertainty in determining when Δn had fallen to zero. Small discrepancies in this quantity gave rise to large errors in A_2. An alternative procedure was to compute A_2 from the measured relaxation time. This area is expressed by the equation

$$A_2 = \int_0^\infty \Delta n \mathrm{d}t$$

where $t = 0$ represents the start of the decay and Δn is given by eq 2. Thus

$$A_2 = \Delta n_0 \tau \qquad (5)$$

Before using this equation it was verified that only one relaxation time was detectable. Equations 5 and 3 were then employed to obtain the values of r shown in Figure 4 for different acidity. r was always very close to zero. Curves obtained for samples A and B both show a continuous decrease in the magnitude of r with pH. Negative values of r signify that the calculated ratio A_1/A_2 was less than unity and that the axes of the permanent and induced moments were in quadrature. Owing to the large uncertainty in evaluating A_2 and hence r, it was difficult to stipulate the true position of the abscissa axis relative to the curve so that we conclude little from Figure 4 except to point out that even though permanent dipolar effects may be important,[5] the *changes* in the induced moments play a more important role during the transitions than permanent dipolar variations.

It has been mentioned that the amplitude $(\kappa)^2$ of the birefringence trace (Figure 1b) was proportional to Δn^2. Figure 5 shows the variations of κ which accompanied the pH changes. From eq 1 it is seen that provided the changes in the refractive index of the solution were negligible and that the applied electric field strength was maintained constant, variations in κ reflected alterations in K_{sp} for solutions of different

---

(13) J. T. Edsall and J. T. Foster, *J. Am. Chem. Soc.*, 70, 1860 (1948).

acidity. The changes indicated in Figure 5 closely resemble the variation of the specific Kerr constant with pH as given by Krause and O'Konski. They were not obtained however, under conditions of constant ionic strength, the increase of which was estimated from the quantity of HCl added and is shown in the same figure. Flow birefringence studies[13] have demonstrated that the optical anisotropy of the solutions does not alter with acidity. Thus if we assume that, as mentioned above, changes occur more readily in the induced rather than permanent dipolar effects, then the considerable rise in κ suggests a very large increase in the electrical anisotropy of the molecules in solutions below pH 4.

Discussion

The abrupt changes in relaxation times which occur at pH 4.1 and 3.6 are compatible with the original view of Harrington, *et al.*,[14] that the BPA molecule consists of subunits which, when subjected to acid solution, can rotate independently yet without completely dissociating. The presence of two such sharp transitions, however, gives direct evidence for the more specific four-member model suggested by Foster.[2]

A number of workers have reported a molecular configuration change as the acidity of BPA solutions has been increased through pH 4. The physical techniques employed have been fluorescence depolarization,[14,15] optical rotation,[16,17] centrifugation,[18] intrinsic viscosity,[17] and X-ray scattering.[19] On the other hand, Ridgeway[20] interpreted his results on the variation of the optical rotatory dispersion parameter λ_0 as indicating that a possible molecular transiton occurred close to pH 3.5. Only the viscosity experiments of Tanford, *et al.*,[21] and perhaps the electrophoresis data of Aoki and Foster[22] have indicated previously by physical methods the possible occurrence of two configuration changes between which the molecule assumes a discrete intermediate form. Both samples studied in this investigation have demonstrated the two-stage configuration changes by their sudden reductions in relaxation times as smaller, or more compact, molecular units were free to rotate in the electric field. Both transitions were very sharp with changes of pH (Figure 3). This observation refutes the suggestion by Bloomfield[23] that the N globules undergo a slow separation as the acidity of the solution is increased. Moreover, his suggestion that the F′ form consist of three members *tightly* held in juxtaposition does not explain the present results where we have observed sudden reductions in relaxation times as smaller units rotate in response to the applied electric fields. A more extended tightly bound structure would require an increase in τ.

At the isoelectric point we assumed that the molecules were rigid in solution and analyzed the relaxation times in terms of prolate ellipsoids by employing Perrin's equation.[24] For this we used the value of Champagne, *et al.*,[25] 130,000 A^3, for the hydrated molecular volume of sample A and obtained a major axis of length 196 A as has often been observed for nonfractionated BPA. The relaxation times observed for each sample at the isoelectric point have been reported previously, that of 172 nsec by the Kerr effect[1,4] and flow birefringence,[13] while the value of 123 nsec was earlier demonstrated in this laboratory[3] using the Kerr effect and well supports the results of Harrington, *et al.*[14] (128 nsec), Weber[15] (127 nsec), and Oncley[26] (124 nsec), from depolarization fluorescence and dielectric dispersion, respectively. However, Moser, *et al.*,[5] have shown that both sets of values are probably high owing to the presence of aggregated material (dimers, trimers, etc.) and the concentration dependence of the birefringence for BPA preparations. The value of 123 nsec for sample B agrees well with the data of Moser, *et al.*, for the pure N form at this concentration. However, it does seem to be more than fortuitous that the other set of result from three separate workers are in close agreement. It is thus possible that the larger material present in these cases is not a haphazard quantity of aggregate but a specific amount of a certain type or types, depending upon the product and its prehistory.

For either sample, Figure 3 shows that the changes in magnitude of the relaxation times throughout the transition at pH 4.1 were not very large. This is in agreement with the suggestion that the N to F′ transition occurs by the cleaving of the four-member N globule into the two doublet pairs along a plane containing the major axis of the particle. A much greater change in τ would have been expected for other types of division.

The increase of τ for solutions with pH less than 3.4 indicates the formation of large particles. Other workers have observed this and attributed the particles either to dimers or other aggregates of the BPA or to coalesced fatty acid impurities which are liberated when the albumin molecules are fully extended. The liberation of these fatty acid impurities has been demonstrated by Champagne.[27] Bro and Sturtevant[28] have shown that such impurities are always present at low pH in BPA samples which have not been previously maintained at low pH during their preparation. No such precaution was taken with our samples prior to this investigation. Champagne[27,29] has moreover concluded from light scattering experiments that the behavior of the large particle impurity was not reproducible, a conclusion that is strongly demonstrated by our data. However, Bro, *et al.*,[30] and Williams and Foster[31] noticed that dimerization occurs in the pH range 2.5 to 4.0 with pH 3.3 the optimum condition. All of their observations indicated that the formation of dimer was a slow process, contrary to the present authors' data, which were all obtained within minutes of changing the solution conditions. Unless the dimerization is

(14) W. F. Harrington, P. Johnson, and R. H. Otterwill, *Biochem. J.*, **62**, 569 (1956).

(15) G. Weber, *ibid.*, **51** 155, (1952).

(16) B. Jirgensons, *Arch. Biochem. Biophys.*, **39**, 261 (1952).

(17) J. T. Young and J. F. Foster, *J. Am. Chem. Soc.*, **76**, 1588 (1954).

(18) P. A. Charlwood and A. Ens, *Can. J. Chem.*, **35**, 99 (1957).

(19) V. Luzzati, J. Witz, and A. Nicolaieff, *J. Mol. Biol.*, **3**, 379 (1961).

(20) D. Ridgeway, *Proc. Natl. Acad. Sci.*, **48**, 1482 (1962).

(21) C. Tanford, J. Buzzell, D. Rands, and S. Swanson, *J. Am. Chem. Soc.*, 77, 6421 (1955).

(22) K. Aoki and J. F. Foster, *ibid.*, **79**, 3385 (1957).

(23) V. Bloomfield, *Biochemistry*, **5**, 684 (1966).

(24) F. Perrin, *J. Phys. Radium*, **5**, 497 (1934).

(25) M. Champagne, V. Luzzati, and A. Nicolaieff, *J. Am. Chem. Soc.*, **80**, 1002 (1958).

(26) J. L. Oncley, *Chem. Rev.*, **30**, 433 (1942).

(27) M. Champagne, *J. Chim. Phys.*, **54**, 393 (1957).

(28) P. Bro and J. M. Sturtevant, *J. Am. Chem. Soc.*, **80**, 1789 (1958).

(29) M. Champagne, *J. Polymer Sci.*, **23**, 863 (1957).

(30) P. Bro, S. J. Singer, and J. M. Sturtevant, *J. Am. Chem. Soc.*, **80**, 389 (1958).

(31) E. Williams and J. F. Foster, *ibid.*, **82**, 3741 (1960).

accompanied by a drastic increase in particle size, our relaxation times correspond to other much larger particles. Williams and Foster[31] demonstrated, however, that dimers and possibly higher order associates are formed through hydrophobic bonding so that, as the hydrophobic bonds of the F′ form break to give the F state, conditions for association are very favorable. If one concludes that the isoelectric relaxation time of sample B is in approximate agreement with the value for a pure sample at this concentration,[5] then the initial presence of fatty acid must be excluded, and the low pH increase of τ attributed to molecular association alone. Nevertheless, although we observed only a single relaxation time in this pH region, it is possible that our detection system was not sensitive enough to display the presence of both coalesced impurities and associated BPA which might have coexisted in the solutions.

Both samples A and B demonstrated the molecular transitions and at very low pH, either the liberation of lipoprotein impurities or molecular associations. Of interest, also, was their difference in behavior, both at the isoelectric point and throughout the whole low pH range. At the isoelectric point, each sample had a relaxation time which corresponded to one or other of the two hitherto reported values. The sigmoidal variations of τ through each transition observed with sample B were not noticed during either of the runs on sample A. If the isoelectric point relaxation time of sample B is taken as indicative of a purer sample by comparison with the results of Moser, *et al.*,[5] then the variations of τ for this sample might indicate the presence of molecular interactions. If so, true relaxation times and hence geometric dimensions of the rotating molecular units would only be obtained after careful isolation of the material at each pH for investigation and that one would be obliged to ensure that, even after undertaking such isolation, associates were not readily formed. Such work, although tedious, would be of interest.

The variation of the specific Kerr constant (which is directly proportional to κ in Figure 5), was similar to that previously published[1] except in two minor ways. Firstly, Figure 5 shows a minimum at pH 4.0. This is below the isoelectric point where the earlier reported minimum occurred. The slight changes in ionic strength or even the experimental error could possibly have accounted for this. Secondly, we observed an inflection in the curve at very low pH which was not recorded by Krause and O'Konski for their previously deionized sample. Thus the decline in κ reported here might have arisen from either the presence of impurities which could have been liberated during the pretreatment of Krause and O'Konski's deionized sample or the rapidly increasing ionic strength of our solutions. An addition of these factors is not excluded.

Conclusions

Observations on the relaxation time of BPA solutions at low pH indicate two abrupt molecular configuration transitions at pH 4.1 and 3.6. The presence of two transitions lends support to the four-member molecular model proposed by Foster.[2] Foster has suggested that as the acidity of the solutions is increased from the isoelectric point the increasing electrostatic repulsion between the subunits reaches the magnitude when it overcomes the electrostatic bonding forces between the subunit pairs. Continued acidity results in the forces overcoming even the hydrophobic bonding between the subunits constituting the pairs. Our results are consistent with this interpretation in that as the molecule assumes its more extended configuration so the changes in the electrical properties of the molecule are manifested in terms of a large increase in the electrical anisotropy of the particles.

The two samples investigated demonstrated the same qualitative behavior throughout the pH range studied. At the isoelectric point each had a different relaxation time, yet both values could be substantiated by other experimental results given throughout the literature. The difference between these two types might be explained in terms of the presence of associated material.

From this investigation it is clear that detailed electric birefringence studies of solutions of globular proteins should prove fruitful, not only in yielding information about the electric and geometric parameters of the molecules, as has often hitherto been undertaken, but also in indicating *changes* in these parameters. In particlular, reversible changes can be followed in the configuration of multimember molecules and some assessment made of the properties of the molecular components without any destruction of the composite molecule.

Acknowledgments. The authors are pleased to thank Dr. H. G. Jerrard in whose research group this work was carried out. C. L. R. thanks the Science Research Council for the award of a research studentship, and B. R. J. thanks the Imperial Chemical Industries Ltd. for a postdoctoral research fellowship during the tenure of which this work was performed. Both wish to acknowledge the facilities of the Department of Physics.