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On the Interpretation of Dielectric Results on Sodium Thymonucleate. I. On the Relations Between Increment, Dipole Moment and Molecular Weight

GUNNAR JUNGNER and INGMAR JUNGNER

Biochemical Department, Karolinska Institutet, Stockholm, Sweden

In recent years, dielectric measurements have become of increasing importance for the elucidation of the structure and mode of action of various substances. Special difficulties are, however, encountered in applying dielectric methods to physiologically important substances that are often poorly soluble in non-polar media. These difficulties are mainly of two kinds. Firstly, these substances often possess too high an electric conductivity that necessitates the use of special methods for the determination of the dielectric constant (DC). Secondly, the interpretation of the results is made more difficult by the fact that no generally applicable theory can be put forward that gives an absolute measure of the polarity of the molecules for solutions in polar solvents.

The object of the present and subsequent studies in this series is to correlate the dielectric results obtained on sodium thymonucleate, in the following denoted as DNA, with the conceptions obtained by other methods. This is all the more necessary since the structure of DNA, although very simple in principle, is of a very individual nature. Except for the nucleic acids it does not appear to be closely analogous to any other substances. The dipolar structure of DNA differs very considerably from that assumed for molecules in dielectric theories, owing to a pronounced molecular asymmetry with moment-bearing groups (in combination with electrolytic properties) superficially located. We shall, however, confine ourselves to a few simple aspects that appear to be of interest for the understanding of DNA in aqueous solution.

In our work on polynucleotides in this laboratory, we have chosen the ellipsoid method of R. Fürth[1]. We refer to our other publications[2,3] for the choice of method, the technique of measurement as well as the results. The method is a force method and was elaborated for use at a number of varying frequencies in the 0.03—38 Mc/s range. In principle, the method is fairly independent of the electric conductivity of the solution and its application to strongly conducting solutions is mainly limited by the heat convections. In some experiments a special technique has been used on solutions with relatively high electric conductivity [4].

The majority of studies were made on DNA from calf thymus prepared according to Hammarsten's procedure [5]. It is the best-defined thymonucleic acid preparation available, although it is not altogether ideal owing to some low protein content. Nevertheless, other specimens, produced with different methods and of varying origin, all proved to have the same dielectric regularity. Thus, the empirical relations found should be generally applicable to thymonucleic acid.

THE DIELECTRIC INCREMENT OF DNA

There is some contrast between the difficulties of calculating the dipole moment *in vacuo* from measurements in an aqueous solution and the simplicity and regularity often found in dielectric measurements. There is often very good agreement between various substances in a similar group of substances. The systematic study of those groups that can be varied in a definite way, for example by polymerization, was therefore found to be a suitable procedure for the investigation of the relation between the dielectric properties and the molecular structure, etc. The most appropriate investigations appear to be those made by J. Wyman Jr. and co-workers on amino acids and peptides. The conformity found has been of fundamental importance for all dielectric research, in particular for substances in aqueous solution. It is beyond the scope of the present paper to survey the results obtained by Wyman's school. We refer instead to the monograph "Proteins, Amino Acids and Peptides" by Cohn and Edsall (1943).

In aqueous solution, DNA shows an extremely high increment [2,3,6]. The increment is very dependent on the concentration and even in solutions of less than a few hundredths per cent, it deviates from linearity. Different preparations behave differently in this respect, and in extremely dilute solutions an increment is obtained, characteristic for the degree of polymerization of the specimen of DNA. We explain the deviation from linearity as an "association" in the general meaning of the term. This will be discussed further in a subsequent paper in the present series.

Table 1 gives some figures both for the increment per gram per litre and for the molar increment. Some of the results obtained by Wyman and coworkers are included for comparison. This shows, *inter alia*, the interesting relations between the increment and the molecular weight.

Table 1.

	$M = n \cdot M_0$	n	I_g	δ	μ_K	$\mu_K \cdot n^{-x}$	μ_D	$\mu_D \cdot n^{-x}$
Amino acids and peptides				Temp. 25° C		$x = ½$		$x = ½$
Glycine	75	1	0.30	22.6	15.7	15.7	0.61	0.61
Di-glycine	132	2	0.53	70.6	28	19.7	0.91	0.64
Tri-glycine	189	3	0.60	113	32	20.2	1.04	0.60
Tetra-glycine	246	4	0.65	159	43	21.5	1.08	0.54
Penta-glycine	303	5	0.71	215	48	21.4	1.13	0.50
Hexa-glycine	360	6	0.65	234	50	20.4	1.14	0.47
Hepta-glycine	417	7	0.70	290	56	21.2	1.17	0.44
β-alanine	89	(2)	0.39	34.6	19.4	13.5	0.64	0.45
γ-aminobutyric acid	103	(3)	0.50	51	23.8	11.9	0.83	0.48
δ-aminovaleric acid	117	(4)	0.54	63	26.2	13.1	0.88	0.44
ε-aminocaproic acid	131	(5)	0.59	77	29.0	13.0	0.94	0.42
ς-aminoheptylic acid	145	(6)	0.60	87	30.1	12.5	0.97	0.39
Desoxyribo-nucleate				Temp. 20° C		$x = 1$		$x = 1$
DNA, from thymus[1]								
in $1.5 \cdot 10^{-3}$ N KCl	31 000	94	9.2	$2.9 \cdot 10^5$	1 750	18.7	76	0.81
in $8 \cdot 10^{-4}$ N NaCl	53 000	160	17.4	$9.2 \cdot 10^5$	3 150	19.7	137	0.85
in $4 \cdot 10^{-4}$ N NaCl	81 000	245	27	$2.2 \cdot 10^6$	4 850	19.8	205	0.84
in pure water	120 000	360	45	$5.3 \cdot 10^6$	7 600	21.3	315	0.88
in pure water	180 000	545	70	$1.3 \cdot 10^7$	11 700	21.6	495	0.90
DNA, from salmon[2]								
in pure water	390 000	1 200	160	$6.2 \cdot 10^7$	26 000	22.0	1 100	0.92
in pure water	540 000	1 600	210	$1.1 \cdot 10^8$	35 000	21.5	1 500	0.92
DNA, from thymus[3]								
in pure water	610 000	1 850	240	$1.5 \cdot 10^8$	40 000	21.4	1 700	0.92
DNA, from thymus[4]								
in pure water	840 000	2 500	350	$2.9 \cdot 10^8$	56 000	22.3	2 400	0.94

[1] Prepared according to Hammarsten (1924).

[2] Prepared according to Mirsky and Pollister (1942).

[3] Prepared according to Gulland, Jordan and Threlfall (1947).

[4] Recent measurements by Allgén (unpublished) for a specimen prepared by Signer and Schwander (1949) in 0.0025% aqueous solution.

The δ-values for amino acids and peptides are taken from Cohn and Edsall's review and refer to Wyman's measurements. The calculations and measurements on DNA are published by I. Jungner[3].

M is the molecular weight, M_0 the weight of the "monomer", n is the degree of polymerization (or number of carbon atoms between the charged groups). I_g is the dielectric increment per gram per litre (Edsall's $\Delta D_0/g$) and δ the molar increment. μ_K and μ_D are the dipole moments calculated according to Kirkwood ($\mu_K = 3.3 \cdot \sqrt{\delta}$) and Debye respectively.

For glycine peptides, Wyman found that the molar increment (δ) is proportional to the number (n_p) of the constituent amino acids. For the different monoaminomonocarboxylic acids (AA) the molar increment is proportional to the number of carbon atoms (m_{CH_2}) between the momentbearing groups. This gives the following equations

$$\delta_p = const. \cdot n_p \qquad \delta_{AA} = const. \cdot m_{CH_2} \tag{1a, 1b}$$

i.e.

$$\delta_p = const. \cdot M \tag{2}$$

As a result, a solution of a certain concentration in weight/volume of peptides gives approximately the same increment irrespective of whether the amino acids are coupled as peptides or not, *i.e.* the increment per gram per litre (I_g) is constant. It must be pointed out that, in the case of the peptides, the increment was calculated from the DC of the solvent, whereas for DNA it was computed according to $\frac{(\varepsilon_l - \varepsilon_h)}{c}$ where ε_l and ε_h are the DC at respectively lower and higher frequencies than the actual dispersion range at the concentration c. Extrapolation to zero concentration according, for example, to Hedestrand[7] was not made, since the measurements can be made in such dilute solutions that the difference can be disregarded.

The relation given by Wyman between the increment and the molecular weight in peptides appears to be fairly generally applicable. Approximately the same order of magnitude for the increment is also obtained for proteins with considerable molecular weights and very appreciable differences in geometric shape. It would be tempting to draw conclusions from this fact in respect of the length of the chain of polypeptides in a protein molecule. Nevertheless, this connexion is presumably considerably more complicated than implied by the apparent simplicity and, in any event, more difficult to interpret than in the case of DNA.

The conditions for DNA are in contrast to these data obtained for analogous amino acids and peptides. For the former we find

$$I_{DNA} \simeq const \cdot M \tag{3}$$

For DNA, the increment per gram per litre is thus a direct function of the molecular weight. This can be utilized in studying, for example, enzymatic degradation[3,8] and is in good agreement with the fact that the dielectric increment per gram per litre follows the increment of viscosity.

It is difficult to establish the connexion experimentally down to very low molecular weights. A solution of lowmolecular nucleotides has a very considerable electric conductivity which appreciably limits the possibility of obtaining exact values. Moreover, with a decreasing degree of polymerization, the increment becomes very low. On the other hand, the relation is established in a large molecular weight range from approximately 30 000 to 800 000. The regularity of the dielectric increment definitely implies a corresponding regularity in the structure which presumably applies to the whole DNA molecule.

There is found the following formula for the molar increment of DNA

$$\delta_{\mathrm{DNA}} \cong const\ M^2 \tag{4}$$

THE DIPOLE MOMENT OF DNA

Debye's theory and computation of permanent dipole moments, founded on the equation of Clausius and Mosotti for the relation of molecular polarization, is actually only valid for gases but possibly applicable to cubic crystals as well. Experience has nevertheless shown that it can also be used for solutions of permanent dipoles in non-polar media. On the other hand, calculations with Debye's formula do not give the correct order of magnitude for the dipole moment in a polar solvent and particularly not for such a strongly polar solvent as water.

The best-known of the attempts to extend the applicability of Debye's theory is that of Onsager[9]. The pre-requisites for Onsager's theory, *i.e.* a spherical molecule with a centrally located dipole moment, are not fulfilled by DNA, but the conception of a dielectric reaction field must be borne in mind. The theory has been of considerable importance for the interpretation of the empirical relation for the dielectric increment. It has found a widespread use, for example, in the study of proteins. Certain of the conclusions drawn from the theory will therefore be discussed in the following.

Wyman interprets his results obtained on amino acids and peptides to imply that a simple relation also exists between the dipole moment and the increment. This is also apparent to some extent from Debye's theory in the expression for the dipole moment, μ_D, (T = the absolute temperature, c = the weight fraction, d = the density, k = Boltzmann's constant, N = Avogadro's number):

$$\mu_D^2 = \frac{9 \cdot k \cdot T \cdot M}{4 \cdot \pi \cdot N \cdot c \cdot d} \left(\frac{\varepsilon_l - 1}{\varepsilon_l + 2} - \frac{\varepsilon_h - 1}{\varepsilon_h + 2} \right) \quad \text{e.s.u.} \tag{5 a}$$

$$\mu_D = 0.0128 \cdot 10^{-18} \cdot \sqrt{\frac{M \cdot T \cdot 3\ (\varepsilon_l - \varepsilon_h)}{c \cdot d \cdot (\varepsilon_l + 2)\ (\varepsilon_h + 2)}} \quad \text{e.s.u.} \tag{5 b}$$

in which, as a good approximation, we can insert

$$\mu_D \cong const \cdot \sqrt{\frac{M \cdot (\varepsilon_l - \varepsilon_h)}{c}} \tag{6}$$

As pointed out in the foregoing, the increment per gram per litre (I_g) for DNA is proportional to the molecular weight. From it we find the important relationship

$$\mu_{\mathrm{DNA}} \cong const. \cdot M \tag{7}$$

In comparison with this, Wyman's result implies that

$$\mu_{\mathrm{peptide}} \cong const. \cdot M^{\frac{1}{2}} \tag{8}$$

Under ideal conditions, the dipole moment can be determined in absolute units. Since, according to the definition, $\mu = e \cdot l$, this is an expression both of the size of the charge (e) and of the dipole length (l), *i.e.* the distance between the centroids of the positive charges and the corresponding negative charges. The mutual relationship between e and l cannot be deduced from the dipole moment. Whereas a polar solvent can be expected to have an effect on the charge that is hard to determine, a simple relation between the dipole length and the dipole moment can be anticipated. If μ_s denotes the dipole moment calculated according to Debye for a substance in a polar solvent and e_{eff} denotes the effective charge, and, correspondingly, l_{eff} the effective dipole length, we can write as a generalization

$$\mu_s = l_{\mathrm{eff}} \cdot e_{\mathrm{eff}} < \mu_{\mathrm{v\,ac}} \tag{9}$$

where μ_{vac} denotes the dipole moment valid for the vacuum.

For analogous substances we can assume that e_{eff} is influenced in a uniform way by a given solvent. The relation found by Wyman[10] for peptides thus implies that the dipole length of a polypeptide is not the sum of the dipole lengths of the constituent amino acids. Nor will the distance between charges of the different monoamino-monocarboxylic acids be the greatest possible (*i.e.* as in an extended chain). Nor is this to be anticipated, since an attraction between opposite poles must be taken into consideration. The relations given by W. Kuhn[11] and Eyring[12] are attempts to find a measure of the effective dipole length on a statistical basis or from theoretical computations. We shall not enter into these relations which are not of importance for DNA, but only note that they show good agreement with empirical data.

It is shown in Fig. 1 how the effective dipole lengths arise in principle and the conditions are compared in dipoles placed side-by-side and end-to-end. Since the dipole length of a polymerized molecule is proportional to the molecular weight, the relation between the dipole moment and the molecular weight in polymeric substances can be expressed generally as

$$\mu = a \cdot M^{x} \tag{10}$$

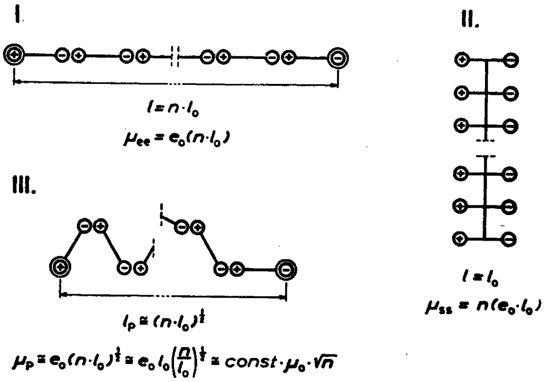

Fig 1. Dipole length in different kinds of polymerization

- *I. End-to-end with a completely extended chain*
- *II. Side-by-side (as in DNA)*
- *III. End-to-end with a folded chain (as in peptides).*

where a is a constant and the exponent x can vary between 0 and 1. $x = 0$ then corresponds to a ring formation in which the partial dipole moments cancel each other, whereas $x = 1$ corresponds to a fully stretched chain of dipoles or one in which the dipoles are linked side-by-side polarly. For peptides and different monoamino-monocarboxylic acids, $x \simeq 1/2$.

There is no reason to assume that the exponent for amino acids and peptides should be altogether constant at different dipole lengths. A deviation can be noted for both low and high glycine peptides[13]. It is interesting to note that the exponent for DNA lies in the immediate vicinity of 1. The exponent falls under the influence of various agents but only a small deviation can be noted on variation of the molecular weight by means of minimal quantities of salts.

From a dielectric aspect, it is irrelevant whether a dipole moment *in vacuo* is composed of an entirely straight chain of similar dipoles (end-to-end) or whether the dipoles lie side-by-side. The exponent x equals 1 in both cases. To sum up, it can thus be noted that, *as regards DNA, the calculations of the dipole moments show that the theoretical extreme case is present with the dipoles parallel and in the same direction.*

It must be pointed out that the formula (10) for the generalized relation between the dipole moment and the molecular weight corresponds to that given by Staudinger and co-workers [14] for the viscosity of long chain molecules. Dielectric measurements show on the whole many conditions that are analogous to the viscosity. The dependence of the increment (and thus also of the dipole moment) on the concentration appears to be of particular interest. As Huggins and co-workers [15] pointed out in respect to viscosity, a formula such as that given is usually an expression of whether the molecule is coiled into a ball-shape or not. By analogy, the formula for the dipole moment would then mainly be an expression of the effective dipole length.

The simple conditions of the dipole length in DNA indicate a possibility of ascertaining the effect of the solvent on the charges in the individual dipoles. For glycine, a dipole moment of 0.6 *D* (Debye units) in an aqueous solution is found by a calculation according to Debye. This is only a fraction of the dipole moment of approximately 15 *D* that could be established by Kirkwood [16] both from the structural formula and experimentally, although indirectly.

From the results obtained for DNA, we get a dipole moment of approximately 0.9 *D* per average mononucleotide. If the conditions were the same as for glycine, the individual average mononucleotide would have a dipole length of only approximately 4 Å. There is, however, a considerable difference between the placing of the charges in DNA and, for example, a polypeptide. In DNA, all the moment-bearing groups are in more or less intimate contact with the solvent, whereas in the polypeptide only the extreme charges can be affected by it. The fact that the dipole moment of DNA is relatively small in comparison with, for example, glycine, although the effect of the solvent should be relatively greater indicates that it should be possible, as a generalization, to express the effect of the solvent as a decrease in the charge (and the dipole moment). We can assume that the function for this effect includes a dependence both on the dipole moment of the solute (μ_1) and of the solvent (μ_2), as well as on the effect of the Na ions (α_{Na}), the intensity of the outer field and other factors. If, on the basis of this simple assumption, we compare the dipole moment μ_{ss} of substances with dipoles arranged side-by-side and μ_{ee} of dipole molecules arranged end-to-end, we find that

$$\mu_{ss} = n \cdot \mu_0 - n \cdot f\,(\mu_1 \cdot \mu_2 \cdot \alpha_{\mathrm{Na}}) = n\mu_0 - n\Delta\mu \qquad (11)$$

$$\mu_{ee} = n\mu_0 - \Delta\mu \qquad (12)$$

When $\mu_{ss} < \mu_{ee}$, it is explicable on the basis of this conception that the dipole moment computed according to Debye for DNA will be relatively low even in comparison to other dipoles in aqueous solution.

In this connexion, we can mention attempts to determine the dipole moment *in vacuo* from determinations on aqueous solutions according to other methods of calculation. Wyman's formula for molecular polarization, P,

$$P = \frac{M}{d} \cdot \frac{\varepsilon + 1}{g} \qquad \mathrm{cm}^3 \qquad (13)$$

has been of great importance. The factor g is obtained empirically. It results in the following expression for the dipole moment (μ_w) of a substance in a solution

$$\mu_W = const \cdot \sqrt{\frac{M \cdot T}{c \cdot d} \cdot \frac{\varepsilon_l - \varepsilon_h}{g}} \qquad (14)$$

This empirical relation has been borne out by Onsager's theory. The value of the factor g must, however, be determined. Wyman, on the basis of a large number of substances, found 8.5 to be the most useful mean figure, although with considerable variations. The relatively low figure of 5.8 was obtained for amino acids (Oncley[17]).

A prerequisite for the calculation according to Wyman is an approximate knowledge of the dipole moment *in vacuo* of some substance within the intended group. A value for g obtained in this way can then be used for similar substances. The true dipole moment for DNA can only be estimated very roughly and with a number of assumptions from the structural formula for the mononucleotides. Provided that the mononucleotide can be interpreted as a simple dipole ion with a charge that does not differ essentially from the elementary charge, the moment will depend on the length of the dipole. From the structural formula, the distance from the oxygen atoms in phosphoric acid to, for example, N_3 can be estimated as approximately 10 Å. The distance does not presumably fall below 7—8 Å nor exceed 16 Å. Under these conditions — which thus require a number of assumptions — the dipole moment can be estimated as between 35 D and 80 D with a value of approximately 50 D as the most probable.

If these values are tested in Wyman's formula, very low values are obtained for g, 50 D corresponding to approximately 2. Such a figure deviates very considerably from all others and gives reason for much doubt. If, in order to obtain an idea of the physical implication of such a value of g, the value for the moment *in vacuo* is tested according to Onsager's theory, the "internal refractive index" is found to be less than 1. The test shows that the assumptions are not valid. Since a mononucleotide presumably has a high dipole moment of the stated order of magnitude, the testing indicates that DNA — as could be anticipated — does not correspond to the prerequisites for Onsager's theory.

Kirkwood[16] in a general statistical theory of dielectric polarization, extended Onsager's theory. He then used a dipole moment, μ_K, which also includes the effect of a moment induced by the adjacent molecules. The theory leads approximately to the expression

$$\mu_K = 3.30 \cdot \sqrt{\delta} \qquad \text{e.s.u.} \tag{15}$$

This gives very good agreement with the values of, for example, peptides. A similar dipole moment as with Wyman's calculation is, however, obtained for DNA. Other empirical relations, for example according to Cohn[18] also give the same low figures of the order of magnitude of 20 D. For the present we refrain, in accordance with the views put forward by Arrhenius[19], from attempts to calculate a dipole moment that is correct as regards the order of magnitude as given by Wyman, Onsager and others and confine ourselves to the calculation according to Debye. The results of the different theories only differ essentially in the attempts to obtain absolute values for the dipole moment. The calculation according to Debye is serviceable for obtaining *relative* values.

THE DIELECTRIC METHOD FOR THE DETERMINATION OF THE MOLECULAR WEIGHT

The molecular weights used for DNA were calculated, as were the dipole moments, from the dielectric dispersion curve. In principle, the way in which the molecular weights were obtained is of no importance. As, however, the measuring technique is thus common to both the values compared, there is reason to discuss the extent to which the calculations are mutually dependent.

There is obviously some uncertainty when it is necessary to deviate from the simple principles for the computation of molecular weights. In the case of amino acids and known polypeptides, these are obtained with great accuracy from their chemical structure. The accuracy in these cases depends mainly on how well defined the preparation is. In the case of DNA, it is a question of obtaining — in addition to the determination of the polarity — as exact a value as possible for the molecular weight with the use of a cumbersome method. The dielectric method for the determination of molecular weight of DNA is based on the following formulae

$$M = \frac{N \cdot k \cdot T \cdot \lambda_c}{3 \cdot v \cdot \eta \cdot 2\pi \bar{c} \cdot \psi_2} \cdot \frac{\varepsilon_h + 2}{\varepsilon_l + 2} = const. \frac{\lambda_c (\varepsilon_h + 2)}{(\varepsilon_l + 2)} \tag{16}$$

(N = Avogadro's number, $6.02 \cdot 10^{23}$, k = Boltzmann's constant, $1.38 \cdot 10^{-16}$ ergs per molecule, $\bar{c}$ = velocity of light, $3 \cdot 10^{10}$ cm/sec., ψ_2 = Perrins' shape-factor, 4/3, η = viscosity of water, 0.01 Poise, v = specific volume of DNA = 0.50 cm^3/g).

The random errors in the determination thus derive essentially from the error in λ_c since the variations in the expression $\left(\frac{\varepsilon_h + 2}{\varepsilon_l + 2}\right)$ are of minor importance.

From equation (5) we can derive

$$\mu = const. \cdot \sqrt{\frac{M \cdot (\varepsilon_l - \varepsilon_h)}{(\varepsilon_h + 2)(\varepsilon + 2)}} \tag{17}$$

for the dipole moment. The insertion of the expression for the molecular weight from the dielectric data then gives

$$\mu = const.\, \sqrt{\frac{\lambda_c \cdot (\varepsilon_l - \varepsilon_h)}{(\varepsilon_l + 2)^2}} \cong const.\, \sqrt{\lambda_c \cdot I} \tag{18}$$

It is then evident that the dielectric molecular weight mainly depends on λ_c, *i.e.* the position of the dispersion curve along the axis of frequency, whereas the dipole moment is dependent on $\sqrt{\lambda_c \cdot I}$. Thus, two different conditions are compared. The characteristic of DNA is the expression $\frac{\mu}{n}$, as is seen from the following

$$\mu = const.\, \sqrt{M \cdot I} = const.\, \sqrt{n \cdot I} \tag{19}$$

$$\frac{\mu}{n} = \bar{\mu}_N = const.\, \sqrt{\frac{I}{n}} \tag{20}$$

On the basis of this computation, the determination of the dipole moment per average mononucleotide ($\bar{\mu}_N$) is made, this being a very good characteristic of the polarity of the molecule as a kind of "specific" polarity.

It must be pointed out in this connexion that the dielectric method for the determination of the molecular weight of DNA gives considerably lower values than the methods generally used. Owing to the uncertainty of Perrin's form factor, used as a correction for deviations from the spherical shape of the molecule, the method cannot be used as an absolute method for globular molecules. It should, however, be stressed that, for globular proteins, the use of molecular weights obtained in other ways gave form factors that were in agreement with the results in respect to the molecular shape. The uncertainty in the form factor is eliminated in the case of thread molecules. The method gives the molecular weight in absolute measures from the specific volume and the dielectric data alone. The determination should refer to the hydratized molecule. We assume, however, that DNA is moderately hydratized and that only a fairly insignificant number of water molecules accompanies it on orientation.

We refer the discrepancies in the results as compared with those by ultracentrifuge, diffusion measurements, streaming birefringence, etc. to the difficulty of following the individual molecule in an isolated state in the case of extreme thread molecules with high "specific" dipole moments.

As will be discussed in more detail in a later paper, the exchange energy of DNA increases so considerably with the degree of polymerization that the mutual interaction becomes too strong even at considerable distances. We therefore feel that the dielectric method for the determination of the molecular weight, based on the effect of an external electric field on dipoles, gives a more accurate magnitude in the case of the individual molecule. Moreover, the method allows a variation in the solvent's content of, *inter alia*, electrolytes, although to a limited extent. This permits a study of the molecular weight under various conditions [3] which is very informative.

It is well known that, for example, the sedimentation *constant* varies with the concentration as an expression of the mutual interaction. The dielectric method also shows a peculiar relation to the concentration [3,6]. With a falling concentration, the molecular weight rises, whereas we should rather have anticipated a very slight shifting of the critical wave-lengths towards higher frequencies. This effect is also certainly caused by a dipole interaction and is explained by the fact that the free orientation of the molecules is prevented in stronger solutions.

An uncertainty in the dielectric method for the determination of the molecular weight appears to be the use of the macro-viscosity of the solvent. The structure of DNA is such that it readily allows hydrogen bonds with the surroundings. We do not in DNA, as for example, in carboxyhaemoglobin [19], find the theoretical relation in solutions containing glycerol. It is true that in the case of DNA (unpublished) it is difficult to obtain exact values with the method used, but it is evident that the use of the macroviscosity is not altogether correct. If this systematic error were considerable, it would nevertheless imply that the molecular weights found were too high. The deviation from the results of other methods is thus not due to the viscosity of the solvent, which presumably gives rise to an error of minor importance. This also appears reasonable since DNA is a long and stiff rod somewhat flattened at the sides and very large in relation to the surrounding water molecules.

The general conception of the shape and appearance of the molecule corresponds to the regularity shown for DNA by the dielectric method. The comparison with, for example, the viscosity is particularly informative. The details of the theoretical calculations are also in very good agreement with experiences with X-ray crystallography and streaming birefringence. They show the reliability of the dielectric method for the determination of the molecular weight and also that the use of a relative value for the dipole moment according to Debye is justified.

Its high increment and marked dielectric regularity appear to render DNA a suitable and in principle simple model substance for dielectric studies under conditions that are theoretically difficult to handle.

SUMMARY

The results of dielectric studies on sodium thymonucleate (DNA) are reported, mainly for comparison with the relations found for peptides and amino acids, in particular by Wyman's school. It is found that the dielectric increment per gram per litre of DNA is directly proportional to the molecular weight instead of being approximately constant, as is the case in peptides and mono-amino-monocarboxylic acids of the type $NH_2(CH_2)_n \cdot COOH$.

As a result, the dipole moment calculated according to Debye shows a direct proportionality to the molecular weight. This is interpreted as an expression of the fact that DNA has a polar structure with simple dipoles (mononucleotides) placed side-by-side in conformity with the generally accepted view. The dependence of the dipole moment on the molecular weight is found to be the theoretically extreme case, implying the maximum of polarity for a multipole.

The results and the comparison with the conditions in amino acids and peptides give reason to generalize the connexion between the dipole moment (μ) and the molecular weight (M) in the following form

$$\mu = const. \cdot M^{x}$$

where $1 \geqq x \geqq 0$. The value for $x = 1$ is thus obtained for DNA, whereas in peptides $x \simeq 1/2$.

This relation is completely analogous to Staudinger's relation between the intrinsic viscosity and the molecular weight. The exponent x varies with the shape of the molecule, *i.e.* with the resulting length of the dipole.

The possibility is discussed of expressing the dipole moment in absolute values with the use of an empirical factor g, in accordance with Wyman's suggestion. For DNA, g would then be of the order of magnitude of 2. This is a very low figure and therefore gives rise to considerable doubt. The calculation according to Debye is therefore accepted instead and the value for the dipole moment is considered to be a relative value.

The calculated dipole moment for an average mononucleotide in poly-DNA is approximately 0.9 Debye. This is low and is explained by the fact that the moment-bearing groups are superficially located and are considerably influenced by the polar solvent.

Finally, the dielectric method for the determination of the molecular weight of stiff thread molecules is discussed. The conclusion is reached that the dielectric method can be expected to give more correct values for thread molecules with high transverse dipole moments than methods in which the mutual dipolar effect plays a considerable rôle and the conditions in the individual

molecule are harder to determine. The possibility of varying the properties of the solvent, particularly as regards its content of electrolytes, makes the method more advantageous in some respects than a number of others.

The results of the measurements in aqueous solution of poly-DNA are in accordance with the general conception of the substance. They therefore support the usefulness of the dielectric method for the determination of the molecular weight and indicate possibilities of using it in fields inaccessible with other methods.

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