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TECHNICAL PROGRESS REPORT

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Title: THE SOLID STATE ELECTRICAL PROPERTIES OF PROTEINS AND BIOLOGICAL SYSTEMS

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This project is to investigate the electrical properties of proteins and biological systems in the solid state, and to evaluate the relevance of these properties to biological function.

I. Compensation Law Behavior:

Our prior work has shown that the conductivity of all biologic materials follows the usual semiconductor equation:

$$\sigma(T) = \sigma_0 e^{-E/2kT} \quad (1)$$

Interaction of proteins and other substrates with water and a variety of other adsorbed molecules is described by the decrease in the activation energy E, by virtue of a change in the effective dielectric constant of the substrate. The equation describing this process is:

$$\sigma(T, K') = \sigma_0 e^{-E_D/2kT} \cdot e^{E^2/2kTR(1/K - 1/K')} \quad (2)$$

where K' is the new dielectric constant of the adsorbate-adsorbent system. For the case of proteins, only E changes, σ_0 remains a constant. We have now investigated a number of systems where this does not hold, i.e. E and σ_0 both change. The pre-exponential factor, σ_0 , is, however, a function of E. The equation describing this phenomenon is now shown to be:

$$\sigma(T) = \sigma_0' e^{E/2kT_0} e^{-E/2kT} \quad (3)$$

where a third constant, T_0 , the 'characteristic temperature' of a substance, must be introduced (or $2kT_0$, a 'characteristic energy'). From the equation, it can be

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seen that as E increases, the temperature independent part of the equation, $\sigma_0' e^{E/2kT_0}$ also increases. Thus the decrease in current expected for a larger activation energy is partially compensated for by the increase in σ_0 . This is a case of a 'compensation law' behavior. This type of behavior is fairly common in a number of phenomena. However, as yet, no general basis for such behavior has been found. Ad hoc ideas, in any given case, have been suggested. In the field of conduction, no one idea is totally free of difficulties.

Compensation law behavior described by equation 3 has been found for phospholipid crystals and bimolecular films; retinals; and nucleic acids. Proteins with the following adsorbents do not follow this behavior; water, ethanol, methanol. However, we have recently found that hemoglobin with adsorbed NH_3 and SO_2 does follow equation 3. This is a peculiarity that we do not understand and are presently investigating. Our previous work has shown that T_0 is a characteristic of the substrate only for all the systems tested. Yet here it appears that T_0 may be a function of the adsorbent as well (see Table I). Thus, two classes of cases have been found. The conductance mechanisms for these two classes may be different and therefore must be investigated.

II. The Nature of the Charge Carriers:

We have developed and tested an apparatus to evaluate the nature of the charge carriers--electronic or ionic--in the conduction of hydrated substances. The sample, between platinum electrodes, is in a vacuum system. A known hydration state is established by allowing a known amount of water vapor into the chamber. A voltage is impressed across the hydrated sample and the total charge passed for a given time is measured. If the charge carriers are electronic, no hydrogen gas is evolved. If the charge carriers are ionic or protonic, an electrode reaction occurs which liberates hydrogen gas in equivalent quantities. The hydrogen evolved is measured by means of a palladium leak valve and a McLeod gauge. The system is

calibrated absolutely by knowing the volumes of the parts; and relatively by using a known protonic conductor, such as oxalic acid, as a standard. The preliminary results so far for Hb, cytochrome C, collagen and lecithin are shown in Table II. In all cases, the carriers are mixed--both electronic and ionic (protonic) carriers contribute at each hydration state. For Hb, it appears the carriers are electronic in the dry state, and as hydration increases, the contribution of protonic carriers increases, but never (till a gel point is reached and liquid water is present) does it go above about 45%. Collagen appears to be the opposite case; in the lower hydration states it is mainly protonic, but as hydration increases, the amount of electronic carriers increases.

The apparatus appears to be adequate to turn out acceptable results in the moderate to high hydration states, but the increased sensitivity of a mass spectrometer system is necessary to probe into the lower hydration states (below 10% adsorbent).

III. Dielectric Measurements

Compacted tablets, 3.5 cm diameter 0.02 - 0.05 cm thick, are measured in a stainless steel dielectric cell (Balsbaugh Laboratories model LD-3). The high electrode is connected to a micrometer drive. Electrode separation, therefore, may be accurately determined. The ground electrode, 2.5 cm diameter, is guarded. Insulation throughout the chamber is of teflon. Regulation of the relative vapor pressure of the adsorbates in the dielectric chamber was effected in the same manner as in our conductivity chamber work.

Capacitance measurements in the frequency range of 30 Hz - 100 KHz were made with a General Radio model 1610-B Capacitance Measuring Assembly.

A 100 pF capacitor was placed in parallel with the sample capacitance. The direct reading method of determining capacitance was used throughout. In this mode, capacitance of the magnitude measured, could be determined to an accuracy of ± 0.8 pF. Dissipation factors could be determined to an accuracy of $\pm 2\%$.

Some extremely low frequency, 0.1 - 10 Hz, measurements of capacitance were

made with a guarded bridge designed by Nakajima and Saito (1958). The assembly was built by the Ando Electric Co. Ltd. of Tokyo, Japan.

The capacitance of the sample was measured in a vacuum, 10^{-2} torr, by enclosing the dielectric sample chamber in an outer brass chamber which was evacuated with a mechanical pump. All insulation in the outer chamber was of teflon and all leads were shielded. The outer chamber was at ground potential.

Dielectric constants are calculated from measurements of the capacitance according to the formula:

$$\kappa = C_S / C_A \quad (4)$$

where κ is the dielectric constant of the material, C_S is the capacitance of the sample and C_A is the capacitance of an equal geometry of air. In general the capacitance, and hence the dielectric constant, of a specimen will vary with frequency. Having made direct current conductivity measurements it appeared most appropriate to make dielectric measurements in the log frequency region of the spectrum.

When only a small quantity of water is adsorbed on the hemoglobin the frequency dispersion is flat. However, as the amount of adsorbed water increases, a considerable low frequency dispersion in the dielectric constant is found. The low frequency capacitance of a hydrated hemoglobin tablet does not vary inversely with the thickness of the tablet. Electrode polarization, which is independent of sample thickness would account for this result. However, the same results were found when capacitance measurements were made with either an air gap or a sheet of teflon placed above the sample. In the case of air gap measurements the moveable electrode is not in contact with the hemoglobin tablet. The equivalent capacitance of the tablet in series with an air capacitor is measured by the bridge. Measurements were made with a variety of air gaps. The capacitance of the tablet is then obtained by extrapolation to the zero air gap condition. An air gap or a sheet of teflon would act as a blocking electrode and should greatly diminish

electrode polarization. If electrode polarization is diminished the low frequency dispersion would be altered. As this was not the case, it is believed that electrode polarization is not responsible for the low frequency dielectric dispersion in hydrated hemoglobin.

A large positive temperature coefficient is found to be associated with the low frequency dielectric dispersion. This is in agreement with studies of O'Konski, Moser and Shirai (1964) on nucleic acids. At relatively low temperatures ($\sim 25^\circ\text{C}$) the low frequency dielectric dispersion is not found and the capacitance of a hydrated hemoglobin pellet is found to vary inversely with the thickness of the sample.

It appears that, at low frequencies, the capacitance of hydrated hemoglobin varies inversely as a power of the frequency.

When the data is plotted on a log-log graph, it is found that the capacitance varies as $\omega^{-0.35}$. This frequency dependence continues to 200 - 500 Hz for the data at 76% relative humidity. At lower hydration states this effect terminates at lower frequencies. An examination of the dielectric data of Maricic, Pifat and Pravdic (1964b) indicates that the same frequency dependence was found, but not discussed by these workers. The adsorption of methanol and ethanol on hemoglobin produces similar results.

The apparent low frequency dielectric dispersion of hemoglobin with various adsorbates is believed to be the result of a polarization effect, probably of the Maxwell-Wagner variety. In the region 10^4 - 10^5 Hz, polarization effects are negligible. Therefore, the dielectric constant has been calculated from the capacitance as measured at 10^5 Hz. The dielectric constant is corrected for the air filled interstices found in compacted tablets. This is done by extending Bottcher's (1962) treatment of a powder to the case of a compacted tablet. One then obtains for the dielectric constant of the crystallite material:

$$\kappa = \frac{3\kappa_p \delta + 2\kappa_p (\kappa - 1)}{3\kappa_p \delta - (\kappa_p - 1)} \quad (5)$$

where κ is the dielectric constant of the crystalline material; κ_p is the dielectric constant of the tablet (given as κ in equation 4) and δ is the packing fraction, or partial volume of crystalline material in the pellet.

The dielectric constants of hemoglobin with adsorbed water, ethanol and methanol are presented in Table III. Water, methanol and ethanol, when adsorbed on hemoglobin increase the effective dielectric constant of the sample. Concomitant with this increased dielectric constant a decrease is found in the activation energy for semiconduction.

The low frequency dielectric dispersions attributed to electrode polarization, in aqueous solution measurements, possess a frequency dependence which is very different from that found for hydrated compacted tablets. Electrode polarization, in the former case, varies as the -1.5 to -1.7 power of the frequency.

Employing Scheider's (1962) model of a lumped polarization capacitance in series with the specimen conductance, which is in turn in parallel with the specimen capacitance:

The admittance of the network is given by:

$$Y = G + i\omega C_S + \left\{ \frac{1}{1 + \frac{\omega^2 C_P^2}{G^2}} \right\} (-G + i\omega C_P) \quad (6)$$

where G is the sample conductance, C_S is the sample capacitance, C_P is the polarization and ω is the frequency of the impressed voltage. The real part of the admittance is the conductance, and the imaginary part is the capacitance. In the case of measurements of biomolecules in aqueous solutions it is assumed that:

$$\frac{C_P^2}{G^2} \gg 1 \quad (7)$$

Under this condition the apparent parallel polarization capacitance, C_A , the

second imaginary term in Equation 6, is:

$$C_A = \frac{G^2}{\omega^2 C_P} \quad (8)$$

According to Wolff's (1936) direct measurement of low frequency polarization capacitance in aqueous solutions C_P varies as the -0.5 to -0.3 power of the frequency. C_A , the apparent polarization capacitance should then vary as the -1.5 to -0.3 power of the frequency, which is found to be the case in the measurement of the capacitance of aqueous solutions of biomolecules. However, in the case of hydrated hemoglobin tablets typical low frequency values would be $C = 10^{-10}$ farad and $G = 10^{-7}$ mho at $\omega = 100\text{Hz}$. Therefore, it is more reasonable to assume:

$$\frac{\omega^2 C_P^2}{G^2} \ll 1 \quad (9)$$

in which case

$$Y = i\omega(C_S + C_P) \quad (10)$$

The measured capacitance should then be the arithmetic sum of the specimen capacitance and the polarization capacitance. If, in the low frequency region the sample capacitance does not exhibit a dispersion, the frequency dependence of the measured capacitance will be that of the polarization capacitance. This is the experimental result which is found, i.e.:

$$C \propto \omega^{-1/3} \quad (11)$$

When these polarization effects are subtracted, the low frequency dielectric dispersion in hydrated hemoglobin tablets disappears.

Low frequency dielectric dispersion is most probably the result of a Maxwell-Wagner type of process. This type of polarization arises at the boundary between two materials which do not possess the same conductivity to dielectric constant ratio.

This boundary polarization may be associated either with the interface between the electrode and the hydrated tablet or with the interface between the crystallites and the interstices.

IV. Comparison of Results with Dielectric Theory

The adsorption of water, ethanol or methanol alters the conductivity of hemoglobin in such a way that the conductivity increases while the semiconduction activation decreases. The pre-exponential factor in the conductivity equation is unchanged by the adsorption process. The theoretical equation 2 predicts a linear dependence of activation energy on the reciprocal of the effective dielectric constant. Since the log of the conductivity varies linearly with activation energy, a similar relation should exist between the log of the conductivity and dielectric constant.

When the reciprocal of the dielectric constant is plotted against the log of the conductivity, for water, ethanol and methanol, they all fit the same straight line. Interpreted in terms of the theory this indicates that, according to Equation 2, R , the polarization radius, is independent of the adsorbate. If the reciprocal of the dielectric constant is plotted against the activation energy a value of R may be calculated. In all cases, $R = 4.3 \text{ \AA}$. The relaxation of the dielectric medium within a region of radius $4.3 \text{ \AA}$ is responsible for the decreased activation energy and hence higher conductivity of hemoglobin samples with adsorbed water, ethanol or methanol.

The effective dielectric constant of hemoglobin with the same mole percent of the various solvents adsorbed does not vary as the dielectric constant of the solvent.

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TABLE I

Compensation Law Behavior in Hemoglobin-Adsorbent Systems

<u>System</u>	<u>T₀ (°K)</u>	<u>$\sigma'_0 (\Omega\text{-cm})^{-1}$</u>
Hb - H ₂ O	∞	$\sim 10^2$
Hb - Ethanol	∞	$\sim 10^2$
Hb - Methanol	∞	$\sim 10^2$
Hb - NH ₃	500	1.5×10^{-12}
Hb-SO ₂	500	1.5×10^{-12}

TABLE II

Contributions of Ions (Protons) and Electrons to Conductivity in Hydrated Substances

<u>Substance</u>	<u>Hydration State</u>	<u>% Ionic (Protonic)</u>	<u>% Electronic</u>
Hb	11.4	32	68
	14.4	28	72
	16.5	19	81
	17.5	27	73
	19.2	31	69
	23.5	36	64
	31	44	56
	31	45	55
	32	43	57
	32	46	54
Collagen	11.5	93	7
	15.5	92	8
	15.5	88	12
	18.5	84	16
	30	70	30
	31	73	27
	36	64	36
Cytochrome C	11.5	72	28
	14	83	17
	16.8	50	50
	20.7	64	36
	23.0	59	41
	30	69	31
	38	90	10
Lecithin	9.0	76	24
	10.4	67	33
	12.4	70	30
	15.2	73	27
	16.8	81	19

TABLE III

Dielectric Constant and Activation Energy of Hemoglobin
with Adsorbed Water, Methanol and Ethanol

Adsorbate	m(mole %)	E (eV)	Dielectric Constant
None	0	2.35	2.3
water	.355	1.95	2.9
	.656	1.77	3.4
	1.01	1.55	4.2
	2.2	1.44	5.5
	---	1.23	7.4
methanol	0.203	1.75	3.2
	0.53	1.45	4.6
	---	1.20	5.9
ethanol	0.073	1.8	3.1
	0.35	1.6	4.5
	---	1.5	4.9

PUBLICATIONS REPORTING ON WORK SUPPORTED BY THIS CONTRACT

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3. Barnett Rosenberg and Elliot Postow, "Semiconduction in Proteins and Lipids and Its Possible Biological Import", Annals, N. Y. Acad. Sci. 1969 - In Press.