# STUDY OF BOUND WATER OF POLY-ADENINE USING HIGH FREQUENCY DIELECTRIC MEASUREMENTS

S. Takashima,* A. Casaleggio,‡ F. Giuliano,‡ M. Morando,‡ P. Arrigo,‡ and S. Ridella‡

*Department of Bioengineering/D3 University of Pennsylvania, Philadelphia, Pennsylvania 19104; and
‡Instituto per i Circuiti Elettronici-C.N.R. 16145, Genova, Italia

ABSTRACT    The high frequency dielectric constant of poly-adenine (poly-A) was measured between 1 MHz and 1 GHz. The purpose of these experiments was to investigate the state of water molecules that are bound to the charged groups of the poly-A molecule. Analysis of the data using the Maxwell's mixture equation revealed the dielectric constant of bound water higher than we expected. Using Onsager's internal field in Debye's equation, we calculated the dielectric constant of water in the vicinity of a charged ion. The result of this computation demonstrates that the dielectric constant of bound water is much smaller than the normal value only in the immediate proximity of charged ions (within 2 Å). The dielectric constant increases rapidly to the normal value as the distance increases from 2 to 4 Å. This observation indicates that charged sites of polyions have only short range interactions with the surrounding water molecules. However, this conclusion pertains only to rotary diffusion of bound water since dielectric measurement is unable to detect translational diffusion.

## INTRODUCTION

The characterization of biological materials by means of dielectric constant measurement is now a well established field of research (1, 2, 3, 4). Of particular interest is the investigation of the physical and/or dynamic properties of water molecules in the vicinity of polyions. The results obtained in previous investigations indicate that the electrical properties of water bound to biological polyions are different from those of normal water in the bulk. The measurements of dielectric constant and conductivity at high frequencies between, ~1 MHz and a few GHz were first performed by Schwan (5), and by Pennock and Schwan (6), using hemoglobin. Also, similar research was done by Grant et al. (7, 8) using human serum albumin. These investigators demonstrated that the relaxation time of bound water is three orders of magnitude larger than that of normal water.

In protein solutions, however, bound water manifests itself in the dielectric measurement only as a small and ill-defined dispersion curve between the principal dispersion due to protein dipoles and that due to water dipole. Under these circumstances, similar studies with other biological polyions, such as DNA and synthetic polynucleotides, are desired that seem to have a well-defined high frequency dielectric behavior (9, 10).

The aim of this paper is to discuss the results of high frequency dielectric constant measurements with a synthetic polynucleotide, poly-adenine (poly-A), which has similar dielectric properties as those of DNA molecule

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This research was performed when S. Takashima was visiting the Universita di Genova, Genova, Italia, in 1984.

(11). The results of these experiments will be interpreted in terms of the relaxation behavior of water that is bound tightly to poly-A molecule.

## EXPERIMENTAL PROCEDURE

Poly-A samples were purchased from Sigma Chemical Corp. (St. Louis, MO), and used without further purification. The molecular weight of the sample is ~100,000. Poly-A was dissolved in dilute phosphate buffer (0.001 M, pH 7.0) and dialyzed overnight before experiments against the same buffer solution. The concentration of poly-A was determined using the Cary 219 spectrophotometer. Separately we prepared another buffer solution mixed with small amounts of KCl. The conductivity of this buffer-KCl mixture was carefully adjusted to that of poly-A solution. This mixture was used to dilute poly-A solution without changing its conductivity. The temperature of the sample was maintained by circulating thermostated water at 23° ± 0.1°C.

## Measuring Technique

The block diagram of the measuring system used for this experiment is shown in Fig. 1. The HP 4191A is a radio frequency impedance analyzer which enables the complex reflection coefficient to be measured between 1 MHz and 1 GHz. The HP 3478A is a digital voltmeter used for the measurement of temperature of the sample using a thermistor. Both HP 4191A and HP 3478A are operated by an on-line HP 85 computer using an IEEE 488 Bus.

The sample holder is shown in Fig. 2. The experimental procedure consisted of three steps: (a) Calibration, (b) Verification, and (c) Measurement.

(a) Calibration: First, we measure the reflection coefficients of NaCl solution of known normality at 31 logarithmic swept frequencies. Complex permittivities $\tilde{\epsilon}$ are computed using Stogryn's equation (see reference 12). (b) Verification: During this step we measure again with a known reference sample, emagel. We determine the complex reflection coefficient $\bar{\rho}$, then using the double ratio expression (Eq. 1) we can find the value of $\tilde{\epsilon}$ of the reference solution.
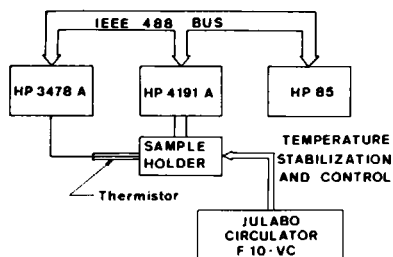
FIGURE 1 The block diagram of the system for high frequency dielectric constant measurements. HP 4191 is RF Impedance Analyzer and HP 3478 is temperature control system. Both are operated by an on-line computer HP 85.

$$\frac{(\bar{p} - P_x)}{(P_y - P_z)} \cdot \frac{(\bar{p} - P_y)}{(P_x - P_z)} = \frac{(\bar{\epsilon} - \epsilon_x)}{(\epsilon_y - \epsilon_z)} \cdot \frac{(\bar{\epsilon} - \epsilon_y)}{(\epsilon_x - \epsilon_z)}, \qquad (1)$$

where $P_x$, $P_y$, $P_x$, $\epsilon_x$, $\epsilon_y$, and $\epsilon_z$ are the stored calibration coefficients. We compute the difference $\bar{\epsilon} - \tilde{\epsilon}$, where $\tilde{\epsilon}$ is the complex permittivity of the solution that was calculated using the Stogryn's equations. If the difference is within the range of accuracy of the instrument (1%) for each frequency, then we can start the measurements with unknown samples. The measurements were performed using the interactive computer program that was written by Bianco et al. for HP 85 to control the Impedance Analyzer (12, 13, Casaleggio et al., unpublished manuscript).

## RESULTS

An exemplary result of high frequency measurements of the dielectric constant and conductivity of poly-A solution
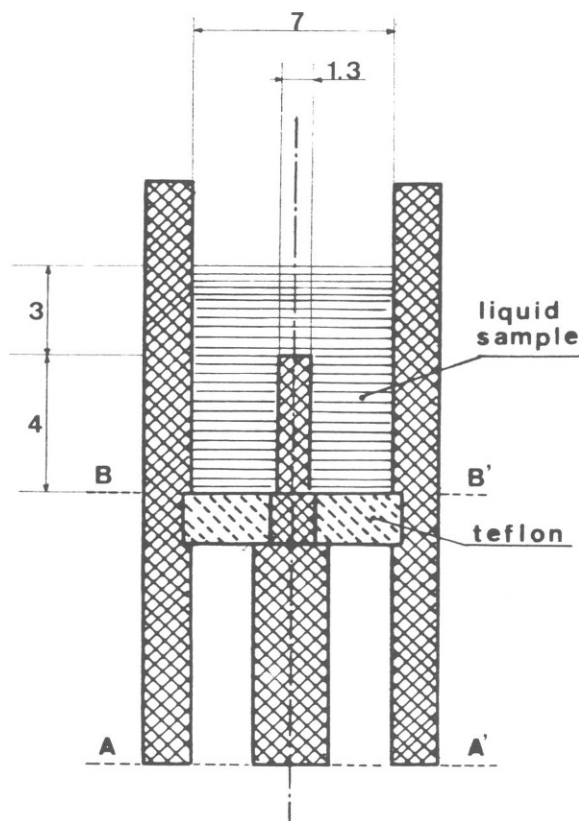


FIGURE 2 A lateral view of the sample holder. Dimensions are in millimeters.

is shown in Fig. 3. As shown, the dielectric constant of poly-A exhibits a well defined dispersion between 2 MHz and 1 GHz with an amplitude of ~10 dielectric units at the concentration of, e.g., 3.6%. A similar dispersion was found with DNA by Takashima et al. (10). Its origin is, however, still unexplained. The dispersion curve begins at the level of 89, and approaches a lower limiting value of 77. Since the dielectric constant of phosphate buffer ($\epsilon_b$) is measured to be 78.8, the high frequency dielectric constant of poly-A is lower than that of water by 1.8 units. It is well known that the high frequency dielectric constant of protein solutions is considerably lower than that of water, and the difference has been attributed to irrotationally bound water (1). Likewise, the difference between the dielectric constants of poly-A solution and phosphate buffer can be atrributed to bound water molecules.

The same measurement was repeated varying the concentration of poly-A. Based on these results, we obtain two pieces of information: (a) The amplitude of dispersion decreases with the decreasing concentration of poly-A. This demonstrates that this dispersion is most likely to be due to poly-A. (b) The high frequency limiting value seems to increase with decreasing poly-A concentration. The high frequency limiting value may be determined fairly accurately by extrapolating the dispersion curve to the higher frequency region. However, the extrapolation can be done with accuracy and reliability by the use of Cole-Cole plot (14). Fig. 4 illustrates one of the Cole-Cole plots we obtained. The imaginary part $\epsilon''$ is calculated using the following equation:

$$\epsilon'' = \frac{\Delta\kappa}{\omega\epsilon_0}, \qquad (2)$$

where $\Delta\kappa$ is the conductance increment of poly-A between low and high frequencies. $\epsilon_0$ is the permittivity of free space ($8.64 \times 10^{-14}$ $F$/cm). $\omega$ is radian frequency ($2\pi f$). The high frequency end of this plot rises sharply, indicating
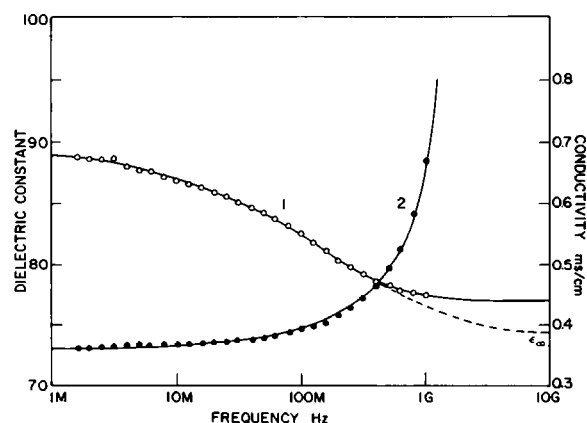


FIGURE 3 The high frequency dielectric constant (curve 1) and conductivity (curve 2) of poly-A solution (3.6% by weight). The solid curve was drawn through measured points. The broken curve was drawn using $\epsilon_\infty$ obtained by Cole-Cole plot. Only the dashed curve is considered the correct high frequency asymptote.
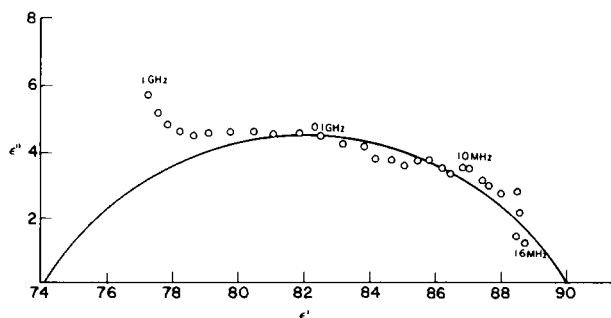
FIGURE 4  The Cole-Cole plot of poly-A (3.6% by weight) The abscissa is the real part and ordinate is the imaginary part of complex dielectric constant. The sharp rise at high frequency end is considered to be due to the dispersion of bulk water.

that the dispersion of bulk water is already beginning at these frequencies. Since this part has no relation to the dispersion of bound water, we can ignore it and draw an arc to obtain the high frequency dielectric constant ($\epsilon_\infty$). This value is somewhat lower than the value obtained from Fig. 3. Looking at Fig. 3, clearly the dielectric constant approaches a high frequency limiting value asymptotically, creating an asymmetric dispersion curve. However, the real asymptote must be the one that is shown by a dotted curve. The values of $\epsilon_b - \epsilon_\infty$ are displayed in Fig. 5 as a function of poly-A concentration. As shown, the plot is found to be linear. Fig. 6 shows a plot of $(\epsilon_b - \epsilon_\infty)/P$ (where $P$ is volume fraction, i.e., dry weight divided by density) against $P$. The straight line intersects with the ordinate at 107. The broken line was obtained by incrementing the volume fraction including the shell of hydration water surrounding poly-A (0.3 g/g). The inclusion of hydration shell decreases the intercept from 107 to 98. These values will be used for the calculation of internal dielectric constant of poly-A molecule with and without hydration shell.
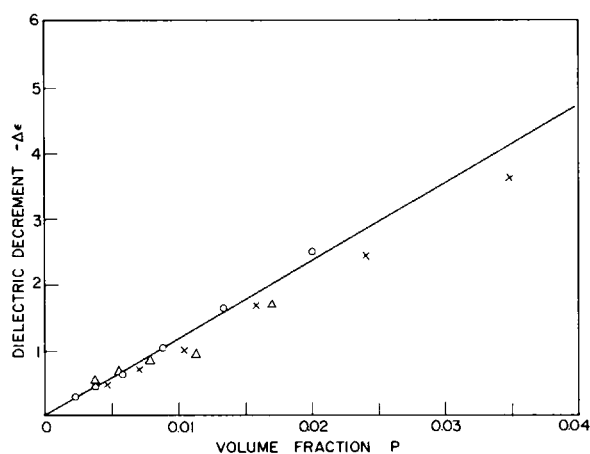


FIGURE 5  The plot of dielectric decrement $-(\epsilon_\infty - \epsilon_b)$ against volume fraction $P$. The volume of poly-A was calculated by dividing weight by its density ($d = 1.4$). The circles, crosses and triangles represent separate measurements.
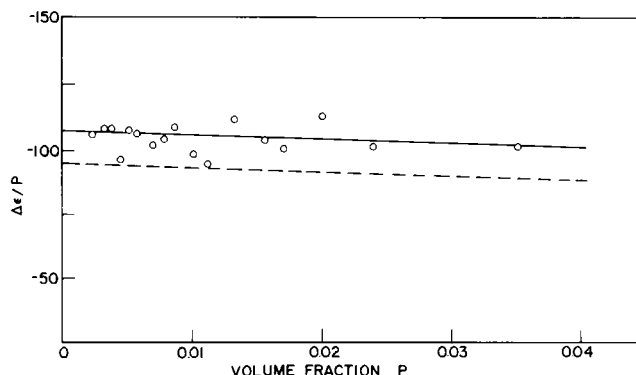


FIGURE 6  The plot of specific decrement against volume fraction. Open circles and solid line were obtained by dividing decrements $-\Delta\epsilon$ by volume fraction without hydration shell. The broken line was obtained by dividing the decrements by volume fraction including hydration shell.

## ANALYSIS AND DISCUSSION

As mentioned in the foregoing, poly-A solution displays a well defined dispersion curve in the frequency range between 1.5 MHz and a few GHz. At present, the origin of this dispersion is unknown and no further information as to its origin is available. We are currently more interested in the analysis of the dynamic properties of bound water surrounding poly-A molecule. This analysis requires a careful examination of high frequency dielectric data.

The dielectric constant of cylindrical molecules dissolved in an aqueous medium is related to the dielectric constants of the solvent and that of the molecule by Maxwell's equation (15) (see also Fricke, reference 16).

$$\frac{\epsilon_\infty - \epsilon_b}{\epsilon_\infty + X\epsilon_b} = P\frac{\epsilon_i - \epsilon_b}{\epsilon_i + X\epsilon_b}, \tag{3}$$

where $\epsilon_\infty$ is high frequency dielectric constant of the sample, $\epsilon_i$ and $\epsilon_b$ are the dielectric constants of poly-A and phosphate buffer, and $P$ is volume fraction. $X$ is shape factor which ranges from 1 for rigid rod to 2 for sphere. If the volume fraction $P$ is very small, then this equation can be rewritten:

$$\epsilon_\infty = \epsilon_b\left\{1 + P(X + 1)\frac{\epsilon_i - \epsilon_b}{\epsilon_i + X\epsilon_b}\right\} \tag{4}$$

After rearranging, we can further change it to

$$\frac{\epsilon_\infty - \epsilon_b}{P} = \frac{\epsilon_i - \epsilon_b}{\epsilon_i + X\epsilon_b}\epsilon_b(X + 1). \tag{5}$$

The left hand side of this equation is the same as the ordinate of Fig. 6. To calculate the dielectric constant of poly-A, we need to assume a value for the shape factor $X$. Since poly-A is a fibrous polymer, the value of 2.0 for sphere is highly unlikely. Also, poly-A may not be a rigid rod in solution. Therefore, the value of $X$ must be >1.0. Perhaps a value between 1 and 1.5 is most appropriate to represent the slightly curved poly-A fibers. Using Eq. 5

TABLE I

MEASURED INTERNAL DIELECTRIC CONSTANT OF
POLY-ADENINE FOR VARIOUS SHAPE FACTORS

| $W_{H_2O} = 0$* | |
| --- | --- |
| $X$ | $\epsilon_i$ |
| 1.0 | 15.5 |
| 1.5 | 8.78 |

| $W_{H_2O} = 0.3$ per one gram of poly-A molecule | | |
| --- | --- | --- |
| $X$ | $\epsilon_i$ | $\epsilon_{H_2O}$ |
| 1.0 | 20.39 | 36.0 |
| 1.5 | 15.59 | 32.2 |

*Poly-A is assumed to be bare in aqueous solution.

twice, we calculated the dielectric constant of poly-A molecule, with and without the water of hydration. Table I shows the results of this calculation. The amount of hydration water was estimated by Tunis and Hearst (17) for DNA using ultracentrifugation and found that ~6–7 mol of water molecules are bound per mole of nucleotide. This amount is translated into 0.3–0.4 g of water per gram of DNA molecule. Assuming that the amount of bound water for poly-A is about the same as those of DNA, the volume occupied by bound water becomes 0.273 cc/1.0 cc of poly-A assuming the density of poly-A to be 1.4.[1] The dielectric constant of bound water can now be calculated using Maxwell's mixture theory (Eq. 3). Table I also shows the results of these calculations for various values of shape factor $X$. As shown, the dielectric constant of water bound to poly-A is found to be very large ranging between 32 to 36 for $X = 1.5$ to 1.0.

The amount of water we used, 0.3 g/g poly-A, represents the hydration water located very near negatively charged phosphate ions. The rotational motion of these water molecules must be restricted substantially because of the electrostatic interaction between charges and water dipoles. The dielectric constant of water molecules in the vicinity of a charge was first calculated by Debye (18) and the method used will be summarized briefly.

The charge of the central ion exerts a force $F$ on water diploe $\mu$. The force and displacement $D( = \epsilon E)$ are related to polarization $I$ by the following equations:

$$F = E + \frac{4\pi}{3} I \tag{6}$$

$$D = E + 4\pi I, \tag{7}$$

where $E$ is external field that differs from the actual field $F$. Polarization $I$ is related to the polarizability $\alpha$ and dipole moment $\mu$ of water molecule by Eq. 8.

[1]Since poly-A is single stranded and the experiments of Tunis and Hearst were performed with double stranded DNA, the amount of bound water in this analysis may be slightly incorrect.

$$I = N\left[\alpha F + \mu L\left(\frac{\mu F}{kT}\right)\right], \tag{8}$$

where $L(x)$ is the Langevin function and $N$ is the number of molecules per cc. Eliminating $E$ from Eqs. 6 and 7, we obtain Eqs. 9 and 10.

$$D = F + \frac{8\pi N}{3} I \tag{9}$$

$$E = F - \frac{4\pi N}{3} I. \tag{10}$$

We apply small radial and tangential field $E_r$ and $E_\theta$, as shown in Fig. 7. The dielectric constants $\epsilon_r$ and $\epsilon_\theta$ are defined by the following equations:

$$\epsilon_\theta = D/E \quad \text{and} \quad \epsilon_r = dD/dE \tag{11}$$

In addition, we can calculate the distance r by

$$r = e/D. \tag{12}$$

Using the method described above, we can calculate the dielectric constant of water around a charge. However, the internal inconsistency of Debye's theory is well known. Actually, numerical calculation revealed that the $E$ in Eq. 10 becomes negative for small values of $F$. This is a clear indication of internal inconsistency of the theory. A similar calculation was performed by Hasted et al. (19). Their calculation was based on the use of Onsager equation as well as Kirkwood theory (20, 21). However, to solve transcendental equations that arise from the inclusion of internal as well as reaction fields, they had to resort to the use of an approximation.

Our attempt is to apply Onsager's equations in Debye's theory (Eqs. 6–11) and solve the resultant transcendental equation using computer iteration technique. Based on Onsager's formalism, the displacement $D$ is expressed by Eq. 13.

$$D = F + \frac{3\epsilon(n^2 - 1)F}{2\epsilon + n^2}$$
$$+ \frac{4\pi(n^2 + 2)(2\epsilon + 1)\mu}{3(2\epsilon + n^2)} L\left[\frac{\epsilon(n^2 + 2)}{2\epsilon + n^2} \cdot \frac{\mu F}{\kappa T}\right], \tag{13}$$

where $n$ is the refractive index of water and is assumed to be 1.3333 at 25°C. The dipole moment of water was assumed to be 1.88 D.U. The field $E$ is related to $F$ by

$$E = \frac{2\epsilon + 1}{3\epsilon} F. \tag{14}$$

The dielectric constant $\epsilon_\theta$ was calculated using Eqs. 13, 14, and 11. The procedure is to assume a value for $F$ and for $\epsilon_\theta$ in Eqs. 13 and 14 and iterate the computation until calculated dielectric constant becomes self consistent. The convergence of computed dielectric constants was reached after 10–15 iterations. Likewise, the same procedure was
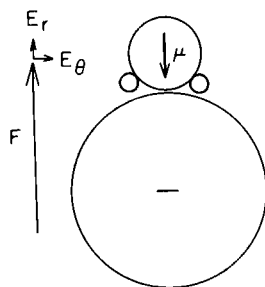
FIGURE 7 The model used for the calculation of dielectric constant of water in the vicinity of a negative ion. $\mu$ is the dipole moment of water, $F$ is the actual field due to the anion. $E_r$ and $E_\theta$ are applied fields for the measurement of dielectric constant. Note that the magnitudes of $E_r$ and $E_\theta$ are much smaller than $F$.

used for the calculation of $\epsilon_r$. Instead of Eqs. 13 and 14, we used the following equations for the calculation of $\epsilon_r$:

$$\frac{dD}{dF} = 1 + \frac{3\epsilon(n^2 - 1)}{2\epsilon + n^2}$$

$$+ \frac{4\pi(n^2 + 2)(2\epsilon + 1)\mu}{3(2\epsilon + n^2)} \frac{d}{dF}\left\{ L\left[\frac{\epsilon(n^2 + 2)}{2\epsilon + n^2} \cdot \frac{\mu F}{kT}\right] \right\} \quad (15)$$

$$\frac{dE}{dF} = \frac{2\epsilon + 1}{3\epsilon} . \quad (16)$$

In Eqs. 15 and 16, we assumed that the magnitude of applied $E$ is so small that the change in $F(\delta F)$ is negligible. Under this condition, $\epsilon$ can be considered independent of $\delta F$. The dielectric constant $\epsilon_r$ was calculated using Eq. 17.

$$\epsilon_r = \frac{\left(\dfrac{dD}{dF}\right)}{\left(\dfrac{dE}{dF}\right)} . \quad (17)$$

The results of these calculations are illustrated in Fig. 8. As shown, the dielectric constant of water remains small only between 0 and 2 Å. $\epsilon_\theta$ and $\epsilon_r$ begin to increase rapidly to a normal value beyond the distance of 4 Å. In other words, the effect of the central ion does not reach beyond 2–3 Å. This result demonstrates that only the first layer and part of the second layer of hydration water should be considered rotationally immobilized. The large value of dielectric constant we determined experimentally indicates that a considerable portion of hydration water (perhaps extends beyond the first layer) is only partially immobilized and has relatively high rotational freedom.
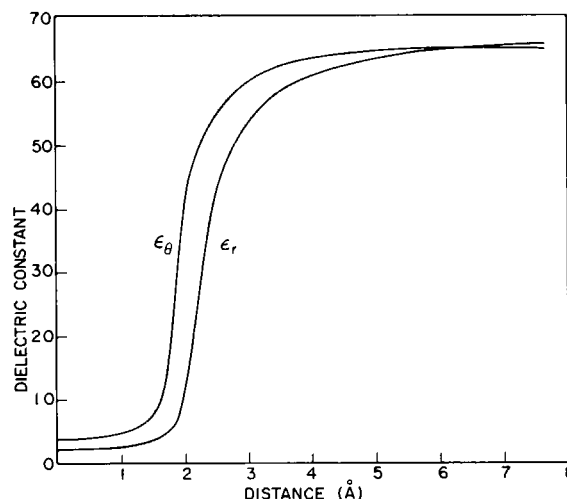
FIGURE 8 The profiles of the dielectric constants of water as a function of distance from the central ion. Curves 1 and 2 were calculated using Onsager's formulae in Debye's theory. $\epsilon_r$ and $\epsilon_\theta$ are the dielectric constants calculated using small electrical fields in radial and tangential directions, $E_r$ and $E_\theta$.

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