errors are so small and their values could not be known with precision, no attempt was made to correct for them.

By the procedure described in this paper it is possible in one experiment to estimate a sedimentation coefficient in the range 0.2 to 1 S to within 0.05 S of its true value. If three or more experiments are carried out and the results are averaged, one could rely on the value obtained to ± 0.03 S with some certainty. This corresponds to an error of 15% in a coefficient of 0.2 S and of 6% in one of 0.5 S.

Such data may be combined with diffusion data to obtain an estimate of the molecular weight of molecules of small and intermediate size such as are often isolated from biological sources. The error in this estimate would be nearly the same as that in the sedimentation coefficient, if the material were monodisperse. In the case of polydisperse or paucidisperse materials the estimate would be much poorer. In cases where such a molecule is known to be polymeric in character and the equivalent weight of the monomeric unit is known, the molecular weight of the polymer could be determined with sufficient accuracy to provide the degree of polymerization to a reasonable degree of certainty.

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MADISON, WISCONSIN

[CONTRIBUTION FROM THE BOTANICAL INSTITUTE, UNIVERSITY OF TOKYO, AND THE TOKUGAWA INSTITUTE FOR BIOLOGICAL RESEARCH]

Dielectric Properties of Hemoglobin. I. Studies at 1 Megacycle

BY SHIRO TAKASHIMA[1]

RECEIVED JULY 29, 1955

Dielectric properties of hemoglobin, oxyhemoglobin, carboxyhemoglobin and methemoglobin were studied by the resonance method using a frequency of 1 megacycle. A characteristic change in the dielectric increment was observed when hemoglobin was subjected to progressive oxygenation, and indeed it was found that at temperatures lower than 30°, the curve relating dielectric increment to oxygen partial pressure showed two distinct maxima. This fact indicates that in the process of oxygenation, there exist four steps having alternately increasing and decreasing effects on the dipole moment of the hemoglobin molecule. At 37°, however, the first maximum of the curve disappeared, thus giving rise to a single peak curve. Assuming that the consecutive steps of increasing and decreasing increment values correspond to the formation of intermediary compounds of progressing oxygenation, an O_2-saturation curve of the whole hemoglobin molecule was constructed based on the data obtained at 15°. It was found that the curve thus drawn coincided almost completely with the O_2-saturation curve which has been determined spectrophotometrically at the same temperature. These results confirm the fact that the four consecutive steps in the increment–O_2 curve correspond to the formation of intermediary compounds of different grade of oxygenation. The effect of dielectric constant of the medium upon the dielectric increment of the hemoglobin oxyhemoglobin system also was investigated.

I. Introduction

Although the dielectric properties of carboxyhemoglobin have been studied by Errera[2] and later by Oncley,[3] no investigation in the same line has ever been attempted for other derivatives of hemoglobin. The acid–base titration studies performed by German and Wyman[4] have shown that in the molecules of hemoglobin and oxyhemoglobin there are some ionizable groups closely linked to hemes. Since the states of these groups are not independent of the chemical state of hemes, we may suspect that the modifications in hemes brought about by their reaction with oxygen or carbon monoxide might possibly lead to a change in the charge distribution of the hemoglobin molecule. It may not be extravagant to speculate that such a change in the charge distribution would be detectable as a modification in the dipole moment of the whole hemoglobin molecule or in its behavior in dielectric dispersion. Thus, we attempted in this work to measure the effect of oxygen and carbon monoxide on the dielectric properties of hemoglobin.

One of the difficulties in measuring the dielectric constants of proteins in aqueous solutions is the elimination of electrolytic impurities which enhance the electric conductivity and render the dielectric measurement at low frequencies difficult or even impossible. It has been found[5-8] that protein solutions usually show anomalous dispersion at frequencies in the neighborhood of a few megacycles, below which the dielectric measurements must be carried out.[9] Therefore, a great deal of effort was made to remove the electrolytic impurities by repeating the purification until the conductivity of the solution was rendered negligible in

(1) School of Chemistry, University of Minnesota, Minneapolis, Minn.

(2) J. Errera, *J. chim. phys.*, **29**, 577 (1932).

(3) J. L. Oncley, THIS JOURNAL, **60**, 1115 (1938).

(4) B. German and J. Wyman, *J. Biol. Chem.*, **117**, 533 (1937).

(5) J. D. Ferry and J. L. Oncley, THIS JOURNAL, **60**, 1123 (1938).

(6) J. D. Ferry and J. L. Oncley, *ibid.*, **63**, 272 (1941).

(7) M. A. Elliott and J. W. Williams, *ibid.*, **61**, 718 (1939).

(8) H. O. Marcy and J. Wyman, *ibid.*, **64**, 638 (1942).

(9) The lower the frequency, the larger is the value of correction term for conductance.

its effect on the accuracy of the determination. Our experiment showed that the dielectric properties of hemoglobin are distinctly affected by the reaction of hemes with oxygen and carbon monoxide. Especially striking and characteristic was the change brought about by oxygenation, which will be dealt with as the main subject of the present paper.

II. Materials and Methods

Hemoglobin was prepared from horse blood cells. Crystallization was repeated two or three times, by adding alcohol to a saturated aqueous solution of hemoglobin at low temperature near 0°. Great care was taken to reduce the electric conductivity by repeating the washing of the crystals with cold water several times. The concentration of hemoglobin was determined either by the determination of nitrogen content using the Kjeldahl method or by weighing the protein after drying to constant weight. The crystals were kept in a refrigerator. Aqueous solutions were prepared using water which had been carefully purified with ion-exchange resins. The specific conductivity of the water was estimated to be as low as 1.0 μmho or less. pH of the solution was in the neighborhood of 6.8. Most of the measurements were carried out within a few hours after the preparation of the solution.

Dielectric measurement was performed, according to Nagakura,[10] by using a resonance type apparatus with a standard condenser of variable capacity between 30 and 1000 $\mu\mu$F., which could be regulated by means of a subdividing dial, with the accuracy of 0.1 $\mu\mu$F. The cell electrodes were concentric nickel cylinders spaced about 3 mm. apart and the cell volume was about 5 ml. with an electric capacity of 3.08 $\mu\mu$F.[11] In the case of oxyhemoglobin and carboxyhemoglobin, 5–6 ml. of oxyhemoglobin solution was placed in the upper cell (B) which was first evacuated and then equilibrated with O_2 and CO or a gas mixture of appropriate composition to attain the desired equilibrium. After spectroscopic observation indicated that the equilibrium had been established, the solution was introduced into the condenser which had been filled with the same gas or gas mixture as that in the upper cell. In the case of methemoglobin, the solution of oxyhemoglobin was treated with potassium ferricyanide, and after being dialyzed in an ice-box until the conductivity had been reduced low enough for the determination, was introduced into the condenser vessel.

In all experiments the frequency of the current applied was 1 megacycle, which, according to Oncley, is just below the margin of the maximum dispersion region for carboxyhemoglobin. The dipole moment (μ) may be calculated by the empirical formula

$$\mu^2 = \frac{9kT}{4\pi N} \times \frac{1000}{b} (\delta_0 - \delta_\infty) \qquad (1)$$

where δ_0 and δ_∞ are the dielectric increments at low and high frequencies, respectively, N the Avogadro number, T absolute temperature, K is the Boltzmann constant, and b is a constant (see Oncley[6]). Since in the case of hemoglobin derivatives, δ_∞ is of the order of -0.05 to -0.09 and δ_0 measured at 1 megacycle of the order of 0.25 to 1.4 (see below), we may approximate, in the following, the dielectric increment at 1 megacycle ($\delta_{1\text{ mc.}}$) as representing the relative magnitude of the dipole moment (μ) of the protein, according to the well-known relationship

$$\delta_{1\text{ mc.}} \approx \delta_0 - \delta_\infty = \frac{a\mu^2}{T} \qquad (2)$$

in which a is a constant. Justification for this approximation may be found in the fact (see below) that the value of dielectric increment of carboxyhemoglobin we have obtained at 1 megacycle coincided well with the value of δ_0 for the same substance determined by Oncley[3] in the same frequency range.

(10) S. Nagakura, *Rep. Inst. Sci. and Technol. Univ. Tokyo*, **4**, 9 (1950).

(11) This capacity is considerably smaller than that of similar apparatus used by other workers. Smallness of capacity was necessary in order to avoid the difficulties caused by high electric conductivity which is inherent to aqueous solutions.

III. Results

1. Dielectric Increments of Hemoglobin, Oxyhemoglobin, Carboxyhemoglobin and Methemoglobin.—The dielectric constants of reduced hemoglobin, oxyhemoglobin and methemoglobin are obtained as functions of their concentrations. In Table I are presented, as an example, some details of the experimental data obtained for oxyhemoglobin, and in Table II, the dielectric increment of hemoglobin and its derivatives. As may be seen, the dielectric increment of carboxyhemoglobin obtained by resonance method coincides well with the value obtained by Oncley[3] using bridge method.

TABLE I

MEASUREMENT OF DIELECTRIC PROPERTIES OF OXYHEMOGLOBIN (AT 15°, AND 1 MC.)

Concn.	Specific conductivity, μmho	Dielectric constant Obsd.	Dielectric constant Corrected[a]	Dielectric increment/g.
3.75	3.5	78.9	79.3	0.28
11.2	17.5	78.6	81.3	.28
12.0	16.0	79.8	82.3	.27
8.3	7.3	79.6	80.7	.26
11.2	11.0	79.8	81.5	.21
7.5	12.0	79.9	81.8	.27
11.2	11.0	79.8	81.5	.21
5.0	6.7	79.1	80.1	.21

[a] Correction (ϵ'') for conductivity was made according to the equation $\epsilon'' = \Delta(1/R)/2\pi f C_x$ where R is the resistance, C_x, the cell constant and f the frequency in megacycle. At 1 megacycle the correction for electrode polarization is practically negligible (see Oncley[2]).

TABLE II

AVERAGE VALUE OF THE DIELECTRIC INCREMENT FOR SEVERAL FORMS OF HORSE HEMOGLOBIN (AT pH 6.8, TEMP. 15°, FREQUENCY 1 MC.)

	Dielectric increment/g.	Molar dielectric increment[a]
Hemoglobin	0.61 ± 0.05	$5.1 \times 10^4 \pm 3400$
Oxyhemoglobin	0.25 ± 0.04	$1.5 \times 10^4 \pm 2700$
Carboxyhemoglobin	0.35[b] ± 0.015	$2.2 \times 10^4 \pm 1000$
Methemoglobin	0.58 ± 0.03	$3.7 \times 10^4 \pm 2040$

[a] The molecular weights of all substances were taken to be 68,000. [b] This value coincides with that obtained by Oncley.

2. Dielectric Increment of Horse Hemoglobin as it Changes with the Degree of Oxygenation.—Of special interest is the question as to whether or not and in what manner the dipole moment of hemoglobin will change with the degree of the oxygenation.

The dielectric increment of hemoglobin equilibrated with CO_2-free air at pressures ranging from 0.1 to 50 mm. was measured at various temperatures (15, 20, 25, 30 and 37°). The results obtained are presented in Figs. 1 and 2, in which the dielectric increment (per gram, measured at 1 mc.), is plotted against the oxygen partial pressure in mm. Quite astonishing was the fact that, except for the results obtained at the highest temperature (37°), the curves showed two maxima with a distinct minimum between them. The double peak nature of the curves suggests that in the process of oxygenation of hemoglobin, there are four steps having alternately increasing and decreasing

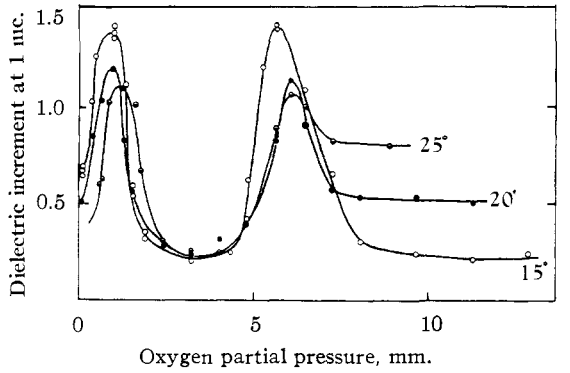

Fig. 1.—Dielectric increments of horse hemoglobin at various oxygen partial pressures (at 15, 20 and 25° and at 1 mc.).

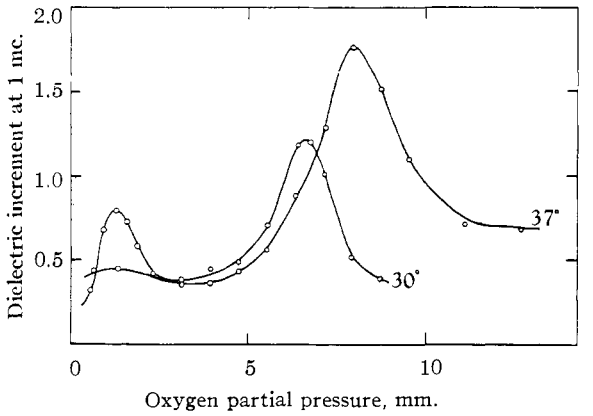

Fig. 2.—Dielectric increments of horse hemoglobin at various oxygen partial pressures (at 30 and 37° and at 1 mc.).

effects on the dipole moment of the protein molecule. Worthy of notice is the effect of temperature on these four steps, which may be summarized as follows.

(1) With the increase of temperature, the first hump of the curve is lowered until it disappears almost completely at 37°.

(2) With the increase of temperature, the second hump is lowered, except at 37° where it is again raised to a considerable degree.

(3) With the increase of temperature, both the first and the second maximum are shifted toward the direction of higher oxygen pressure.

(4) The last low level, which represents the stationary value at sufficiently high oxygen pressure, is the lowest at 15° and increases with increase of temperature (Fig. 1).

The double peak curve mentioned above is also effected by the dielectric constant of the solvent. In Fig. 3 are shown the data obtained with the hemoglobin solution, whose bulk dielectric constant was changed in the range between 78.5 to 70 by adding varying amounts of ethanol (up to 20%) to the solution. The following fact emerges from this experiment.

(5) With the decrease of dielectric constant of the solvent, the maxima of both the first and second humps are lowered and shifted toward the direction of higher oxygen pressure.

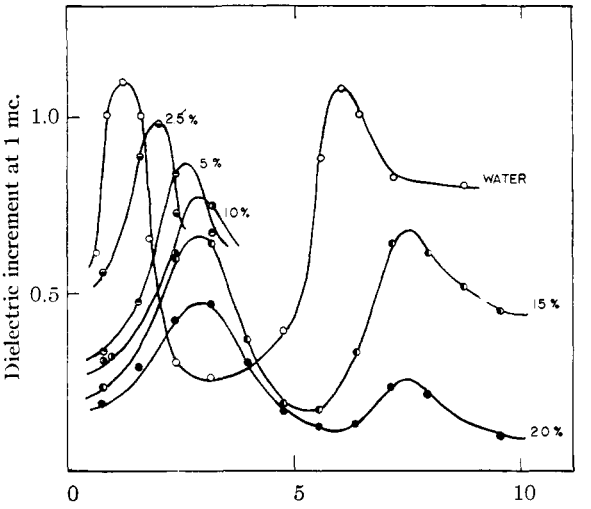

Fig. 3.—Dielectric increments of hemoglobin–oxyhemoglobin system as a function of the bulk dielectric constant of the medium (temp. 25°). The figure given to each curve indicates the percentage of ethanol added to the solution.

3. Analysis and Interpretation of the δ–O_2 Curves.—The fact that the δ–O_2 curve of hemoglobin is distinctly double-peaked suggests that the alternately ascending and descending phases of the curve may correspond to the formation of the four intermediary compounds, O_2Hb, O_4Hb, O_6Hb and O_8Hb, respectively. Using the data obtained at 15°, the relative increase or decrease of dielectric increment at each phase was estimated, and it was plotted against the logarithm of the oxygen partial pressure in Fig. 4. The four sigmoid curves obtained appear to show different grades of steep-

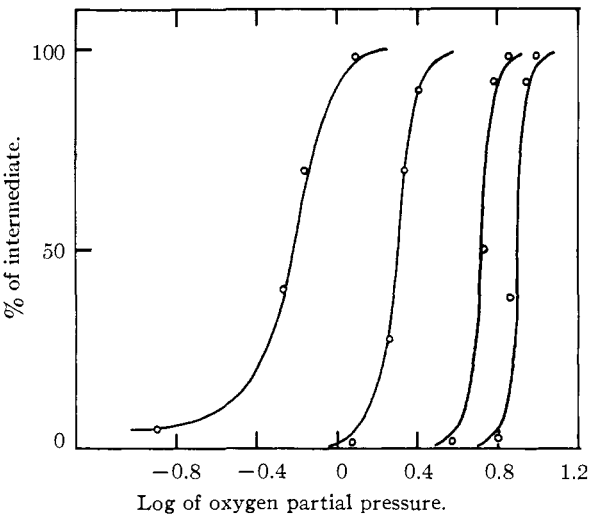

Fig. 4.—Relative increase and decrease of the dielectric increment (as function of oxygen partial pressure) at successive stages of oxygenation shown by the curve for 15° in Fig. 1. The ordinate shows the degree of intermediate "saturation" calculated on the assumption that each maximum or minimum of the curve in Fig. 4, represents the "saturation" point for each intermediate form of oxygenation.

ness which becomes more and more pronounced with the increase of oxygen partial pressure.

The general formula corresponding to sigmoid curves of such forms is

$$Y = \frac{[O_2]^n}{\phi^n + [O_2]^n}$$

where Y is the degree of oxygenation, and n and ϕ are constants. The constant ϕ represents the oxygen partial pressure corresponding to 50% oxygenation and the magnitude of n indicates the steepness of the curve. The values of ϕ and n for each curve in Fig. 4 were estimated to be approximately as follows

	ϕ	n
1st curve	−0.3	About 3
2nd curve	.27	About 5
3rd curve	.71	$\geqslant 6$
4th curve	.87	$\geqslant 6$

By linking the four sigmoid curves in Fig. 4 one after the other, we can construct a curve which may be regarded as an O_2-saturation curve, represented in terms of dielectric increment, for the whole hemoglobin molecule. Such a curve is shown in Fig. 5, in which the O_2-saturation curve of horse-hemoglobin determined spectrophotometrically[12] (at 28°) is also reproduced for comparison. The coincidence not only in the shape but also in the position of the two curves is evidence that the four consecutive steps we have observed in the δ–O_2 curve corresponds to the formation of intermediary compounds with different degree of oxygenation. The gradual increase of n-value with the progress of oxygenation might be interpreted as due to the attracting interaction between oxygen molecules on the hemoglobin molecule. Admitting that there occur interactions between oxygen molecules on the hemoglobin molecule, we have to infer that each maximum and minimum of the curve in Fig. 1 does not strictly correspond to a single intermediate compound (O_2Hb, O_4Hb, O_6Hb and O_8Hb, respectively) although these forms appear to provide

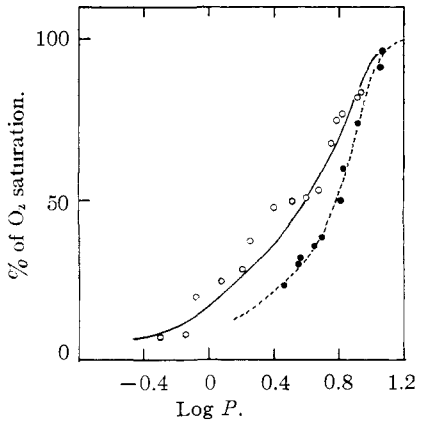

Fig. 5.—Coincidence of the data of dielectric measurement with those of spectrophotometric measurement with respect to the degree of O_2-saturation of hemoglobin as a function of oxygen partial pressure. The continuous line (with open circles) is the result of direct addition of the four shown in Fig. 5, while the dashed curve (with solid circles) represents the results of spectrophotometric measurement. Both sets of data were obtained at the same temperature, 15°.

the major fraction of the compounds existing at respective stages.

The shift of the double peak curve toward the direction of higher oxygen partial pressure, which occurred at higher temperatures or in the presence of increasing amount of ethanol in the solution, indicates the decrease of the affinity of oxygen for hemoglobin under these conditions. The fact that, at 20 and 25°, the final steady value of dielectric increment (see Fig. 4) did not attain the low level such as shown by the curve for 15° may be explained on the basis of Roughton's observation[12] that the fourth heme of hemoglobin cannot easily be oxygenated completely at higher temperatures. Attention should be called to the fact that at 37° the first hump in the δ–O_2 curve disappears, while the second hump becomes markedly higher than at lower temperatures. If we construct the O_2-saturation curve (similar to that shown in Fig. 5) on the basis of δ–O_2 curve at 37°, we obtain a curve which resembles the O_2-saturation curve of urea-denatured hemoglobin which was measured spectrophotometrically by Wyman and Ingalls.[13] As is well known, urea causes a splitting of hemoglobin molecule into two halves having two hemes each. The most plausible explanation for the single-peakedness of the δ-O_2-curve at 37° is, therefore, that the hemoglobin molecule had been split into two or more parts at that temperature.[14,15] Assuming that the half hemoglobin molecule ($Hb_{1/2}$) has a molecular weight of 34,000 the maximum molar dielectric increment observed at 37° is calculated to be 6.05×10^4 ($1.78 \times 34{,}000$). According to our assumption this is the δ-value shown, in the main, by the compound $O_2Hb_{1/2}$, whereas the two maxima observed at lower temperatures (Fig. 4) represent the δ-values shown in the main, by the compounds O_2Hb and O_6Hb, respectively.

The dependence of the first and second maximum δ-values upon the dielectric constant of the solvent is illustrated in Fig. 6. It is apparent that the δ-values of the first maximum decrease are approximately linear with respect to the decrease of the dielectric constant of the solution. Data are insufficient to allow any conclusion about the values for the second maximum. This finding may be important, for it will be recalled that the dielectric increments of simple amino acids and peptides are not influenced by the presence of organic solvent in the solution.[17]

Discussion

Various attempts have been made to elucidate the factors determining the measured dipole mo-

(12) W. H. Forbes and F. W. Roughton, *J. Physiol.*, **71**, 229 (1931).

(13) J. Wyman and E. N. Ingalls, *Advances in Protein Chem.*, Vol. 4 450 (1948).

(14) Recently Nagahisa, *et al.*,[16] observed that horse hemoglobin splits into two halves when it is dissolved for one to two days in low concentration in water solution at low ionic strength. Although we also used dilute hemoglobin solutions with low ionic strength, such a splitting may have been absent at low temperature in our experiments, in which the dielectric determinations were carried out within a few hours after the crystals were dissolved.

(15) Attempt was made to determine directly the δ–O_2 curve of hemoglobin in urea solution, but it proved impossible because of the high electric conductivity of the urea solution.

(16) M. Nagahisa, A. Hattori and A. Takamiya, THIS JOURNAL, **77**, 6173 (1955).

(17) J. Wyman, *ibid.*, **56**, 556 (1934).

ments of protein molecules. At low frequencies the measured quantities are undoubtedly due to some equilibrium state of unbalanced charge distribution plus small polarization contributions. At higher frequencies, approaching those of the dielectric dispersion region, the factors may be considerably more complicated as a result of the mobility of protons among acid and base groups of the protein. Thus Kirkwood and Shumaker[18] suggested that some part of dielectric dispersion is due to hydration rearrangements required as protons migrate from group to group under an applied field. Jacobsen[19] also has been led to believe that the connection between rotational relaxation and dielectric effects is not simple and direct. We thus lack a clear picture of the situation responsible for the dielectric constants measured in our experiments, but by confining ourselves to the classical picture of a rotational relaxation mechanism we may nevertheless draw certain conclusions.

Dissociation of hemoglobin into sub-units appears to be excluded as a source of the changes involved. One would have to postulate several such dissociations perhaps followed by associations of the sub-units, to explain the alternate increasing and decreasing values of dielectric increment on oxygenation. Nor is it easy to explain these changes as the result of increasing total charge on the protein. Assuming the longest dimension of hemoglobin, as determined from X-ray studies of the "solid," the maximum change in dielectric increment observed would require the production at the extreme ends of this dimension of 780×10^{-5} moles of each charge per gram protein, to renew the form with greater moment. The Bohr effect would produce a maximum of 3.0×10^{-5} mole of charge per gram in a total charge of more than 260×10^{-5} mole per gram. So far as is known, there are no other charge changes associated with oxygenation and furthermore the changes are monotonic with oxygenation. Consequently it must be concluded that the charge number alteration known as the Bohr effect cannot in itself be the source of the dielectric increment changes.

Since the dipole moment is determined not only by the number of charges available but also by their distribution, we must, on a classical picture, look for drastic rearrangement of charge on an unchanging protein supporting structure or a large change in structure with unchanging charge position on the elements of that structure or both. There is as yet little evidence to support a rearrangement of charge on a fixed surface during oxygenation but various investigators have produced evidence to suggest structural changes. Keilin[20] has reviewed much of this evidence and in doing so has supported the ideas of Wyman and Allen[21] and St. George and Pauling.[22] Wyman and Allen have suggested that the entropy decrease during the first step of oxygenation indicates a marked change in molecular

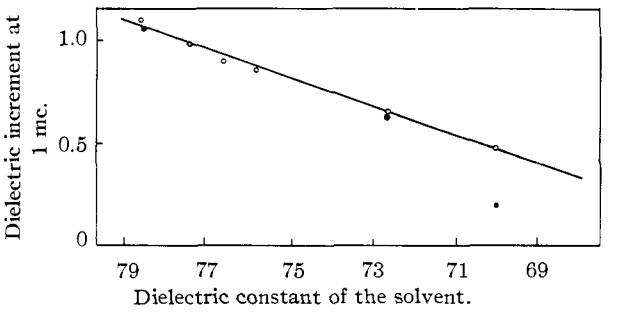

Fig. 6.—Plot of the values corresponding to the first and second maxima shown by the curves in Fig. 3 against the dielectric constant of the solvent. Explanation in text.

configuration. St. George and Pauling have investigated the possibility that the interaction among hemes may be due to changes in the relative positions of the tubes of alpha helix as oxygen is forced between them. Lumry and Eyring[23] have presented a picture in which an increase or decrease of the strain existing between heme and globin as oxygen is added triggers conformation changes in the globin portion of the molecule. In any event there appear to be significant alterations in charge geometry in addition to the changes in ionization of the groups which appear in the Bohr effect and this conclusion must be true no matter what the mechanism responsible for the observed dielectric increment changes. Our studies add additional evidence to suggest that biologically active proteins may undergo considerable reversible conformation change during their reaction.

Our deduction that the compounds O_2Hb and O_6Hb show appreciably higher dipole moment than O_4Hb and O_8Hb leads us to the inference that in their structures, the former are considerably more asymmetric than the latter. This is in contradiction either to Pauling's[24] square model of heme arrangement or to Wyman's[14] rectangular model of hemoglobin molecule, both of which assume asymmetric structures for O_4Hb in respect to the arrangement of oxygen molecules. If we assume for the hemoglobin molecule a rectangular form having two hemes each on both ends of its longitudinal axis, the the simplest picture to account for our observation may be that, in the compound O_4Hb, one heme each on the opposite sides is oxygenated to form a symmetric structure. As was discussed already, our observations also suggest the existence of strong interactions between oxygen molecules in their combination with hemes. The problem of interaction of oxygen molecules on hemoglobin has thus far been discussed only on the basis of Pauling's model, which turned out to be unsatisfactory in the light of our observations. Reinvestigation of this problem from an entirely new angle seems to be highly relevant.

It is a pleasure to the writer to acknowledge his indebtedness to Prof. H. Tamiya, under whose kind guidance this work was performed. The cost of this research has been defrayed from the scientific research expenditure of the Ministry of the

(18) J. G. Kirkwood and J. B. Shumaker, *Proc. U. S. Nat. Acad. Sci.*, **38**, 855 (1952).

(19) B. Jacobsen and M. Wenner, *Biochim. Biophys. Acta*, **13**, 577 (1954).

(20) D. Keilin, *Nature*, **171**, 922 (1953).

(21) J. Wyman and W. D. Allen, *J. Polymer Sci.*, **7**, No. 5, 449 (1951).

(22) R. C. C. St. George and L. Pauling, *Science*, **114**, 629 (1951).

(23) R. Lumry and H. Eyring, *J. Phys. Chem.*, **58**, 110 (1954).

(24) C. Coryell, L. Pauling and R. W. Dodson, *ibid.*, **43**, 825 (1939)

Education, to which thanks are due. It is a great pleasure to thank Dr. Rufus Lumry of the University of Minnesota, for his theoretical advice.

TOKYO, JAPAN

[CONTRIBUTION FROM THE SHELL DEVELOPMENT COMPANY]

Cross-sections of Molecules for Ionization by Electrons

BY J. W. OTVOS AND D. P. STEVENSON

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It is shown that for a wide variety of substances including hydrocarbons and their substitution derivatives, the total relative ionization cross-sections as measured in ion gages or mass spectrometers (low pressures and low energy electrons), or by total ion production in gases (at or near atmospheric pressure) by C^{14} or Sr^{90}–Y^{90} β-particles, are a constitutive molecular property. It is further shown that, particularly for ionization by single electron impact and to a lesser degree for total ion production by β-particles, the atomic ionization cross-sections of the elements are given by the sum of the valence electrons weighted by the mean square radii of those electrons as calculated for hydrogen-like wave functions for the electrons. A table of calculated atomic ionization cross-sections is given for elements with $1 \leq Z \leq 56$, and $Z = 80$, 81 and 82. The utility of the rules for ionization cross-sections in various analytical applications is described and illustrated.

Total cross-sections[1] of molecules for ionization by electrons form the basic calibration data for a variety of analytical instruments such as ionization manometers,[2] ionization chambers[3] and mass spectrometers.[4] Furthermore these quantities have the same basic significance for the initiation of reactions by ionizing radiation as do absorption coefficients for photo-chemical reaction initiation. Although considerable attention has been paid to the manner in which the ionization cross-sections of individual substances vary with the energy of the ionizing radiation,[1] relatively little is to be found in the literature concerning the variation of these cross-sections from substance to substance for a specific ionizing agent. The most detailed discussion of this latter question is that given by Dushman[2] for the variation of the sensitivity of ionization manometers, particularly the VG-1 Gauge, with the substance whose pressure is to be measured. Dushman's discussion is purely empirical, and the conclusion reached is that the best representation of the variation of ionization cross-sections (as measured by relative sensitivity of the VG-1 Gauge) is obtained by taking the cross-section to be linearly related to the total number of atomic or molecular electrons. The nature of Dushman's discussion of his correlation suggests that a theoretical result due to Bethe,[5] was inadvertently overlooked. This result of Bethe is that the ionization cross-section, Q^{i}_{nl}, of an atomic electron with quantum numbers, (n,l) is approximately proportional to the mean square radius of the electron shell, (n,l).[6]

This paper has the dual purpose of presenting evidence that the relative total ionization cross-sections of atoms are given to a good approximation by the weighted sum of the outer, or valence, electrons of the atoms, where the weights are the mean square radii of the electrons, and that the relative total ionization cross-sections of molecules are constitutive molecular properties, *i.e.*, the sum of the atomic cross-sections. The additivity rule of ionization cross-sections is shown to be equally valid for ionization by single electron impact by slow (50 to 100 volt) electrons, such as are employed in mass spectrometry, and for ionization in gases at atmospheric pressure by such energetic β-particles as those emitted by carbon-14 and strontium-90–yttrium-90. This may be considered to be an extension and generalization of the "Bragg Rule,"[7] based on observations of ion production by α-particles from naturally occurring α-emitters.

The paper is divided into three sections: in the first portion there is briefly described the method of calculation of relative atomic ionization cross-sections, and there are tabulated the results of calculations for fifty-nine elements. Comparison of calculated and observed atomic ionization cross-sections is presented. The second section is concerned with evidence of the additivity of ionization cross-sections in molecules with particular reference to mass spectrometric and low pressure ionization chamber data, and the applicability of the calculated (Section I) atomic cross-sections to the computations of molecular cross-sections. In the third section the evidence for the applicability of the additivity rule to ion production in gases at atmospheric pressure by carbon-14 and strontium-90–yttrium-90 β-particles is presented.

I. Relative Atomic Ionization Cross-sections.—For hydrogen-like wave functions the mean square radius of an electron, (n,l), is given by[8]

$$\bar{r}^2_{nl} = a_0^2\, n'^4 (Z - S_{nl})^{-2} - 1 + \frac{3}{2}\left(1 - \frac{l(l+1) - 1/3}{n'^2}\right) \quad (1)$$

where the effective quantum number, n', and the screening constant, S_{nl}, of Slater are computed according to the rules summarized by Eyring, Walter and Kimball.[9] There are given in Table I the mean square radii of the various electrons of the

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