[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING, UNIVERSITY OF CALIFORNIA, BERKELEY]

On the Interpretation of Dielectric Constants of Aqueous Macromolecular Solutions. Hydration of Macromolecules

BY BERTIL JACOBSON[1]

RECEIVED OCTOBER 22, 1953

Two preparations of sodium desoxyribonucleate were investigated with a streaming dielectric technique in which the macromolecules were hydrodynamically oriented in relation to the electric field. For one preparation the dielectric increment of the solutions was independent of the velocity gradient. For the other preparation, which was highly thixotropic, a small decrease in dielectric constant was observed when the electric field was perpendicular to the stream lines. In a second series of experiments the dependence of the dielectric constant upon frequency was determined in mixtures of water and thoroughly washed filter paper. In a mixture containing approximately 20% filter paper the dielectric constant decreased with increasing frequency from 109 at 0.1 Mc. to 98 at 10 Mc. The value for pure water is 80.3 and that for dry filter paper is below 7 at these frequencies. The macromolecular rotation theory for explaining the dielectric properties of macromolecular solutions is evaluated in view of existing experimental data. The theory is found to be invalid for some asymmetric polyelectrolytes which give high dielectric increments, and it is very likely incorrect for the proteins. Other existing theories cannot satisfactorily explain certain streaming dielectric effects, that is a change in dielectric properties with increasing velocity gradient in the solution. Therefore, a new hypothesis is suggested according to which the dielectric properties arise from a structural change in the water lattice. Through hydrogen bonding the macromolecular surface stabilizes the surrounding water to form lattice-ordered (ice-like) hydration shells. In certain cases these shells can have a diameter of at least one fourth of the length of an asymmetric macromolecule. The lattice order in the hydration shells is greater close to the macromolecular surface than it is farther away from it. The hypothesis can give a satisfactory qualitative explanation for experimental dielectric observations mentioned, as well as for certain other data on the viscosity, proton magnetic resonance and X-ray diffraction of macromolecular solutions.

Introduction

Dielectric measurements have been widely used to calculate axial ratios, degrees of hydration and molecular weights of macromolecules.[2,3] To make possible such calculations the assumption is generally made that the dielectric properties arise from rotations of whole macromolecules in the electric field. The purpose of the present work has been to investigate the validity of this assumption. Since it appears that it does not hold, a new hypothesis is suggested to explain the physical significance of dielectric properties of macromolecular solutions.

The following material discusses experimental data published previously by various investigators as well as two new experiments which appear to be of importance for an understanding of dielectric processes in aqueous solutions. The first experi-

ment is a streaming dielectric study of sodium desoxyribonucleate. In these experiments the dielectric properties are measured when the macromolecules are hydrodynamically oriented in relation to the direction of the electric field. In the second experiment the dielectric constant has been determined at various frequencies for a two-phase system containing cellulose and water. This experiment was undertaken in order to extend Meek's[4] data which indicated that such mixtures probably have higher dielectric constants than has pure water.

Several abbreviations are used: DNA = sodium salt of desoxyribonucleic acid (thymonucleate), DNP = desoxyribonucleoprotein (nucleohistone), PPB = poly-4-vinyl-N-n-butylpyridinium bromide, CMC = sodium salt of carboxymethylcellulose and HUA = hyaluronic acid. Since these substances have many properties in common and all give high dielectric increments at low frequencies, they are referred to as "high increment polyelectrolytes" in order to distinguish them from the pure proteins.

(1) Rockefeller Fellow. Permanent address: Karolinska Institutet, Stockholm, Sweden, where the experimental part of this investigation was carried out. Paper presented at the 124th Meeting of the American Chemical Society, Chicago, Illinois, September 6–11, 1953.

(2) J. L. Oncley in Cohn and Edsall, "Proteins, Amino Acids and Peptides," Reinhold Pub. Co., New York, N. Y., 1943, p. 543.

(3) G. Jungner, I. Jungner and L. G. Allgén, Nature, 163, 849 (1949).

(4) G. A. Meek, Thesis, Imperial College, University of London, England, 1950, p. 97.

Experimental Part

Streaming Dielectric Data on DNA.—Two preparations of DNA from calf thymus were studied. Preparation A was prepared according to the method of Hammarsten[5] and contained 7.00% P and 11.99% N. Preparation B, isolated by the method of Gulland, Jordan and Threlfall,[6] contained 7.52% P and 12.65% N. The moisture content (after equilibration in an atmosphere of 40% relative humidity at 20°) was 20.2 and 31.1% for preparations A and B, respectively. The moisture content was obtained from the loss of weight when samples were dried to constant weight over P_2O_5 at 90°. The viscosity of dilute salt-free solutions was studied with three Ostwald viscometers of different physical dimensions. Preparation A showed only a low degree of thixotropy[7] whereas preparation B was highly thixotropic. In a 0.01% (w./w.) solution the relative viscosities were 1.5 and 2.2 for preparations A and B, respectively, when measured at 20° in a viscometer having a mean gradient of 370 sec.$^{-1}$. Both preparations showed a strong streaming birefringence when observed in a capillary between two crossed Nicol prisms.

Solutions of the DNA preparations were dialyzed three times at 4° against redistilled water before examining in the streaming dielectric apparatus previously described.[8] The dielectric constant and the conductance were measured at 20° in the frequency range 0.1 to 7 Mc. and at concentrations between 0.01 and 0.12% (w./w.). During measurements the solutions were subject to velocity gradients from 0 to 10,000 sec.$^{-1}$. The electric field was perpendicular to the stream lines in the solutions.

No streaming dielectric effect, that is no change of dielectric constant or conductance with the velocity gradient, could be observed for any solution of preparation A. A small streaming dielectric effect was observed for solutions of preparation B as can be seen in Fig. 1. Both the dielectric constant and the conductance decreased slightly with increasing velocity gradient at frequencies below 1 Mc. At high velocity gradients a saturation value was approached. The difference (ΔI) between the dielectric constant at zero velocity gradient and the saturation value will be called the maximum streaming dielectric effect. This property (ΔI) decreases with decreasing concentration (g). However, the maximum streaming dielectric effect per concentration ($\Delta I/g$) increases with decreasing concentration as shown in Fig. 2.
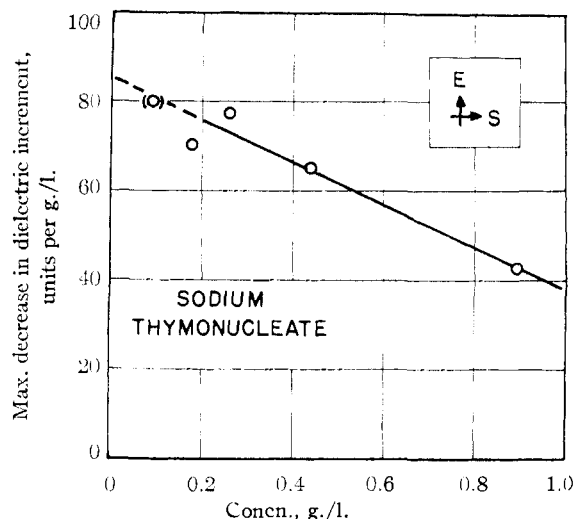
Fig. 2.—Maximum streaming dielectric effect (difference in dielectric constant at 0 and 10,000 sec.$^{-1}$) for DNA, calculated per unit concentration, plotted as a function of the concentration; temperature 20°, frequency 0.2 Mc., electric field perpendicular to stream lines; preparation B.

The streaming dielectric results for DNA closely resemble those for HUA which have been studied previously.[9]

Dielectric Constant of Cellulose-Water Mixture.—Filter paper of type "Munktell No. 3" (a comparatively soft and porous paper intended for average laboratory needs) was washed continuously for four days in flowing redistilled water. The dielectric constant of a mixture of water and filter paper was determined in the following way. The paper was tamped down into a silver cup which formed the outer grounded electrode of the measuring cell. The flat inner electrode was then fastened in place so that the paper was pressed toward the bottom of the silver cup. The cell was then filled with redistilled water in such a manner that no air was left in it or in the paper. The capacitance of the cell was determined at 20° and at frequencies between 0.1 and 10 Mc. Without removing the inner electrode or changing its position the filter paper was removed through a hole in the bottom of the cup. The capacitance, in the same frequency range as before, was then determined for the cell filled with water and with solutions of glycine and of urea having known dielectric constants. From plots of capacitance versus dielectric constant for each frequency studied, the dielectric constants were determined for the filter paper–water mixture. The estimated accuracy of the dielectric constants obtained was ±0.2 unit. The accuracy in the determination of the concentration of filter paper used in the experiments was low due to the geometry of the measuring cell. However, this is of minor importance in the present study since the main interest is in the values for the dielectric constants at various frequencies relative to the corresponding values for pure water.

As can be seen in Fig. 3 the mixture of filter paper and water has dielectric constant values greater than both that for water (80.3) and those for dry filter paper which were determined in a similar way and found to be below 7 units in the frequency range studied. From the frequency dependence of the dielectric constant it can be seen that the mixture
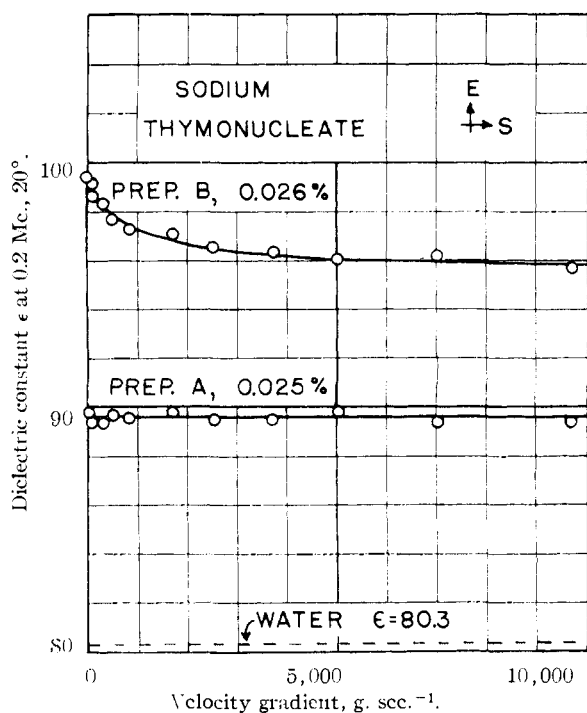
Fig. 1.—Typical streaming dielectric results for two preparations of DNA when electric field was perpendicular to stream lines.

(5) E. Hammarsten, *Biochem. Z.*, **144**, 383 (1924). The sample was received as a gift from Prof. Hammarsten.

(6) J. M. Gulland, D. O. Jordan and C. J. Threlfall, *J. Chem. Soc.*, 1129 (1947). The sample was received as a gift from Dr. Allgén, Stockholm.

(7) This term is used here to indicate that the viscosity was a function of the rate of shear, the time of shear and the time of rest. However, in many cases a true gel with a yield value was never formed in dilute solutions. *Cf.* Pryce-Jones, *Kolloid Z.*, **129**, 96 (1952).

(8) B. Jacobson, *Rev. Sci. Instru.*, **24**, 949 (1953).

(9) B. Jacobson and T. C. Laurent, *J. Colloid Sci.*, **9**, 36 (1954).

gives a dispersion with a broad distribution of relaxation times. Further washing of the paper was found not to remove or change the increment observed.

Evaluation of Macromolecular Rotation Theory

If it is assumed that the dielectric properties arise from rotations of the macromolecules in the electric field, their relaxation times can be calculated from the dielectric dispersion, and the molecular dimensions can then often be estimated.[2] In general it is possible to combine the dielectric data with values for the molecular weights, determined by sedimentation analysis, to give reasonable values for the molecular shape. In this procedure the molecules are arbitrarily assumed to rotate either about the long or the short axis depending upon which gives the best fit with both dielectric and molecular weight data. Now the equations relating molecular shape and relaxation times are such that even a small change in axial ratio corresponds to a large change in relaxation times. Because of this, it is relatively simple to obtain agreement between dielectric data and sedimentation data by adjusting slightly the assumed values for the axial ratio. The agreement obtained[2] therefore, cannot necessarily be taken as a proof for the validity of the theory of rotation of whole macromolecules.

Greater emphasis should, instead, be placed upon cases in which the dielectric data are inconsistent with molecular weight values. Thus it is of interest to observe that for insulin the dielectrically determined relaxation time is too small by a factor of about two, to be explained on the basis of asymmetry of a molecule of 35,000 molecular weight.[10] Therefore, it has been assumed that the rotating unit has a weight of 12,000.[11] In order to investigate these problems further a study was made of Helix pomatia hemocyanin.[12] This protein is especially suitable for testing dielectric theories since its molecular dimensions are accurately known from other methods of investigation. The most favorable relaxation time calculated from known dimensions disagreed by a factor of more than 30 from that obtained from the dielectric dispersion. These results cannot be reconciled with a theory attributing the dielectric properties of protein solutions to rotations of whole macromolecules in the electric field.

DNA[3,8,13] and DNP[14] are probably the substances that have been most carefully investigated by dielectric methods. These highly asymmetric macromolecules have similar dielectric properties. Owing to the large asymmetry, the rotation of the molecules must be assumed to take place about the long axis in order to be consistent with the dispersion in the megacycle range. A rotation of the long axis would give a relaxation time of a completely wrong order of magnitude. This simplifies the treatment of the experimental results and actually

(10) E. J. Cohn, J. D. Ferry, J. J. Livingood and M. H. Blanchard, *Science*, **90**, 183 (1939).

(11) J. L. Oncley, E. Ellenbogen, D. Gitlin and F. R. N. Gurd, *J. Phys. Chem.*, **56**, 85 (1952).

(12) B. Jacobson and M. Wenner, *Biochim. Biophys. Acta*, **13**, 577 (1954).

(13) (a) G. Jungner, *Acta Physiol. Scand.*, **10**, Suppl., 32 (1945); (b) I. Jungner, *ibid.*, **20**, Suppl., 69 (1950).

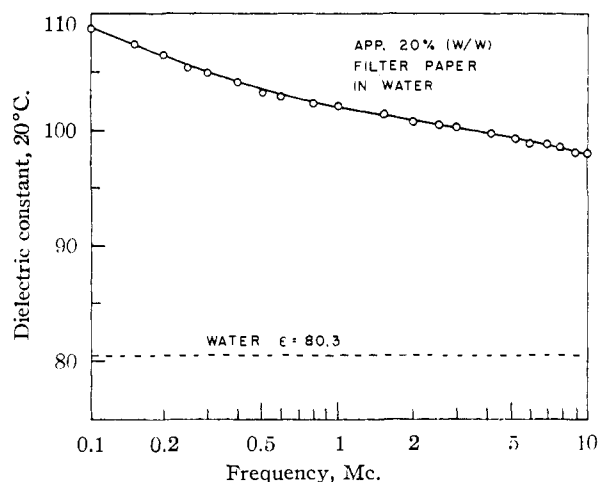(14) L. G. Allgén, *ibid.*, **22**, Suppl., 76 (1950).

Fig. 3.—Dielectric constant of a mixture of filter paper in water, plotted as a function of frequency.

makes possible a calculation of molecular weight from the frequency of the dielectric dispersion. The molecular weights obtained, however, do not agree with those obtained by other methods. DNA is the substance most carefully studied with different methods, and recent investigations with light scattering, electron microscopy and viscosimetry[15] have given a molecular weight of 4.5 million. The weight was mainly independent of the ionic strength in spite of the fact that the length of the molecules decreased by 30 to 40% on increasing the electrolyte concentration to 0.02 N. The highest molecular weight obtained from dielectric dispersion data was about 600,000[13b] and it decreased to about 30,000 on increasing the electrolyte concentration to $10^{-3} N$. These discrepancies between the results obtained from dielectric data and other mutually agreeing results from several other methods also cast doubt upon the correctness of the assumption that the dielectric properties arise from macromolecular rotations.

The streaming dielectric results are also of interest in order to evaluate the macromolecular rotation theory. If the increment of DNA were due to a rotation of the asymmetric molecules about the long axis, which is the only possible type of rotation causing a dispersion in the megacycle range, the dielectric increment should increase when the molecules are oriented perpendicular to the electric field. This is illustrated in Fig. 4. At random distribution the dipole vector component in the plane perpendicular to the electric field (molecule c has its whole dipole moment in this plane) cannot contribute to the dielectric constant through a rotation about the long axis. When the molecules instead are hydrodynamically oriented with their long axis perpendicular to the electric field this is no longer the case, and the increment should be larger than at random distribution. No such increase in increment was ever observed with increasing velocity gradient in the experiments described above, and it must, therefore, be concluded that the dielectric increment of these asymmetric polyelectrolytes

(15) J. W. Rowen, M. Eden and H. Kahler, *Biochim. Biophys. Acta* **10**, 89 (1953); J. W. Rowen, *ibid.*, **10**, 391 (1953).

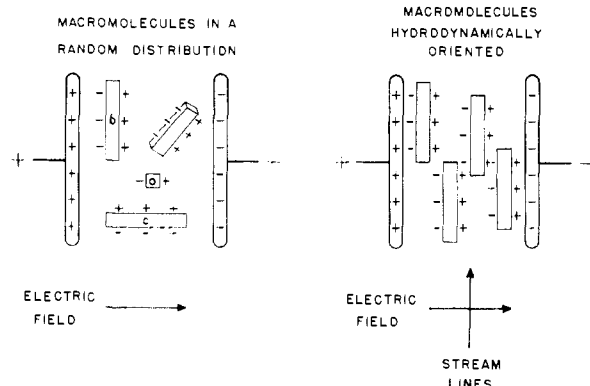cannot be due to a rotation of the whole macromolecules in the electric field.

Fig. 4.—Schematic diagram of electric field and molecular orientation in the streaming dielectric cell.

Conclusion.—Considering these various sets of experimental data it would appear that the macromolecular rotation theory has serious disadvantages and is certainly incorrect in the case of some asymmetric "high increment polyelectrolytes" and very likely incorrect in the case of the proteins. From this it would follow that molecular weights, axial ratios and degrees of hydration cannot be calculated from dielectric data according to the methods previously employed.[2,3]

Other Theories

Three other theories need be mentioned here, since all three involve mechanisms that might be able to contribute, at least to some extent, to observed experimental effects.

Internal Rotation of Molecular Groups.—At the low field strengths (1 to 10 volt/cm.) generally used in the measurement of dielectric properties no complete orientation of the dipoles occurs. On the contrary only very small displacements take place.[16] Therefore, the possibility exists that the dielectric properties might arise from orientations of charged groups or from displacements of internal dipoles within the macromolecules. Since this was found to be a possible mechanism for low molecular weight substances,[17] it cannot be excluded in the case of macromolecular compounds. However, the contribution to the increment is presumably small and should not be much larger in a macromolecule than in the corresponding monomer, which is often the case. Thus DNA[13b] gives increments per g./l., $I/g = 250$, whereas the mononucleotides hitherto investigated[13a] give increments of less than $I/g = 5$, both values calculated from low concentration data.

Through mechanical resonance mechanisms the effect might be larger at some frequencies than at others.[18]

Mobile Proton Theory.—It has been suggested by Kirkwood and Shumaker[19] that fluctuation of

(16) H. Frölich, "Theory of Dielectrics," Clarendon Press, Oxford, England, 1949, pp. 20, 137.

(17) E. Fischer, Z. Naturforschg., 4a, 707 (1949).

(18) I. Hausser, Sitzber. Heidelberg. Akad. Wiss., Math.-Naturw. Klasse, No. 6 (1935); B. Jacobson, Arkiv Mat. Astron. Fysik, 34A, No. 25 (1947).

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

the dipole moment due to mobile proton distribution is of importance for the dielectric increment of macromolecular solutions. The theory is not yet fully developed as far as the dielectric dispersion is concerned and, therefore, it is not possible to decide which dispersion data can be explained by it. It is to be expected, however, that according to this theory the dielectric increment should be less sensitive to molecular orientations than according to the macromolecular rotation theory. This agrees with some of the results obtained from streaming dielectric experiments.

Maxwell–Wagner Theory.—Since this paper was originally submitted for publication Dintzis, Oncley and Fuoss[20] have reported that the Maxwell–Wagner theory can explain dielectric dispersion data of some polyelectrolytes. Through the action of charged groups, ions are concentrated close to the surface of the macromolecule. As a result, the solution contains regions of high and low conductivity which can cause the dielectric properties. Dintzis, et al., assume that the theory does not apply to the proteins since they have different dielectric properties than the polyelectrolytes which give high dielectric increments. However, it is not clear why a charged group on a protein molecule should behave differently from one on a "polyelectrolyte" in its power to attract an ion and to produce regions of varying conductivity. Furthermore, this theory seems to be of somewhat limited applicability. Thus it is difficult to understand from it why a two-phase system such as cellulose–water (and wool–water[4]) gives a large dielectric increment even though there are very few charged groups on the fibers.

None of these three theories can explain satisfactorily why certain preparations of some polyelectrolytes, such as DNA and HUA,[9] show a streaming dielectric effect, whereas other preparations do not give this effect. Such differences exist even though the molecules in both cases are almost completely oriented as indicated by streaming birefringence experiments. Therefore, another hypothesis is suggested here, founded on an interaction process with the water lattice. A similar mechanism has recently been discussed in order to explain the rheological properties of clay systems.[21]

Water Structure Hypothesis

Basic Principle.—The fundamental principle in the hypothesis is that certain physical properties of aqueous systems are caused partly or mainly by a structural change in the water lattice rather than by particular properties of the non-aqueous component itself. The structural change in the water is due to hydrogen bonding between the four-coordinated water lattice and the hydrogen bonding atoms on the surface of the non-aqueous component, be it a soluble macromolecule or the insoluble part of a two-phase system. The coupling can be more or less specific depending on the degree of structural similarity between the four-coördinated ideal water lattice and the stabilizing surface. Through this

(20) H. M. Dintzis, J. L. Oncley and R. M. Fuoss, Proc. Nat. Acad. Sci. U. S., 40, 62 (1954).

(21) E. Forslind, "Proc. Ind. Internat. Congr. Rheology," Oxford, 1953, p. 50.

coupling to the surface the thermal vibrations in the lattice are reduced, even far from the interphase, leading to a greater order in the water lattice. This involves a change in the physical properties of the whole system qualitatively similar to the changes obtained in the properties of pure water when the temperature is decreased.

Originally this hypothesis was developed to explain the dielectric properties of macromolecular solutions, as has been reported in preliminary communications.[8,9,22] However, viscosity and osmotic pressure data can also be explained on the same basis.[22] Furthermore, proton magnetic resonance[23] and X-ray diffraction[24] data of DNA solutions have recently contributed strong support to the hypothesis. Actually, the latter two experimental techniques have definitely shown that the water structure is markedly affected by the presence of certain macromolecular compounds. This justifies a detailed analysis of the applicability of the hypothesis to dielectric processes even if it cannot be treated quantitatively at the present time.

The applicability of the hypothesis to dielectric processes is made plausible by certain experimental observations on two-phase aqueous systems. Several investigators have observed recently that the dielectric constant of the aqueous phase is apparently determined by the nature of the non-aqueous phase as well as by the physical dimensions of the water layers. Thus Palmer, Cunliffe and Hough[25] have found that water films, 2 to 5 μ thick, separated by mica plates, have dielectric constants between 10 and 20 depending on the thickness. The experimental results reported here on cellulose–water mixtures cannot easily be explained without assuming that the dielectric constant of the water phase has changed. Meek[4] has found similar results on wool–water and cellulose–water systems.

These changes in the dielectric constant of the aqueous phase are explained by the assumption of a structural change in the water lattice. With increasing order in the four-coördinated lattice the low frequency dielectric constant increases, as is evident from the fact that ice has a higher dielectric constant than water.[26] Furthermore, the dielectric constant of ice increases rapidly with decreasing temperature as is seen in Fig. 5. The frequency of the dielectric dispersion of ice and water is also highly dependent on the structural order, as will be described below. The effect of the solids in the two-phase systems discussed can be described by saying that they reduce "the structural temperature" of the aqueous phase.

In order to treat these dielectric processes quantitatively one must know the exact nature of the dielectric processes in ice and water. Unfortunately, this is far from being known. Many theoretical explanations have been suggested, and recent investigators have diverging opinions about such a fundamental question as whether the orientations
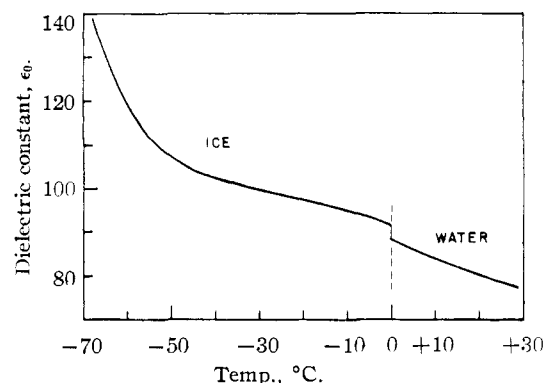
(22) B. Jacobson, Nature, 172, 666 (1953).

(23) B. Jacobson, W. A. Anderson and J. T. Arnold, ibid., 173, 772 (1954).

(24) B. Jacobson, Acta Chem. Scand., in press.

(25) L. S. Palmer, A. Cunliffe and J. M. Hough, Nature, 170, 796 (1952).

(26) R. P. Auty and R. H. Cole, J. Chem. Phys., 20, 1309 (1952).

Fig. 5.—Dielectric constant of ice and water.

of the dipoles depend on a rotation of the water molecules,[27] or on proton displacements,[28] or on a bending of the bonds in the water lattice.[29] The Forslind theory of water will be used here since it is based on a simple model and since it has been applied to studies of different properties such as dielectrics, viscosity and thermodynamical entities. This makes it of more general interest than certain other theories which might have been used instead, such as that of Pople.[29]

According to Forslind,[28] who has enlarged upon some of the ideas of Bernal and Fowler,[30] of Pauling[31] and of others, water consists of a mixture of a four-coördinated lattice with Frenkel defects and of lone interstitial water molecules placed in the open spaces in the lattice. The number of Frenkel defects and of lone interstitial molecules depends on the temperature. During the melting process the number of interstitial molecules becomes greater than the lattice defects, which explains the increase in density. Thermodynamic data can be treated as a function of the proportion of lone interstitial molecules and that of lattice defects.[28]

Interaction between Macromolecules and Water.—In Fig. 6A an illustration is given of the water lattice at room temperature showing the pseudo-crystalline order with the thermal vibrations, the Frenkel defects and the lone interstitial molecules. When a macromolecule is dissolved in water, the lattice can be stabilized to different extents. If the macromolecule has many oxygen and nitrogen atoms on the surface in such positions that they fit into the ideal water lattice, a very pronounced ordering effect is obtained and results in an almost ideal four-coördinated structure. This is seen in Fig. 6B where the coupling between the macromolecular surface and the water lattice has greatly reduced the thermal vibrations, especially close to the macromolecular surface, resulting in fewer Frenkel defects; that is, a "lattice-ordered" hydration shell has been formed.

On the other hand, the hydrogen bonding atoms on the macromolecular surface can be in such positions that the bonding with the water lattice is hindered. In this case, no ordering effect is ob-

(27) G. Haggis, J. B. Hasted and T. J. Buchanan, ibid., 20, 1452 (1952).

(28) E. Forslind, Acta Polytechnica, 3, No. 5 (1952).

(29) J. A. Pople, Proc. Roy. Soc. (London), A205, 163 (1951).

(30) J. D. Bernal and R. H. Fowler, J. Chem. Phys., 1, 515 (1933).

(31) L. Pauling, THIS JOURNAL, 57, 2680 (1935).

WATER MOLECULES IN LATTICE   LONE INTERSTITAL WATER MOLECULE   F = FRENKEL LATTICE DEFECT
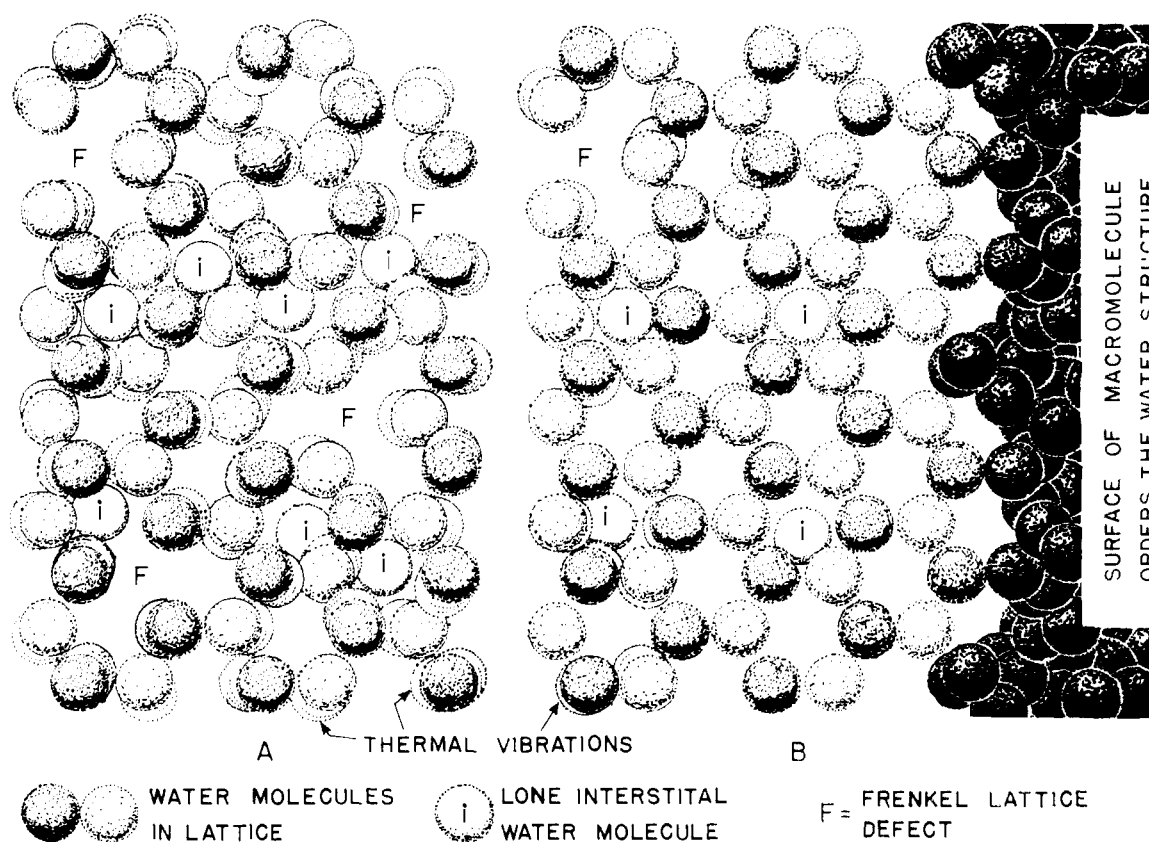
Fig. 6.—A, water structure at room temperature; B, stabilization of water lattice by a macromolecular surface through hydrogen bonding.

tained and the water lattice is unchanged or even more disordered.

In actual cases, the fit between the macromolecular surface and the water lattice is seldom as perfect as has been indicated in Fig. 6. The bonding with the macromolecule could involve every second or third one, or even fewer, of the nearest water molecules and still produce an increased regularity in the lattice.

The power of a particular substance to order the water lattice depends on two factors. First, the actual fit is of importance as just described. Secondly, the absolute size of each macromolecule is of importance in determining the capacity to order a large volume of water far from the interaction surface. Per unit volume of substance a highly asymmetric molecule has a much larger effect than one which is globular and an elongated molecule has a greater effect than a flattened one. Flexibility of the macromolecules might be of less importance since a mutual stabilization can occur between a flexible chain and the "crystallized" water if the fit otherwise is good. Actually, the water structure could be of importance for, and might even determine, the shape of macromolecules in solution through such a mutual interaction.

Previously,[9,12,22] the term "ice-like" has been used for what is generally called "lattice-ordered" in this paper. The former term has caused some confusion as to the nature of the hydration shells. The shells are ice-like in the sense that the number of lattice defects are fewer than the number in pure water. But the density and the other thermodynamic properties of the hydration shells resemble the properties of water more than those of ice. Therefore, the term lattice-ordered is preferable.

Many experimental results indicate that some macromolecules mutually disturb the formation of hydration shells of each other. This might arise from a saturation effect, in the sense that all the available water is tied up, and/or from a disability of different hydration shells to combine into one crystalline structure.

According to this hypothesis, the solubility of macromolecular compounds should be determined partly by the fit of the molecular surface into the water lattice. If the number of hydrogen bonds that can be formed between the macromolecular surface and the water lattice is few compared to the number of bonds between the macromolecules, the substance is likely to be insoluble (e.g., insoluble starch). If the fit is slightly better or fairly good (soluble starch, proteins) solutions can be formed, even of very high concentrations. If the fit is very good (DNA[22]) the substance is miscible with water in all proportions, though the immobilization of large amounts of water in the hydration shells (gel formation) might make the dissolving process very slow. The viscosity is high in the latter type of solution and comparatively low in the former type.

Applicability of Hypothesis

The following is a discussion of the specific appli-

cability of the hypothesis for the interpretation of dielectric processes in macromolecular solutions.

Low Frequency Increment.—Many macromolecular substances give a low frequency dielectric increment. The increment per g./l. varies from 0.1 to 2.0 for proteins[2] to 10 to 2000 for the "high increment polyelectrolytes."[13b,14,20] The increment is usually larger for viscous substances (DNA, DNP, HUA, CMC, PPB) which tend to form gels in dilute solutions. This agrees with the hypothesis since the formation of large hydration shells should produce a high viscosity[21] as well as a large increment. The "high increment polyelectrolytes" are highly asymmetric macromolecules, which agrees with the fact that large extended interaction surfaces facilitate the formation of large lattice-ordered hydration shells.

For the "high increment polyelectrolytes" (DNA,[13a,13b] DNP,[14] PPB,[20] CMC[32]) the increment per g./l. is constant only at very low concentrations, and then decreases with increasing concentration. This happens when the concentration has reached a value where the hydration shells interfere with each other's formation. The non-linearity should be more pronounced the larger the increment per g./l. and this is roughly true. Similar non-linearities exist with other properties such as proton magnetic resonance.

From known molecular dimensions for DNA[15] (length 6000 Å., diameter 20 Å.) and from the fact that the mutual interference between the hydration shells occurs at concentrations of less than 0.05%, it can be estimated that the stabilizing action on the water in this case can extend some 500 to 1000 Å. from the surface of the macromolecules. That is, the diameter of the hydration shells is approximately one fourth of the length of the macromolecules. Of course, at the greater distances the lattice order is only very slightly greater than that in pure water.

It has been observed for DNP[14] and hemocyanin[12] that freeze-dried preparations give higher increments and more viscous solutions than samples prepared in other ways. This fact suggests that during the freezing a slight denaturation occurs so that the structure of the macromolecular surface changes into one having a better fit with the ice–water lattice, and on redissolving larger hydration shells are formed than before. This would explain both the high viscosity and the high dielectric increment.

There is often a considerable effect of time on the dielectric properties of solutions of the "high increment polyelectrolytes."[13b,14] These time effects cannot all be due simply to denaturation or depolymerization, since the effects are strictly reproducible on a sample after it has been precipitated and redissolved. Instead, it is reasonable to assume that such time effects are caused by a slow crystallization or breakdown of the lattice-ordered hydration shells.

Dielectric Dispersion.—Water has a dielectric dispersion at 10^{10} cycles/sec. and ice at 10^2 to 10^4 cycles/sec. depending on the temperature, as shown in Fig. 7. A lattice-order hydration shell should

(32) L. G. Allgén and S. Roswall, J. Polymer Sci., **12**, 229 (1954).

have a dispersion in the frequency range between that of ice and that of water. All macromolecules which give an increment have one dispersion between 10^5 and 10^7 cycles/sec. which would be reasonable values for dispersions in lattice ordered hydration shells.
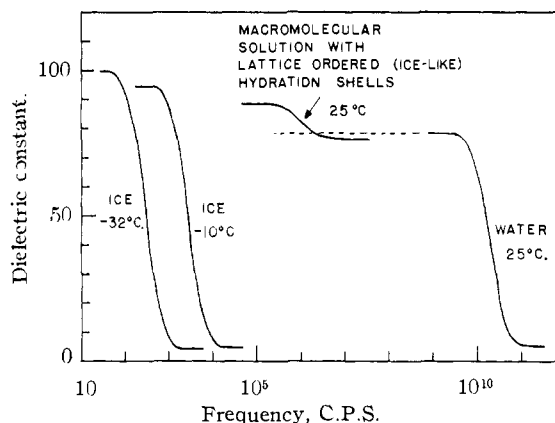

Fig. 7.—Dielectric dispersions in ice, water and a macromolecular solution.

A broad distribution of relaxation times could result either from hydration shells of varying size and order, due to polydispersity of the substance, or from regions of varying lattice order in large hydration shells, depending on the distance from the interaction surface. This can explain why several dispersions or broad distributions are observed in many cases.

The critical frequency for the "high increment polyelectrolytes" increases with increasing concentration.[13b,14,20,32] Thus, for instance, in one preparation of DNA[13b] the critical frequency shifted from 0.3 to 0.9 Mc. upon increasing the concentration from 0.03 to 0.25 g./l. This behavior is explained by the assumption that with increasing concentration the size and the lattice order of the hydration shells decreases due to their mutual disturbing interaction.

It has been pointed out[13b,14] that for different preparations of the same substance (DNA, DNP) a linear relationship exists between the dielectric increment and the "molecular weight" calculated from dielectric dispersion data. However, molecular weights cannot be calculated from dielectric data, as discussed above. Therefore, what has actually been shown experimentally is that for those preparations of a substance which give high increments per g./l. the critical frequency is generally lower than for those with a lower increment. This fact is to be expected from the hypothesis, since large hydration shells give larger increments and lower critical frequencies than do smaller hydration shells. The difference in the various preparations, is, therefore, probably a difference in hydration due to varying surface structure of the macromolecules. More profound differences, such as varying molecular size of the preparations, can also give the same correlation between increment and critical frequency.

High Frequency Dielectric Constant.—At frequencies above the dielectric dispersion region the

proteins give a decrement of 0.06 to 0.1 unit per g./ l. which is larger, by a factor of two, than the decrement calculated from the water volume displaced by the protein.[2] This behavior is to be expected, since the lattice-ordered structure should have a lower dielectric constant than water at frequencies above the dispersion range.

This is so, however, only if small hydration shells are formed, and the main part of the water structure remains unchanged, with a normal and uniform dielectric constant. If a large part of the water structure is affected, as is the case with the "high increment polyelectrolytes," areas of varying structure, depending on the distance from the macromolecular surface, complicate the situation. Thus, even though a highly ordered water structure close to the interaction surface would have a low dielectric constant at frequencies above its dispersion, other areas with another degree of lattice order might have a dielectric constant greater than that of normal water. The over-all dielectric constant might, therefore, be larger than that of water. The fact that cellulose–water mixtures show a broad distribution of relaxation times and that even at 50 Mc. the mixtures have a larger dielectric constant than water,[4] indicates that this is a plausible mechanism. This is probably the explanation for the fact that the "high increment polyelectrolytes" give dielectric increments at frequencies above their dispersion in the megacycle region (DNA,[13b] DNP,[14] CMC[32]).

Streaming Dielectric Effects.—For hemocyanin,[12] CMC[33] and certain preparations of DNA and HUA the dielectric increment is independent of the velocity gradient when the electric field is perpendicular to the stream lines. This is in agreement with the hypothesis, since it is probable that the dielectric constant is approximately the same in all directions of the lattice-ordered hydration shells.[34]

The frequency of the dispersion is also independent of the velocity gradient, which would also be expected.

However, certain other preparations of DNA and HUA[9] show a small decrease in dielectric constant with increasing velocity gradient. This can be explained by assuming that in these solutions, which were all highly viscous and highly thixotropic, exceedingly large hydration shells are broken down through the action of the velocity gradient. As a matter of fact, this also explains why there is a correlation between streaming dielectric effect and thixotropy, which itself can be caused by the same breakdown of shells.

This explanation for the streaming dielectric effect is further supported by the fact that the maximum streaming dielectric effect per concentration $(\Delta I/g)$ increases with dilution. The larger the hydration shells are (at high dilution), the more sensitive they should be to the disturbing action of the velocity gradient. With increasing concentration the shells become smaller and less sensitive to hydrodynamical forces.

A less likely explanation for the streaming dielectric effect is that it arises from dielectric constants which differ in the directions of the a-axes and the c-axis[34] of an oriented water lattice or which differ in the various directions of oriented macromolecules.[35] The experimental observation that the dielectric increment increases with the velocity gradient when the electric field is mainly parallel with the stream lines[8,9] points toward such an explanation. However, no great emphasis can be placed on these results, since the electric field conditions are not ideal in the cell used in this type of experiment, and the whole effect might, therefore, be due to experimental error.

Concluding Remarks.—The water structure hypothesis proposed, according to which the dielectric properties of macromolecular systems should arise from the formation of lattice-ordered hydration shells, can, on a qualitative basis, explain satisfactorily the majority of experimental observations. The hypothesis is strongly supported by data on proton magnetic resonance[23] and on X-ray diffraction,[24] which have shown that macromolecules actually can change the structure of water.

The water structure hypothesis is not unreasonable from a thermodynamic point of view. This is because the dielectric properties are mainly dependent on the long range order in the water lattice, whereas thermodynamic properties such as heat of solution and density are dependent principally on the proportion of lone interstitial water molecules relative to the proportion of lattice defects.[28] Therefore, large hydration shells can be formed without large heats of reaction.

Certain macromolecules order the water lattice to a more regular four-coördinated structure, while the electrolytes[36] are known to produce a decreased order in the lattice. This antagonism could be of biological importance in the maintenance of the proper degree of water lattice order in living cells and tissues.

BERKELEY, CALIFORNIA

(33) B. Jacobson and L. G. Allgén, unpublished data.

(34) J. G. Powles, J. Chem. Phys., 20, 1302 (1952).

(35) D. A. G. Bruggeman, Ann. Physik, 24, 636 (1935); A. Vöri, J. Phys. Colloid Chem., 51, 1037 (1947); 53, 597 (1949).

(36) G. W. Stewart, J. Chem. Phys., 7, 869 (1939).