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Dielectric and Semiconductive Behaviors of Solvated Biomacromolecules

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Introduction

The physical state of water often has a profound effect on both the structure and function of biological systems. Polar molecules, e.g., water, should be found at polar, or polarizable, sites on the macromolecules within the cell. Ling[1] has argued that all intracellular water is so oriented. Recent nuclear magnetic resonance studies on protein, Fuller and Brey[2] and Brey, Evans and Hitzrot,[3] muscle, Hazlewood, Nichols and Chamberlain,[4] and Cope,[5] and nerve, Chapman and McLauchlan[6] and Cope[5] indicate that at least two, if not three, varieties of water exists in cells. One form of the water is believed to be tightly bound to the biomolecules. Klein and Phelps,[7] also using the sophisticated deuterium magnetic resonance technique, however, could not detect oriented water in the rat phrenic nerve.

Crystallographic studies of protein have shown that all polar amino acids are located either on the exterior surface or in large internal cavities. In either case the polar amino acids are available to water. Pauling[8] predicted that monolayer coverage would be achieved when each polar site was occupied by one molecule of water. Isotherms for the adsorption of water on biomolecules are generally Brunauer Emmett Teller (BET) isotherms.[9]

Of the protein properties which change with hydration perhaps the most striking is conductivity. The conductivity of a protein may increase by more than 10 orders of magnitude upon hydration. Protein, as well as several other biological materials (e.g., lipids and nucleic acids) exhibit a positive exponential temperature dependence of conductivity, i.e., proteins follow:

$$\sigma = \sigma_0 \exp(-E/2kT) \tag{1}$$

where σ_0 is a constant; E is the activation energy for semiconduction; k is Boltzmann's constant; and T is the temperature on the Kelvin scale. Therefore, they are classified as semiconductors. The effect of polar adsorbates on protein conductivity has been explained by Rosenberg.[10] The adsorption of polar molecules increases the effective dielectric constant of the medium. This serves to increase the polarization relaxation energy which decreases the activation energy for semiconduction.

Experimental

All experiments were performed on dialyzed, twice crystallized bovine hemoglobin processed by Servac. A compacted tablet was formed by slowly pressing the hemoglobin

crystallites to a pressure of 10^3 kg per cm^2 in a teflon coated die. Compacted tablets, of surface area 4 cm^2 and thickness 0·03–0·05 cm, were fastened between solid metal electrodes with the aid of spring loaded clips. Teflon insulation was used throughout. The sample was placed in a brass chamber as illustrated in Fig. 1 where it completed an electric circuit between a battery and a vacuum tube electrometer.

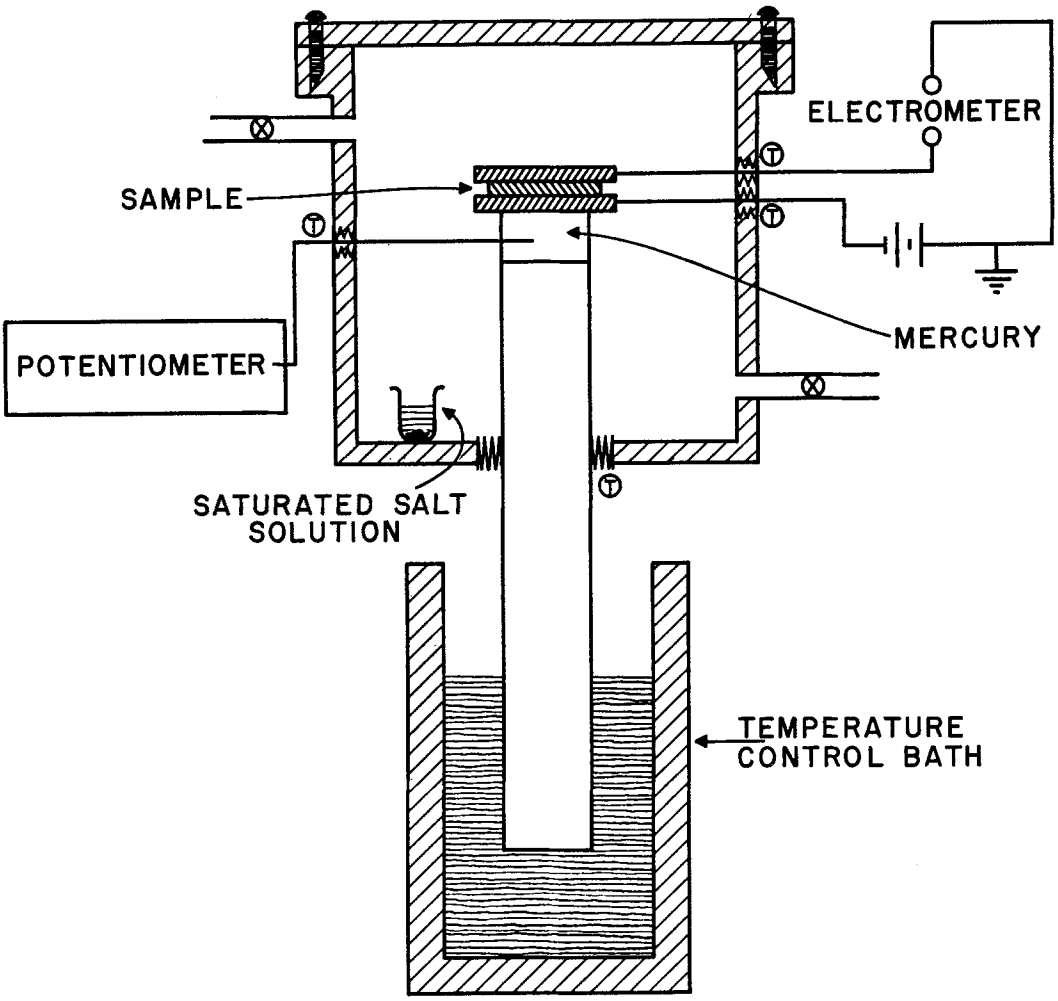

Figure 1. Schematic diagram of the apparatus used to measure conductivity and semiconduction activation energy. The sample makes thermal contact with the temperature control rod via a cup of mercury. All insulation indicated by the symbol Ⓣ is of teflon. Current is measured with a Keithley model 610 BR electrometer.

Drying was achieved by heating the sample for a minimum of 12 hours in a dry nitrogen atmosphere. (This procedure was found to be fully equivalent to vacuum drying as far as electrical parameters were concerned.) After the sample was cooled to room temperature a fixed partial pressure of an adsorbent was introduced into the chamber. A variety of relative humidities was established with the aid of saturated salt solutions.[11] At high relative humidities several days were required for the establishment

of equilibrium between the saturated salt solution, the atmosphere in the chamber and the sample. The atmosphere in the conductivity chamber was regulated to various partial pressures of ethanol or methanol by slowly exchanging the nitrogen atmosphere in the chamber for the nitrogen atmosphere over a thermostatically controlled reservoir of the desired alcohol.

As the sample was cooled its temperature and conductivity were recorded simultaneously. This dynamic technique of determining activation energy was chosen because it disturbs the sample's adsorption state less than the static method of measuring conductivity after the sample has equilibrated at each measuring temperature. The reproducibility with which the system could be recycled indicated that the dynamic method of determining the activation energy does not alter the adsorption state of the sample. The relative vapor pressure of the adsorbates in the stainless steel dielectric cell (Balsbaugh Laboratories model LD3) was regulated in the same manner as in the conductivity chamber. In the region, 30 Hz–100 kHz, capacitance was measured with a General Radio capacitance measuring assembly. Measurements in the 0·1–10 Hz region were made using a bridge manufactured by the Ando Electric Co. Ltd. of Tokyo, Japan.

A portion of the compacted tablet weighing 30 mg was placed on a Cahn model RG electro-balance which was enclosed in an evacuable glass chamber. The relative humidity in the microbalance chamber was regulated by the introduction of small quantities of the vapor from over a degassed solvent into the previously evacuated microbalance chamber. Adsorption isotherms were measured at room temperature, 24° C. Greater experimental detail has been published elsewhere.[12]

Results

Hydrated hemoglobin tablets closely approximate ohmic behavior in the region of 2–600 V. The temperature dependence of the conductivity of hemoglobin has been found to follow Eq. (1), which is the operational definition of semiconductors. The adsorption of water or alcohol on hemoglobin serves to alter its semiconductive property. Both constants in Eq. (1), E and σ_0 can be obtained by plotting the log σ vs $1/T$. Typical data of the temperature dependence of the conductivity of hemoglobin with various quantities of adsorbed water, or alcohol, are illustrated in Fig. 2. Values of E and σ_0 calculated from these graphs are given in Table I. The relative invariance of σ_0 with changes in the adsorption state of the hemoglobin is atypical of organic materials.[13]

Capacitance measurements on hydrated hemoglobin are seen in Fig. 3 to exhibit a large low frequency dispersion similar to those reported by Maričič, *et al.*[14] and O'Konski.[15] As can be seen in Fig. 4, a log-log plot of capacitance versus frequency at low frequencies, the capacitance of hydrated hemoglobin tablets varies as $\omega^{-0.35}$. It is believed that this is due to a polarization effect, probably of the Maxwell-Wagner variety.[16] A more complete discussion of this appears elsewhere.[12] This effect decreases with increasing frequency and is negligible in the region 10^4–10^5 Hz regardless of the hydration state. Dielectric constants were calculated at 10^5 Hz and correction was made for the vapor filled interstices according to the suggestion of Böttcher:[17]

$$K = \frac{3K_p\delta + 2K_p(K_p - 1)}{3K_p\delta - (K_p - 1)} \tag{2}$$

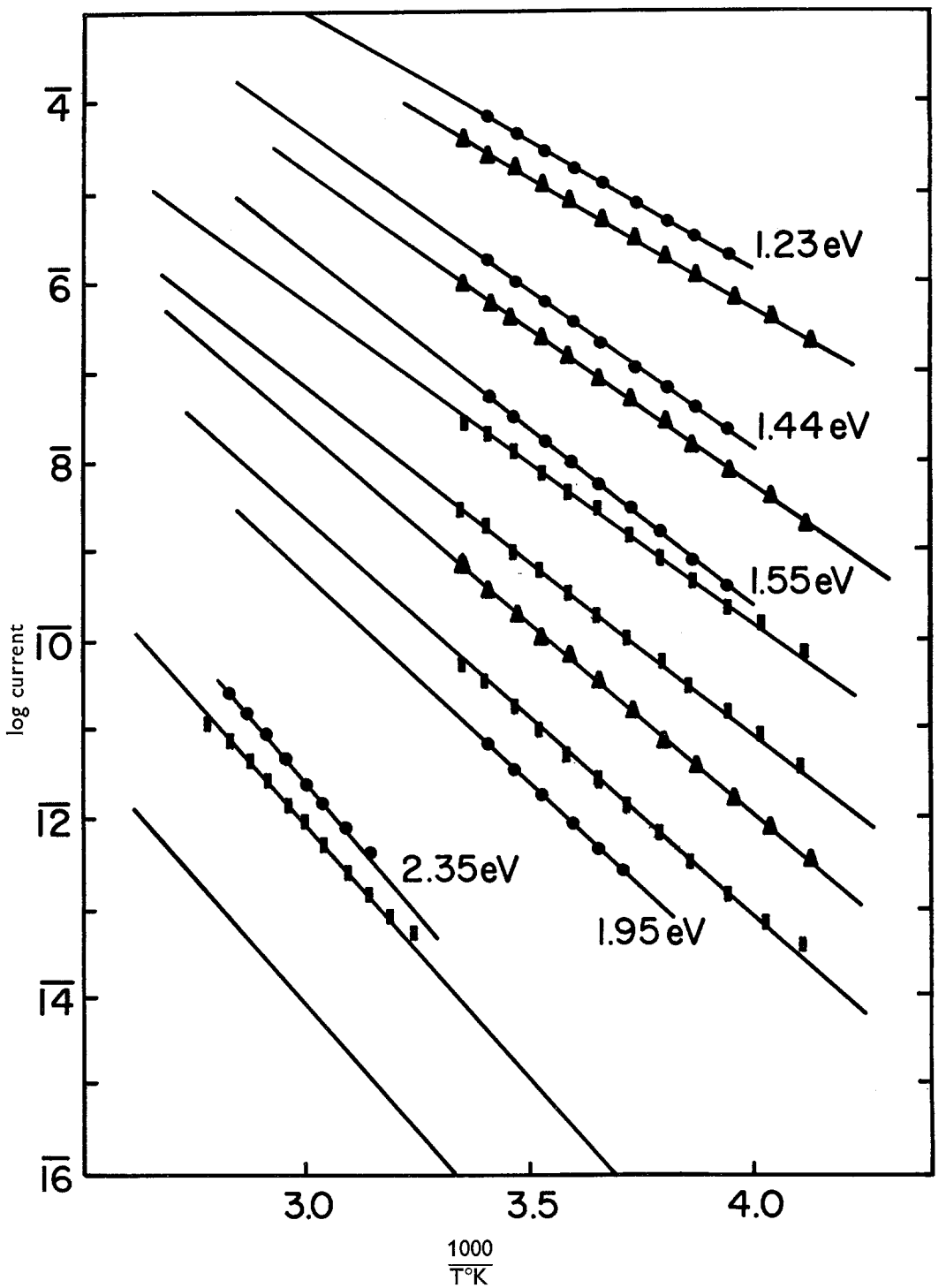

Figure 2. Dependence of the conductivity on the temperature for hemoglobin with water (circles), ethanol (triangles) and methanol (squares) adsorbed.

TABLE I. Conductivity parameters of hemoglobin-adsorbate systems

p/p_0 (%)	m (mole %)	Activation energy (eV)	σ_0 (ohm-cm)$^{-1}$	Dielectric constant
0 water	0	2·35	60	2·3
12 water	0·355	1·95	60	2·9
33 water	0·656	1·77	180	3·4
53 water	1·01	1·55	49	4·2
75 water	2·2	1·44	140	5·5
90 water	—	1·23	84	7·4
21 methanol	0·203	1·75	30	3·2
59 methanol	0·53	1·45	100	4·6
~100 methanol	—	1·20	30	5·9
23 ethanol	0·073	1·8	70	3·1
58 ethanol	0·35	1·6	25	4·5
~100 ethanol	—	1·5	35	4·9

Where K is the dielectric constant of the crystalline material; K_p is the dielectric constant of the tablet and δ is the packing fraction or partial volume of the crystalline material in the tablet. The dielectric constant calculated under several adsorbate conditions can be found in Table I.

The adsorption isotherms for hemoglobin are Type II or BET isotherms as illustrated in Fig. 5. When the isotherm data is plotted according to the BET equation,[9] in the region $p/p_0 < 0{\cdot}3$, a straight line is obtained. Using the BET equation the monolayer coverage, in moles of adsorbate per 100 g of protein, has been calculated and is given in Table II.

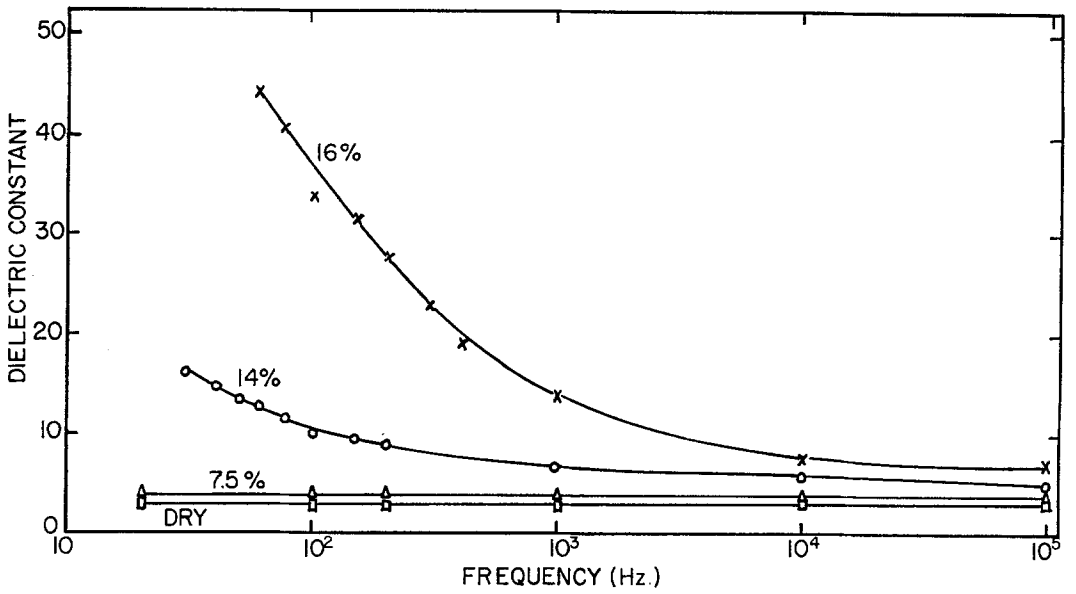

Figure 3. Plot of the frequency dependence of the apparent dielectric constant of hemoglobin with the quantities of adsorbed water indicated.

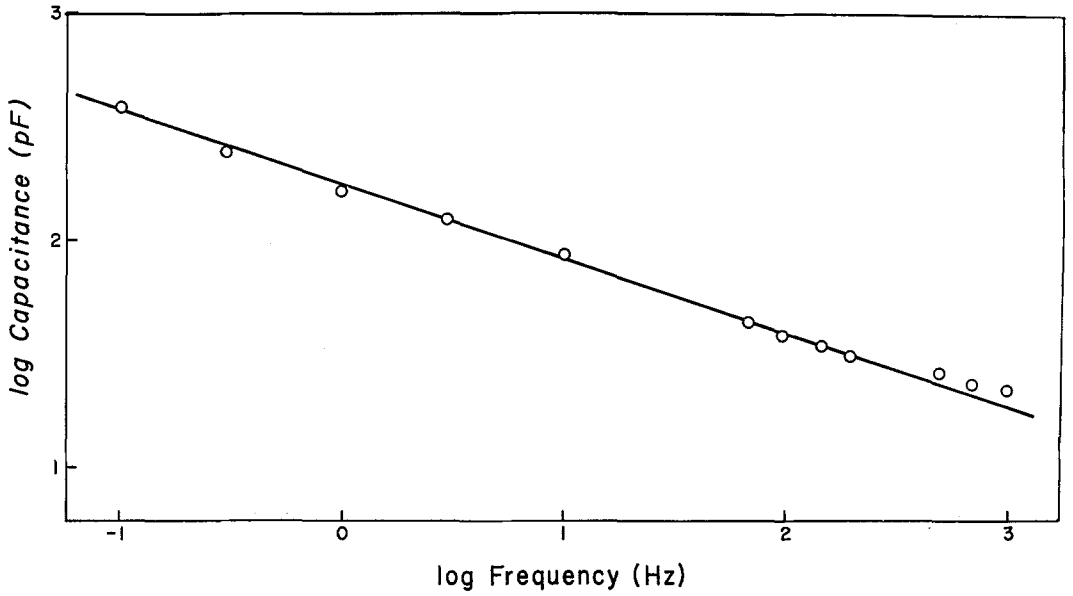

Figure 4. Log-log plot of the frequency dependence of hemoglobin maintained at 76% relative humidity. The slope is −0·35.

Discussion

The conductivity of hemoglobin-adsorbate systems is not an impurity dominated process.[18] Adsorption alters the conductivity of hemoglobin in such a way that the conductivity increases while the semiconduction activation energy decreases. The pre-exponential factor in the conductivity is unchanged by the adsorption process, as can be seen in Table I. Conductivity increases resulting from the adsorption of vapor are given by:

$$\sigma(m) = \sigma_D \exp(\alpha m) \tag{3}$$

Where σ_D is the conductivity of the dry specimen; m is the weight percent vapor adsorbed onto the specimen and α is a constant. Both dry proteins and protein adsorbate systems are operational semiconductors, i.e., their conductivity can be given by Eq. (1). Therefore their conductivity is given by:

$$\sigma_D = \sigma_0 \exp(-E_D/2kT) \tag{4}$$

$$\sigma_H = \sigma_0 \exp(-E_H/2kT) \tag{5}$$

respectively, where E_D is the activation energy of the dry protein and E_H is the activation energy of the protein adsorbate system. Combining Eqs. (3) and (4) we obtain:

$$\sigma(T,m) = \sigma_0 \exp\frac{-E_D}{2kT} + \alpha m \tag{6}$$

By comparison with Eq. (5) we observe that

$$E_H = E_D - 2kT\alpha m \tag{7}$$

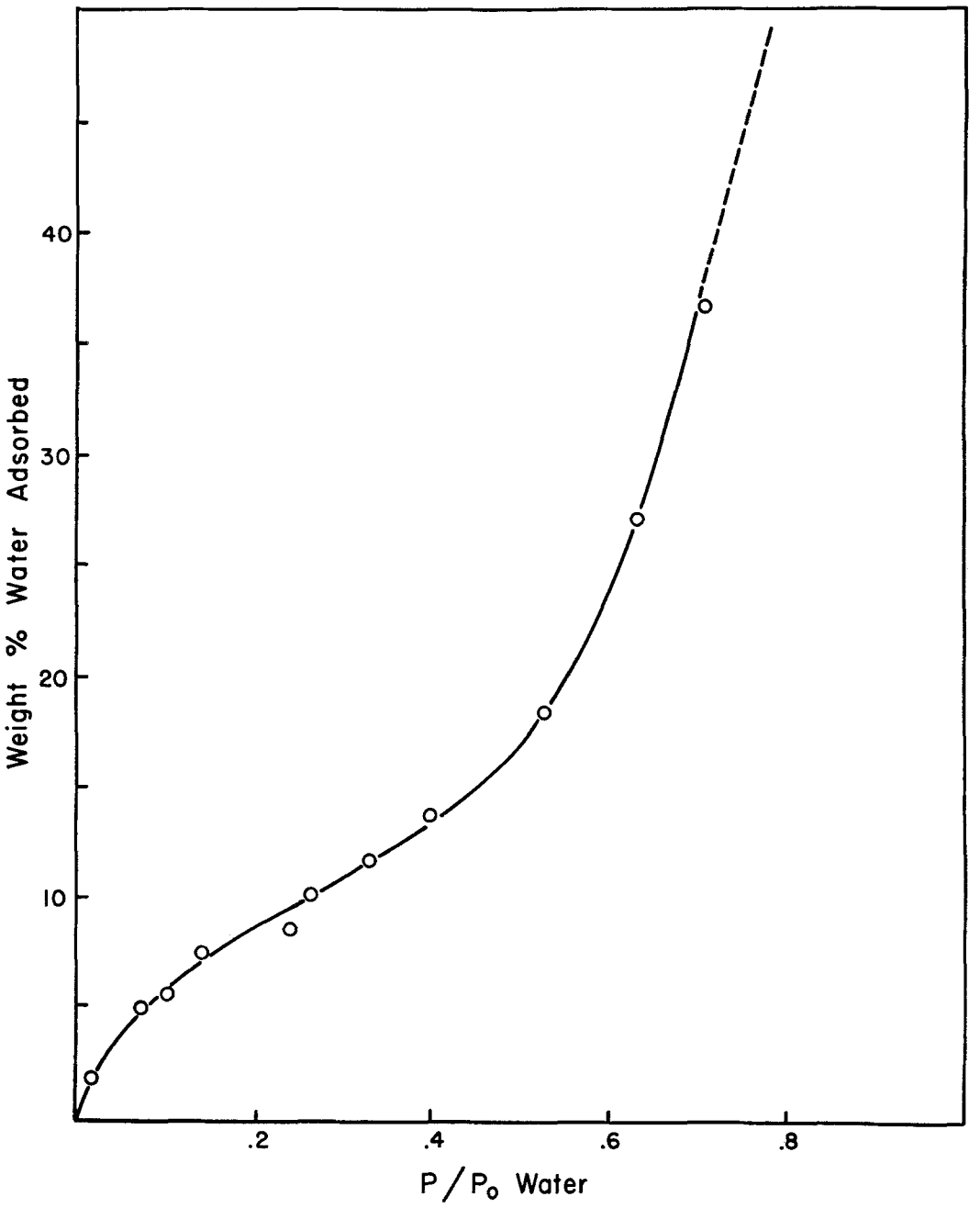

Figure 5. Adsorption isotherm for the adsorption of water on hemoglobin measured at 24° C.

As a first approximation the solvated crystalline protein is considered as a continuous medium describable by a single dielectric constant K. In this medium the work necessary to relocate a charge from a neutral part of the protein molecule to a previously neutral

TABLE II. Adsorption parameters for water, methanol, ethanol, and adsorbed on hemoglobin

Adsorbate	V_m (g/100 g protein)	V_m (moles/100 g protein)	α in (mole %)$^{-1}$
Water	8·5	0·47	17
Methanol	13·5	0·43	25
Ethanol	7·5	0·16	16

part of another, or distant part of the same, molecule can be calculated. If the charge moves a considerable distance the coulombic interaction between the charges may be neglected and the charges considered as essentially free. The energy required for such a process is:

$$E_D = I_g - A_g - 2P \tag{8}$$

where I_g is the gas state ionization potential of the substance; A_g is the gas state electron affinity and P is the stabilization resulting from polarization relaxation at each state of ionization as discussed by Lyons.[19] The polarization stabilization is the result of the relaxation of the dielectric media around a spherical region of the two newly created charges and is given by:

$$P = \frac{e^2}{2R}(1 - 1/K) \tag{9}$$

where R is the radius of the spherical region around which the relaxation occurs and K is the effective dielectric constant of the medium considered as a bulk property. Combining Eqs. (8) and (9) we obtain:

$$E_D = I_g - A_g - \frac{e^2}{R}(1 - 1/K) \tag{10}$$

Solvation cannot alter either the gas state values of the ionization potential or the electron affinity of the protein. Therefore, in the case of the solvated specimen:

$$E_H = I_g - A_g - \frac{e^2}{R}(1 - 1/K') \tag{11}$$

Combining Eqs. (10) and (11) we obtain:

$$E_H = E_D - \frac{e^2}{R}\frac{1}{K} - \frac{1}{K'} \tag{12}$$

Introducing this result into Eq. (5) provides

$$\sigma(T, K') = \sigma_D \exp\frac{-E_D}{2kT} \exp\left[\frac{e^2}{2kTR}(1/K - 1/K')\right] \tag{13}$$

In Eq. (12 we predict a linear relation between the activation energy and the reciprocal of the effective dielectric constant (K') which is verified in Fig. 6. Similarly Eq. (13) demands a linear relationship between the log of the conductivity and the reciprocal of the effective dielectric constant. This data is plotted in Fig. 7. Since the data for water, ethanol and methanol all fall on the same lines in Figs. 6 and 7 it would appear that the

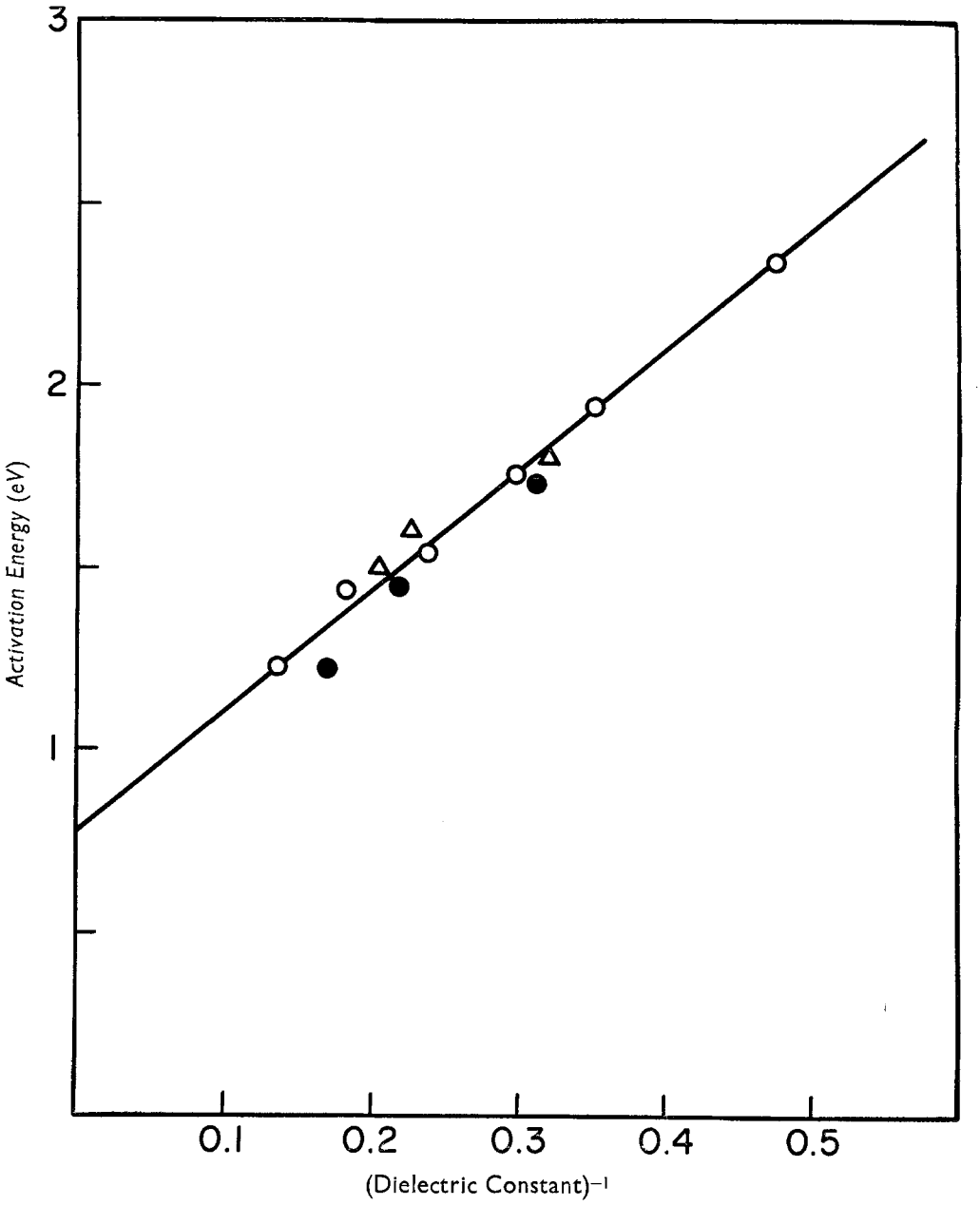

Figure 6. Variation of activation energy with dielectric constant. The circles indicate water; the triangles, ethanol; and the filled circles, methanol adsorption on hemoglobin respectively.

mechanism by which the dielectric constant influences conductivity is independent of the adsorbate. The region defined by the polarization cavity radius R is a property of the substrate alone as it does not change when the adsorbate is changed from water to

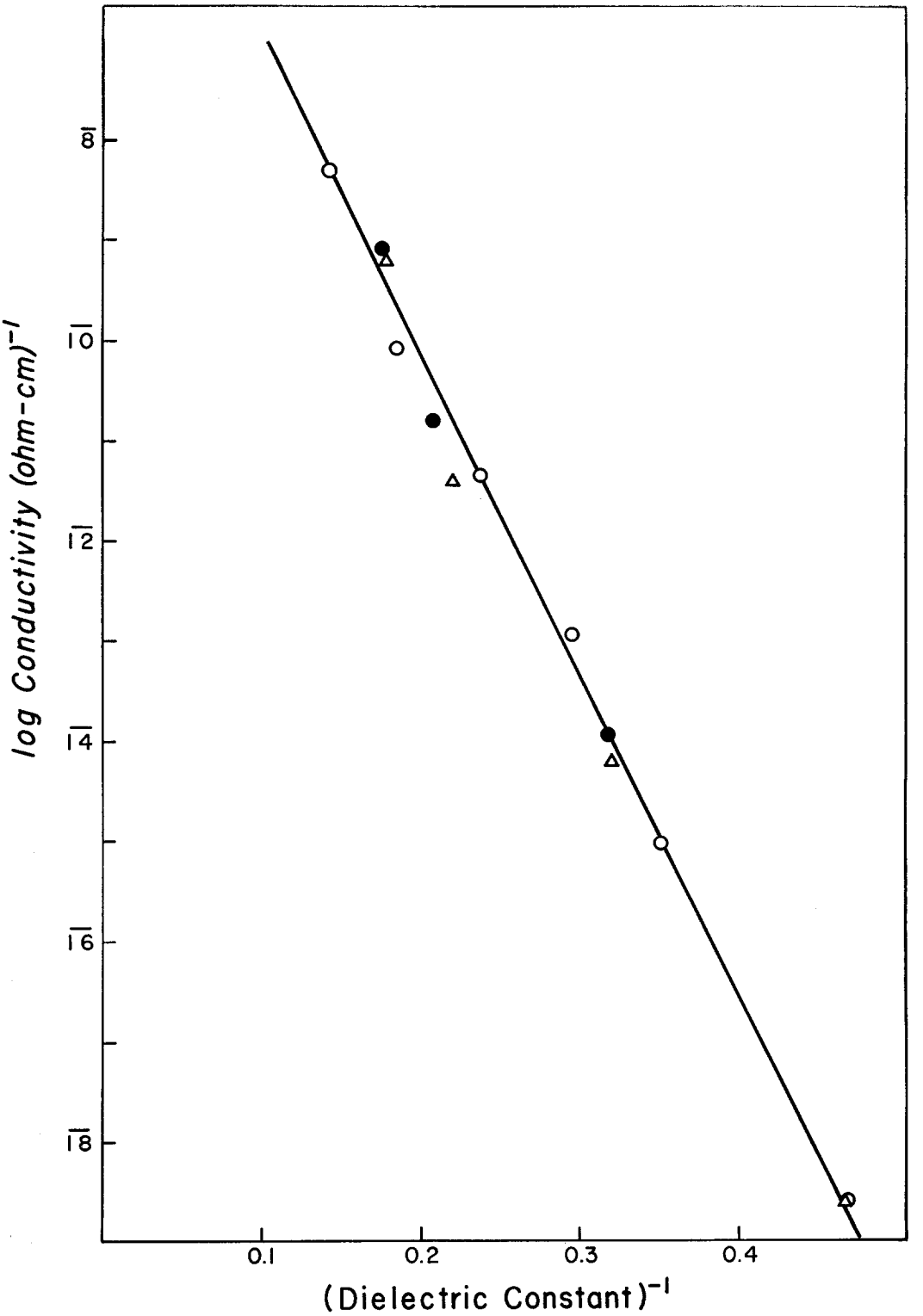

Figure 7. Variation of conductivity with dielectric constant. The circles indicate water adsorption, the triangles indicate ethanol adsorption and the filled circles indicate methanol adsorption on hemoglobin respectively.

ethanol to methanol. From Fig. 6 we can calculate the value of R as 4·3 Å. The relaxation of the dielectric medium outside a region described by a radius of 4·3 Å from the newly created charge is then responsible for the decreased activation energy, and hence higher conductivity of the hemoglobin-adsorbate system.

The effective dielectric constant of the hemoglobin-adsorbate systems does not vary as a simple function of the dielectric constant of the adsorbate. Comparing Eqs. (7) and (12) we find that:

$$2kT\alpha m = \frac{e^2}{R}(1/K - 1/K') \tag{14}$$

Among the solvents tested, R remains constant. This requires that the constant α is a function of the effective dielectric constant of the system and not a function of the adsorbate's dielectric constant. Therefore it is not to be expected that an adsorbate's efficacy in increasing the conductivity, or lowering the activation energy, of the protein should vary as the adsorbate's dielectric constant as has been proposed.[20] The activation energy for semiconduction, and hence the conductivity, varies as the reciprocal of the effective dielectric constant of the protein-adsorbate system as has been shown in Figs. 6 and 7.

Calculations of V_m, the monolayer coverage for water, methanol and ethanol are given in Table II. The monolayer coverage of water is found to be somewhat larger that the 5·76% reported by Cardew and Eley[21] the 5·30–6·35% reported by Brausse *et al.*[16] or the 6·1% found by Powell.[22] Differences between the values herein reported and those found by other investigators cannot be attributed to variation in hemoglobin samples or to protein denaturation. Cardew and Eley[21] and Eley and Leslie[23] have shown that denaturation has a small effect on the value of V_m. This is to be expected because essentially all polar amino acids are on the "outside" of the protein molecule in both the native and the denatured states. The values of V_m reported here for water and methanol adsorption 470 and 430 moles/10^5 g hemoglobin are very close to the number of polar amino acids, including proline, found in hemoblogin, i.e., 435 moles/10^5 g hemoglobin.[24] This is consistent with the model in which water, or methanol, is adsorbed on the polar sites of the hemoglobin molecule completing one BET layer when each polar site entertains one adsorbed molecule. A yet unexplainable anomaly exists in the case of ethanol adsorption on hemoglobin.

If the cross-sectional area of water is taken as 14 Å^2 [25] and the surface area of a dry spheroid of hemoglobin as 8350 Å^2 [26] then at a coverage of one BET monolayer of water 39% of the surface area of the hemoglobin molecule is covered by water. Similarly if the cross-sectional area of methanol is calculated according to Emmett and Brunauer[27] as 14 Å^2 then at one BET of methanol 46% of the protein surface is methanol covered. Assuming the polar sites are distributed uniformly on the surface of the protein molecule, then associated with each polar site is an area of 29 Å^2. When one BET monolayer of water is adsorbed the average nearest neighbor (center to center) distance will be 6 Å. The diameter of the water molecule is 3·8 Å; therefore, a single water molecule can, on the average, bridge the gap between nearest neighbor water molecules. When these gaps are bridged a contiguous path of water molecules may be found. At this time it is expected that protonic conductivity through the adsorbent water molecules will dominate the conductivity process. This may occur when less than two BET monolayers are adsorbed

which is consistent with the reports of Maričič and Pifat[28] who found 90% ionic conductivity in hemoglobin with 15% adsorbed water. Powell,[22] however, has found only 25% ionic conductivity in hemoglobin with 15% adsorbed water. This argument cannot be used to distinguish between these two reports.

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