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The Nature of the Charge Carriers in Solvated Biomacromolecules

Michael R. Powell and Barnett Rosenberg

Department of Biophysics, Michigan State University
East Lansing, Michigan 48823

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Abstract

Solid state electrolysis experiments were performed on the biomolecules, hemoglobin, cytochrome *c*, collagen, lecithin and melanin at various hydration states; and for hemoglobin at various solvation states with methanol adsorbate. The evolved hydrogen was measured and compared with theoretical (Faraday's Law) expectations for the known amount of charge passed through the adsorbents. The difference between the theoretical and actual is a measure of the contributions of electronic charge carriers to the total current. Thus the protonic/electronic conduction ratios are determined.

All biomolecules tested appear to be mixed semiconductors. That is, both electronic and protonic charge carriers make significant contributions to the currents over hydration ranges from 6% to above 50%. The constant temperature conductivity increases exponentially with hydration (solvation) but the ratio of protonic to electronic conduction increases linearly with hydration for the globular proteins, hemoglobin and cytochrome *c*. The fibrous protein, collagen, may be a protonic semiconductor in the "dry" state, with an electronic component that increases linearly with hydration. The hemoglobin-methanol system shows only electronic conductivity below 2 BET monolayers, with a sharp onset to 70% protonic conductivity above this value. This result is similar to the DNA-water system previously reported. The protonic/electronic ratio in hydrated hemoglobin may be a function of the applied voltage; being predominantly electronic below 30 volts (300 volts/cm), and a constant mixed value above 100 volts (1000 volts/cm). Our results suggest that both electronic and protonic conduction are intrinsic processes in these substances and subject to control by a number of techniques.

I. *Introduction*

All biologically interesting molecules in the solid state are semiconductors. It is natural therefore to speculate that electronic semiconduction may be a possible step in energy transfer in many biological processes. Rosenberg and Postow[1] have suggested that hydrated enzymes could have electrical conductivities high enough to act as electron pathways in redox reactions. This problem has also been treated from a kinetic point of view by Cope.[2] Electron transfer at liquid helium temperatures has been found in bacteria by Calvin and Androes.[3] Digby[4] has shown that the cuticle of various crustacea are electron conductors and has suggested that this may be important in the calcification process. Rosenberg[5] has suggested its function in the vision process, and Misra *et al.*[6] have proposed it as a part of the olfaction process. Recent experiments by Tien[7] and Rosenberg and Pant[8] have provided some evidence for electronic conduction in bimolecular lipid membranes.

In general, it is not known whether the dominant charge carriers in such substances are electronic, protonic or ionic. We have therefore undertaken a study to investigate the

nature of the dominant charge carriers in some solvated (water and methanol) biomacromolecules. We report here of our results on hemoglobin, cytochrome-*c* (globular proteins); collagen (fibrous protein); lecithin (lipid); and melanin (a biopolymer). Our results on DNA (nucleic acid) are reported elsewhere.[9]

The first studies on the nature of the dominant charge carriers in hydrated systems were made by Baxter.[10] He determined that the activation energy for electrical conduction in hydrated fibers was greater than the activation energy for conduction in water. He concluded that conduction by water channels in the fiber was not possible, and he proposed an electron tunnelling mechanism.

King and Medley[11] later studied the keratin-water system at 18% hydration and found that the hydrogen evolution yield during solid state electrolysis was 92% of the theoretical amount. From this they concluded that the conductivity was by an ionic mechanism, although they found very little concommitant oxygen evolution.

Maricic *et al.*[12] studied the hemoglobin-water system and concluded that there was no protonic conduction detectable by them at hydration states below 1·5 BET monolayers, but that a very large fraction of the current carriers were protonic at coverages of four BET monolayers. The DNA-water system was also investigated by Maricic and Pifat.[13] They found a large protonic component (about 80%) at hydrations greater than 37%; no hydrogen evolution was detectable with their apparatus below this hydration state.

Electrolysis experiments were performed by Murphy[14, 15] on dry cellulose and he found almost quantitative yields of hydrogen evolution. He concluded that cellulose and fibrous proteins were ionic conductors. Seanor[16] found that dry Nylon-66 was an electronic conductor at temperatures below 90° C and it became increasingly protonic as the temperature was increased.

II. *Experimental Methods*

The compounds which we used were commercial preparations (with the exception of melanin) and they were not further purified. The sources are as follows: (i) hemoglobin (Gallard-Schlesinger), (ii) cytochrome-*c* (Sigma Chemical Co.), (iii) collagen (Ethicon Corp.), (iv) lecithin (synthetic, Nutritional Biochemical Co.), and (v) melanin (synthetic, made by E. Postow of this laboratory by auto-oxidation of DOPA, and extensively dialysed).

Figure 1 shows the apparatus used to determine the adsorption isotherms. It consists of a mechanical and oil diffusion pump (the system was pumped initially to about 3×10^{-4} torr), a mercury manometer and Dubrovin gauge, water reservoir, Cahn RG vacuum electrobalance, conductivity cell, and dielectric cell. For adsorption isotherm measurements, approximately 50 mg of a compound were placed on the pan of the Cahn balance; this was then counterweighted so that full scale readings would be 10 mg. With this arrangement, weight changes of $\pm$ 0·01% of the sample weight could be detected. The samples were dried on the balance pan under a vacuum, by heating with an electrical heating tape to about 50° C, until subsequent weight changes were less than 0·01% after four hours. The sample was maintained at 26·0° C, thermostatically controlled by a water bath. Buoyancy corrections were small for water vapor and not included in the data.

In the pressure range of 0 to 10 torr, the Dubrovin gauge (readable to 0·1 torr) was used. In the pressure range of 10 to 25 torr, the pressure was controlled by thermostating the water in the liquid reservoir. Since the water temperature could be measured to 0·1° C, the vapor pressure could be controlled with good accuracy.

All samples were found to follow adsorption isotherms of the BET type (multilayer adsorption), and the results are shown in Figs. 2 and 3. From the BET equation, the monolayer coverages could be determined and the results appear in Table 1.

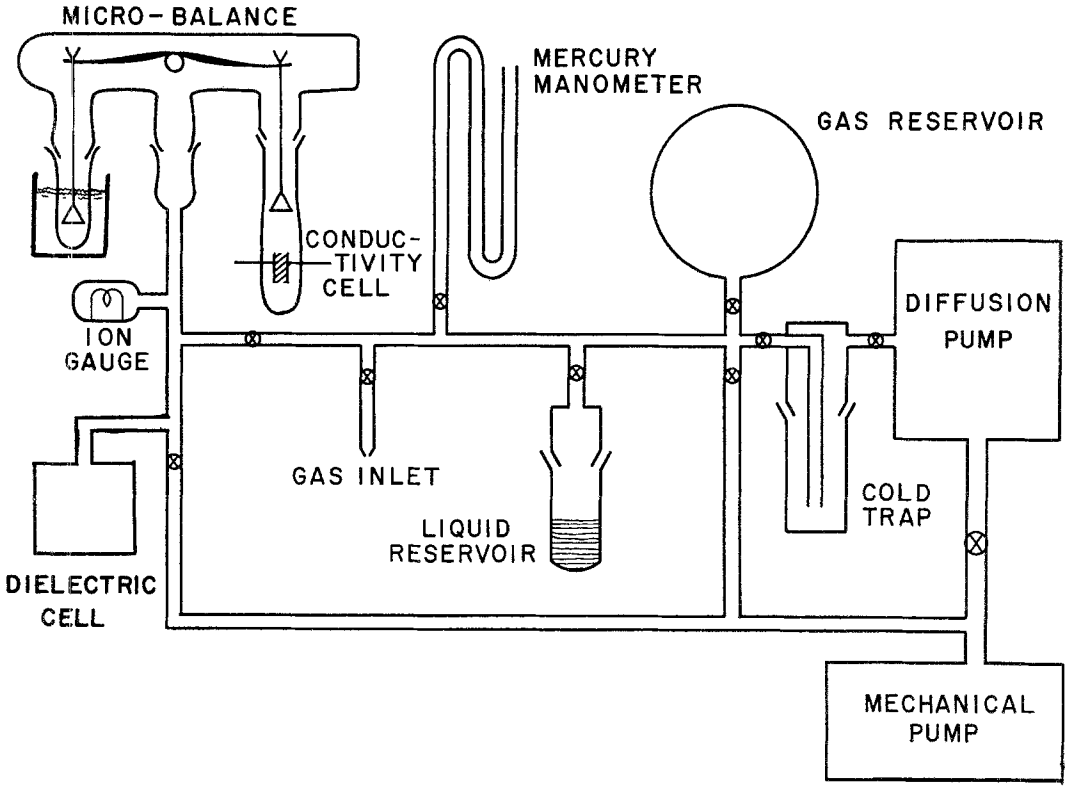

Figure 1. Schematic diagram of the vacuum microbalance apparatus used to determine the adsorption isotherms, and the conductivity and capacitance versus percent hydration.

The constant temperature conductivity of the material as a function of adsorbed water was measured by using the sample in a sandwich cell placed in the vacuum line and connected to a voltage supply and electrometer by tungsten feedthroughs. The collected results are shown in Fig. 4. The amount of adsorbate is given in BET monolayers.

Capacitance measurements were made in the same vacuum line with a Teflon insulated, stainless steel dielectric cell (Balsbaugh LD-3) connected to a General Radio 1610-B Capacitance Measuring Bridge. With this apparatus, the capacitance of the sample as a function of the adsorbed water could be measured simultaneously with the other parameters. The measurements were made at 10^3, 10^4, and 10^5 Hz. The results for the three compounds measured (lecithin, cytochrome-*c*, and hemoglobin) are shown in Figs. 5 to 7.

III. *Electrolysis Measurements*

The apparatus used for the solid state electrolysis measurements is shown in Fig. 8. It was of all glass construction with the exception of the valves (Delmar) which were

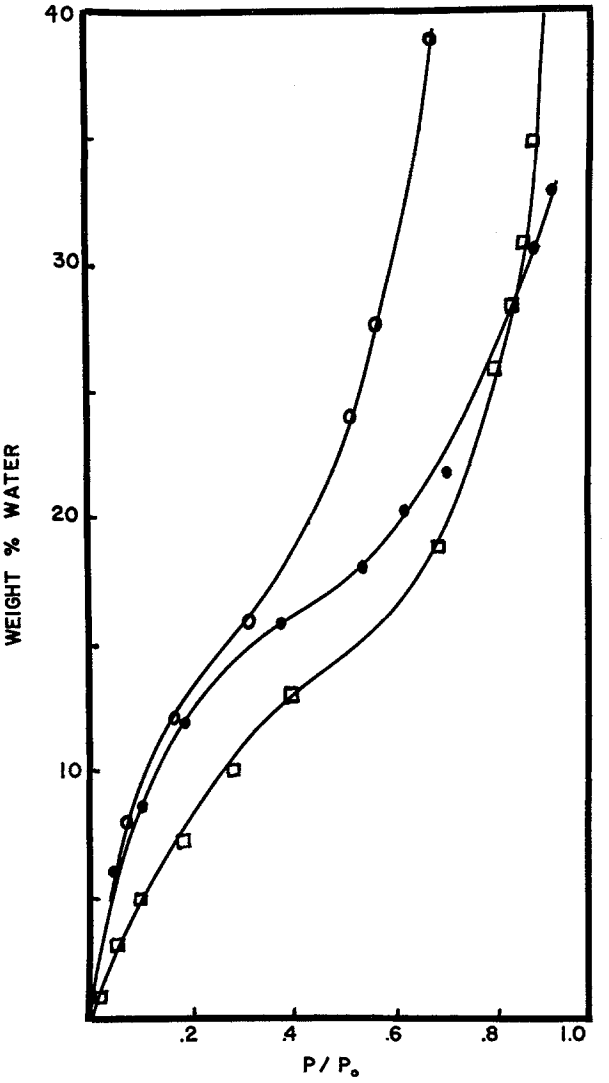

Figure 2. Adsorption isotherm of water on melanin, collagen, and salmon sperm DNA at 26·0° C. The solid circles are melanin, the open circles are DNA, and the squares are collagen.

partly of Teflon. The samples were sealed into the sample chambers and no glass joints were used. Electrical connections were made with Tungsten feedthroughs.

The manifold was evacuated through a mechanical pump and a three-stage oil diffusion pump. A pressure of 5×10^{-6} torr (exclusive of the mercury from the McLeod gauge) could be produced. When the valve to the pump was closed, the system pressure

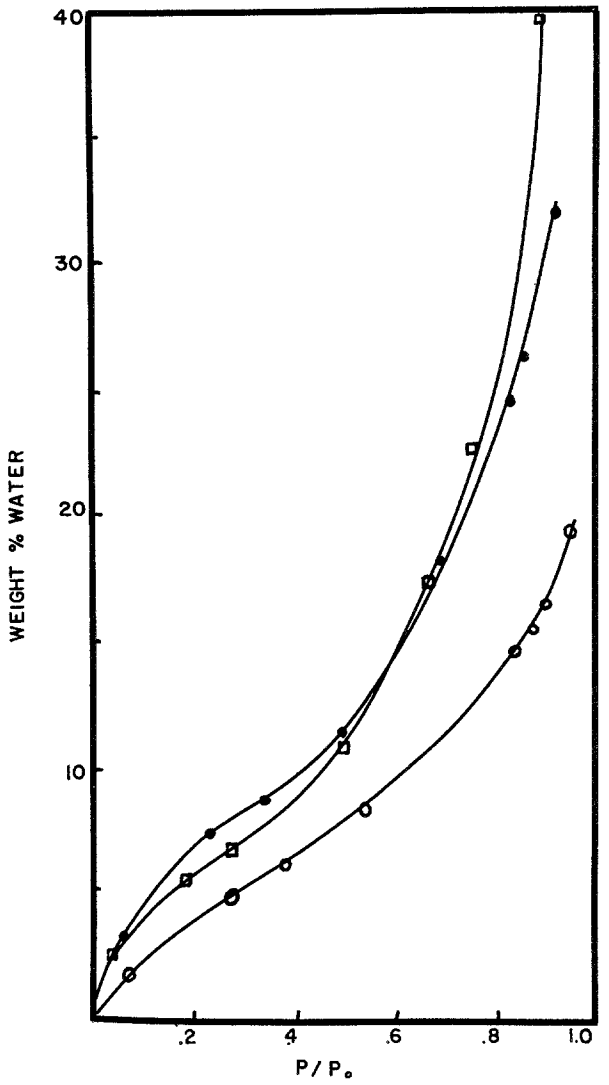

Figure 3. Adsorption isotherm of water on lecithin, cytochrome-*c*, and hemoglobin at 26·0° C. The solid circles are the hemoglobin, the open circles are the lecithin, and the squares are the cytochrome-*c*.

TABLE I

Compound	Adsorbate	BET Monolayer
Collagen (at 26·0° C)	water	7·9%
Hemoglobin	water	6·1%
Melanin	water	12·4%
Lecithin	water	4·6%
Cytochrome-*c*	water	6·3%
Hemoglobin (23° C)*	methanol	13·5%

* Reference 17

would rise with time, because of out-gassing, to about 0·1 millitorr depending on how well the glass had been baked-out.

The samples were placed in a sandwich cell made of platinum foil mounted on a

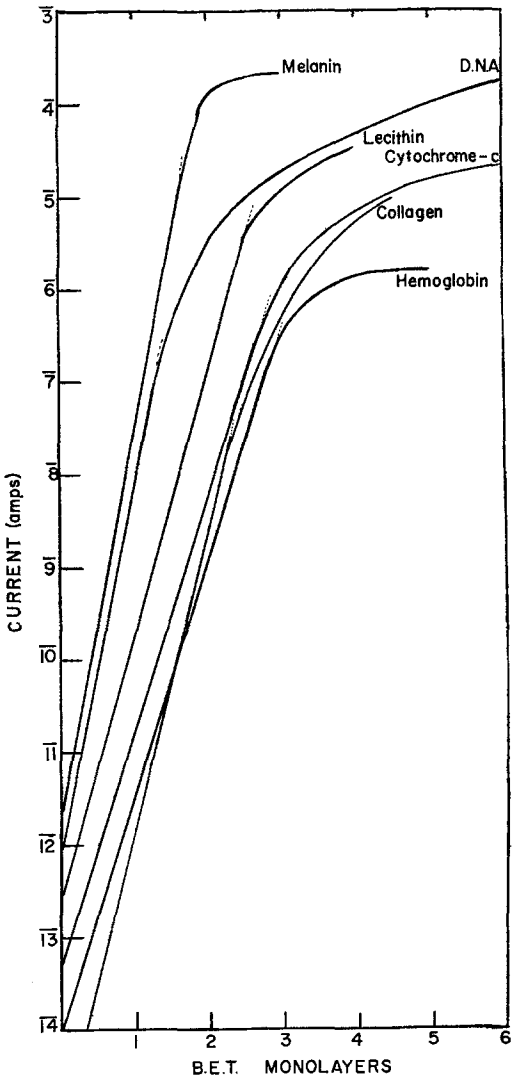

Figure 4. Constant temperature (26° C) conductivity of the biomolecules as a function of the monolayer coverage with water.

Teflon block. The samples were hydrated by closing the valve to the pump and opening the valve to the water reservoir. With the sample at $T_1 = 26{\cdot}0°\,C$ and the reservoir at T_2, the ratio of the vapor pressures of water at these two temperatures (p/p_0) determined the amount of hydration on the sample. The samples used in these electrolysis experiments were from the same source that the adsorption isotherms had been made on.

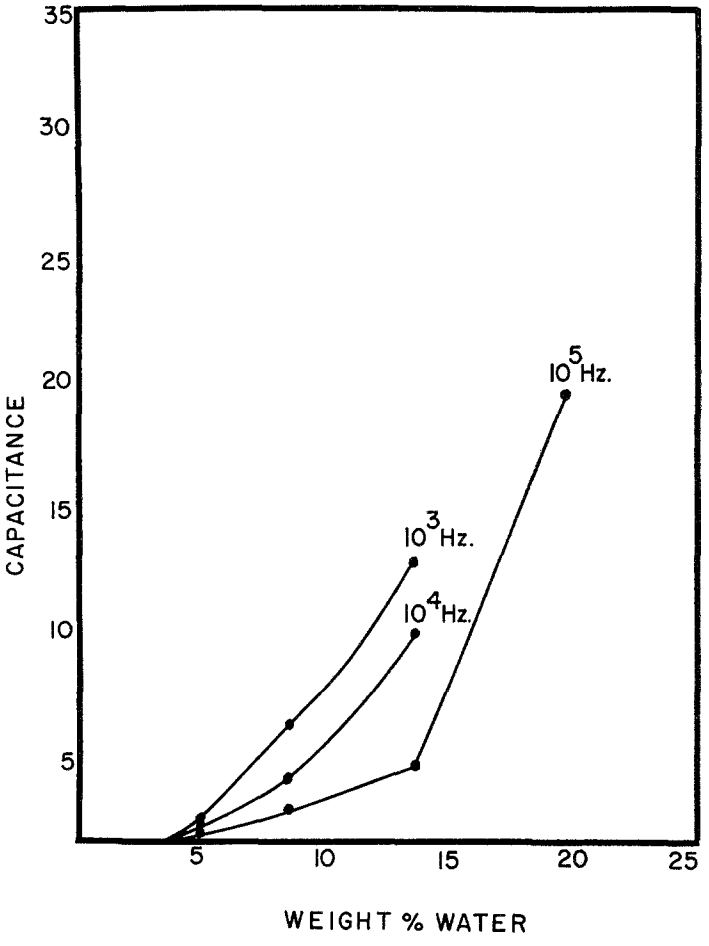

Figure 5. Capacitance vs. hydration for lecithin.

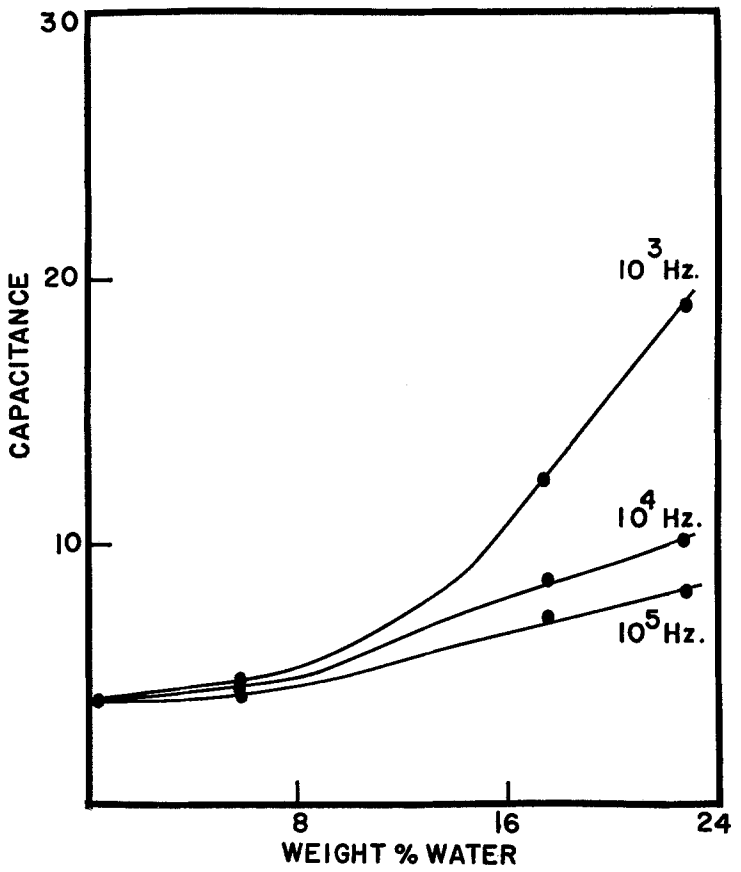

Figure 6. Capacitance vs. hydration for cytochrome-*c*.

The dc voltage for the electrolysis of the hydrated samples was kept between 50 and 200 volts. The number of coulombs passed was determined using an electrometer and strip-chart recorder by graphical integration. From a knowledge of this quantity, the number of equivalents of hydrogen generated (assuming 100% ionic conduction) could be calculated using Faraday's law of electrolysis. With a knowledge of the manifold volume, the number of equivalents generated could be expressed as a pressure change.

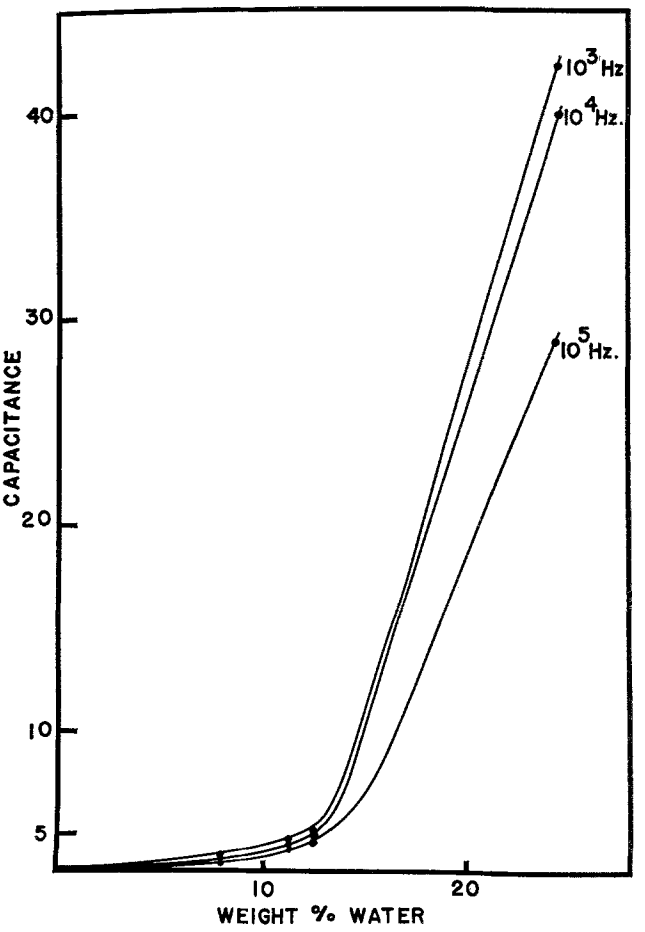

Figure 7. Capacitance vs. hydration for hemoglobin.

At the conclusion of the electrolysis, which generally took about two hours, the gas was analyzed by condensing out the water in the trap and measuring the total pressure with the McLeod gauge. By heating the palladium tube, the hydrogen was allowed to diffuse out and a second pressure measurement with the McLeod gauge was made. The difference between the first and the second readings was the hydrogen pressure evolved. Because of the specific permeability of palladium to hydrogen, no further chemical analysis was needed.

The limit of the detectability was set by the "background" pressure caused by the outgassing of the glass walls. When this was reduced to about 1×10^{-4} torr, the sensitivity of the apparatus with our volume (422 cm^3) was about 10^{-9} moles.

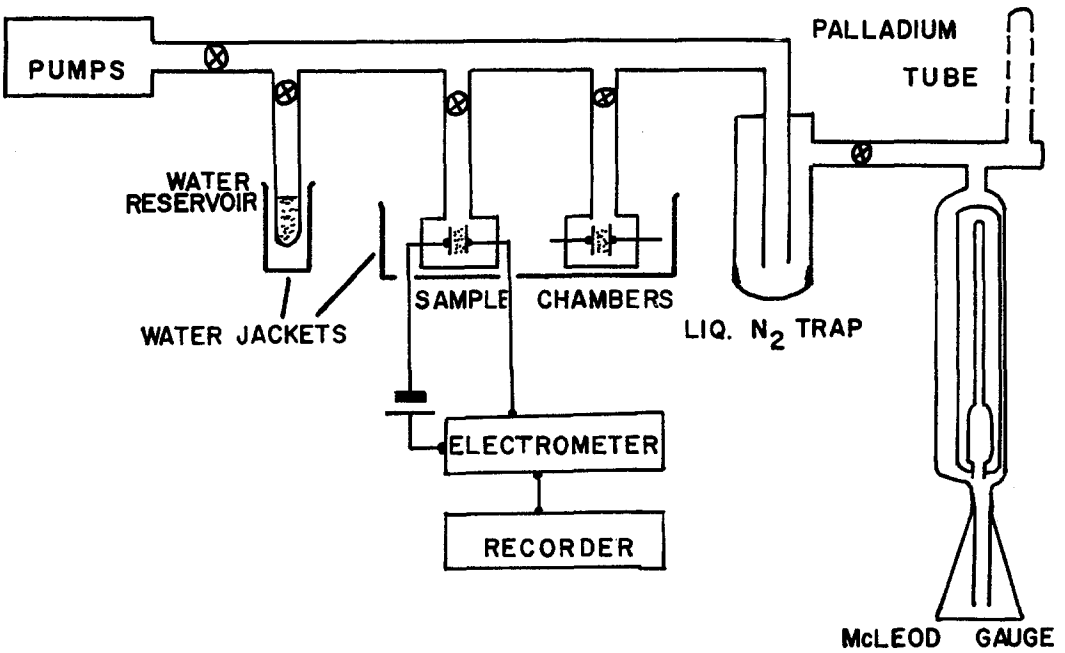

Figure 8. Schematic diagram of the solid state electrolysis apparatus.

The apparatus was designed to operate with fundamental measurements (volume, pressure, temperature), and not need an external calibration. A pressure measurement with a McLeod gauge on a manifold of known volume accomplishes this aim. However, calibration tests were made to insure that small amounts of evolved hydrogen could be detected. Two samples of oxalic acid dihydrate were electrolysed and the resultant yields were 112% and 98% of the theoretically expected. Studies by Pollack and Ubbelohde[18] on the conductivity and activation energy of these crystals, led to the conclusion that these crystals were protonic conductors. When a Teflon spacer was used in the holder in place of the sample, the leakage current was found to be only 10^{-10} amps at 500 volts and $p/p_0 = 1{\cdot}0$. This was less than 10^{-6} of the current which would pass through a sample under the same conditions.

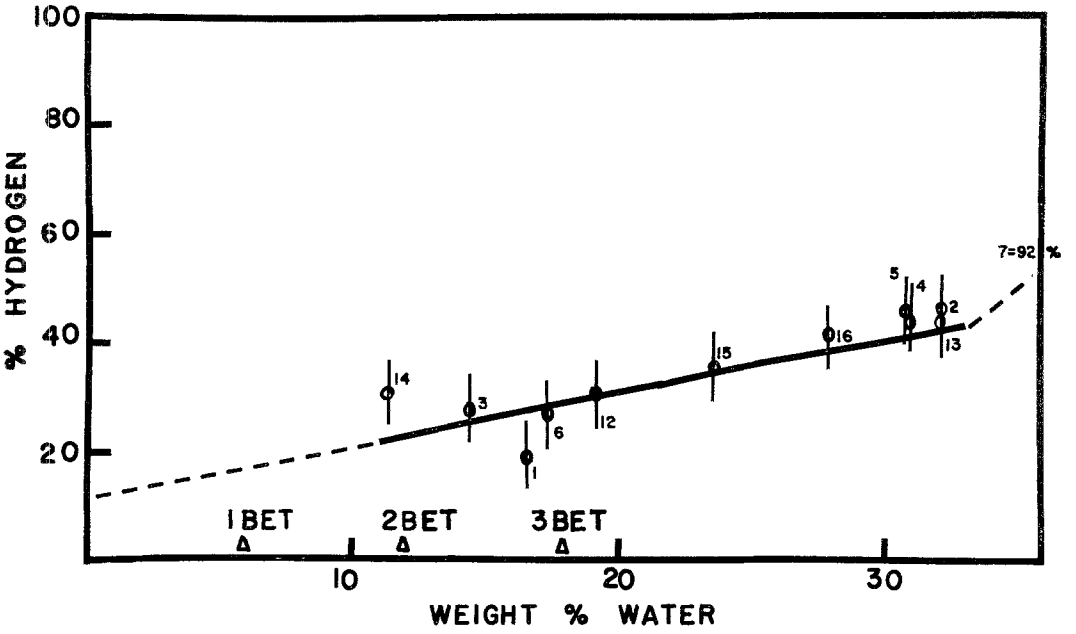

Figure 9. The yield of hydrogen as a function of hydration for hemoglobin.

The yields of hydrogen at various states of hydration for the five materials tested here are shown in Figs. 9 through 13. The hydration level in terms of BET monolayers for each compound is given by the small triangles above the figures for weight percent water adsorbed. The small figures beside each data point indicate the experiment number,

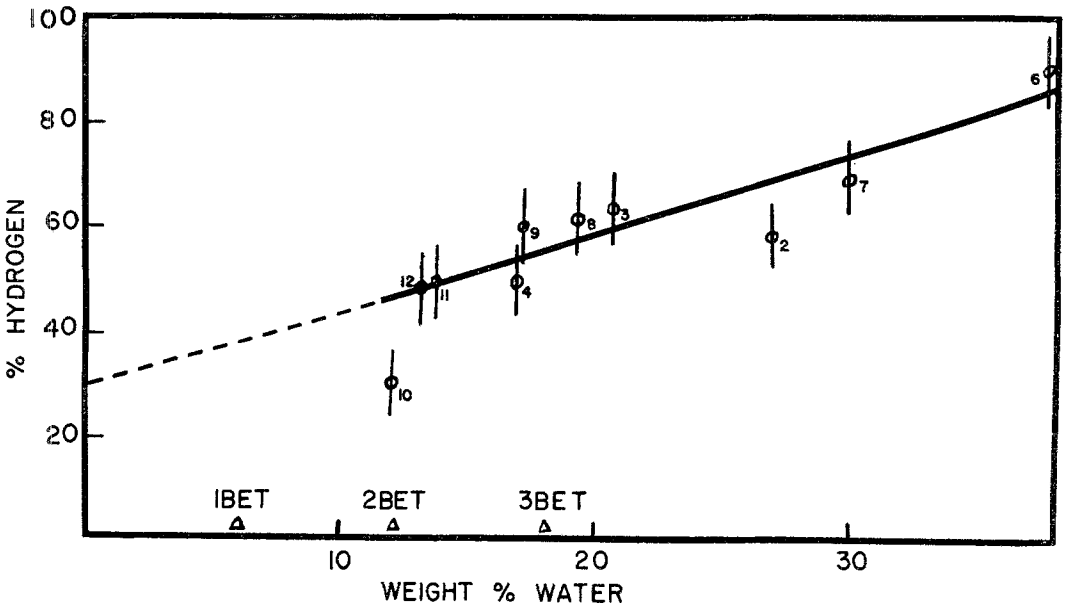

Figure 10. The yield of hydrogen as a function of hydration for cytochrome-*c*.

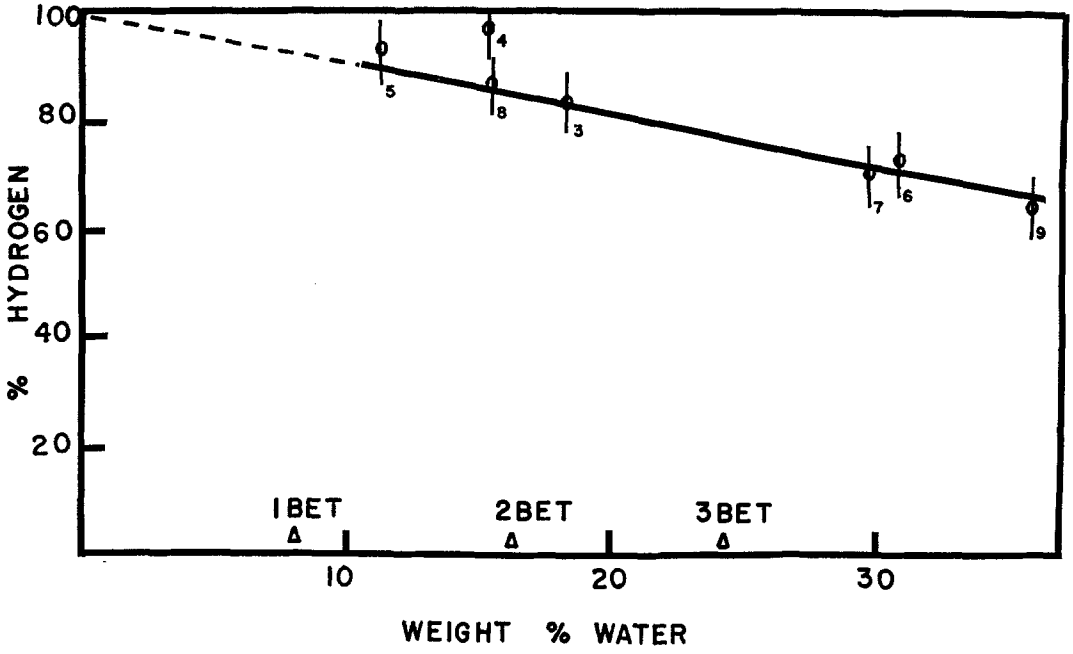

Figure 11. The yield of hydrogen as a function of hydration for collagen strips.

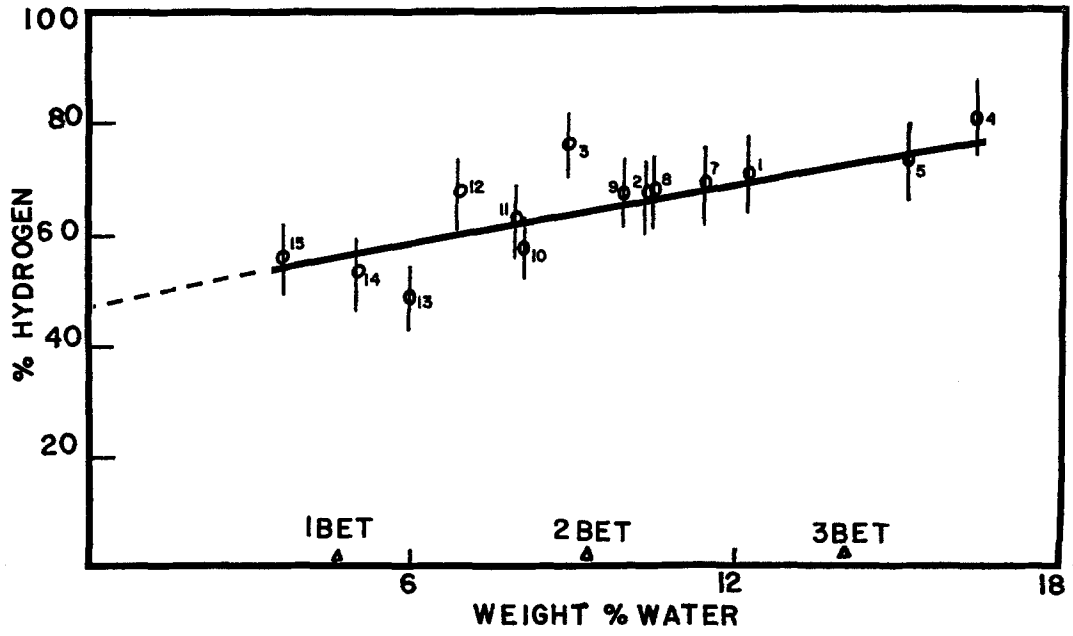

Figure 12. The yield of hydrogen as a function of hydration for lecithin.

and thus the order in which the electrolysis runs were made. The hydration states were randomly varied. In all cases, except for methanol on hemoglobin, the hydration, dehydration, and rehydrations were reversible.

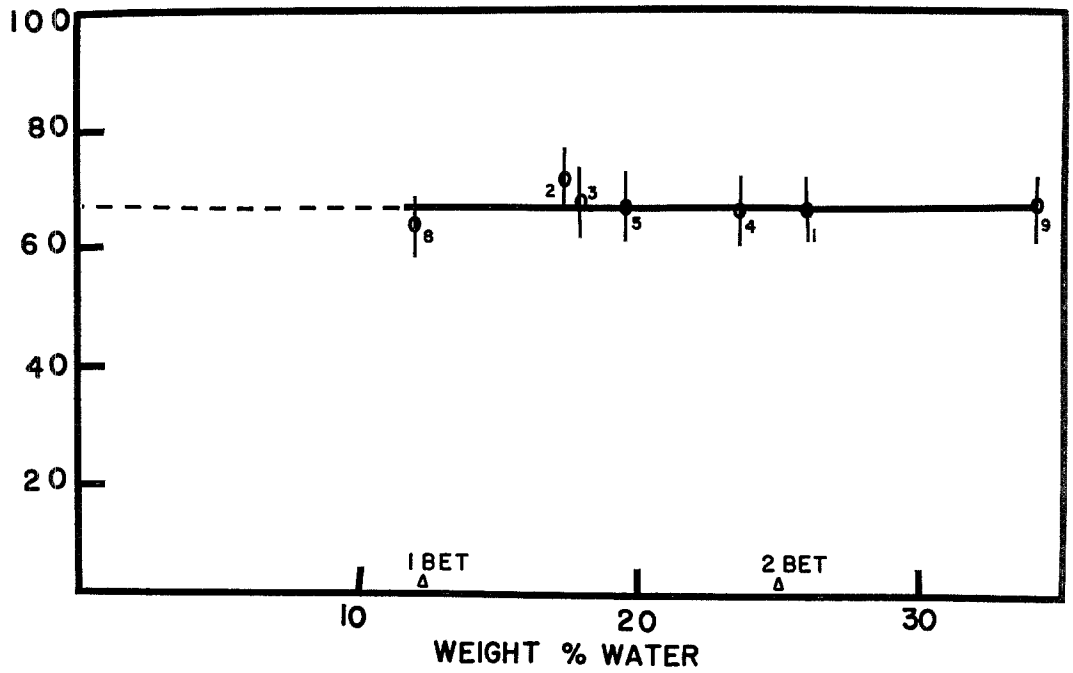

Figure 13. The yield of hydrogen as a function of hydration for melanin.

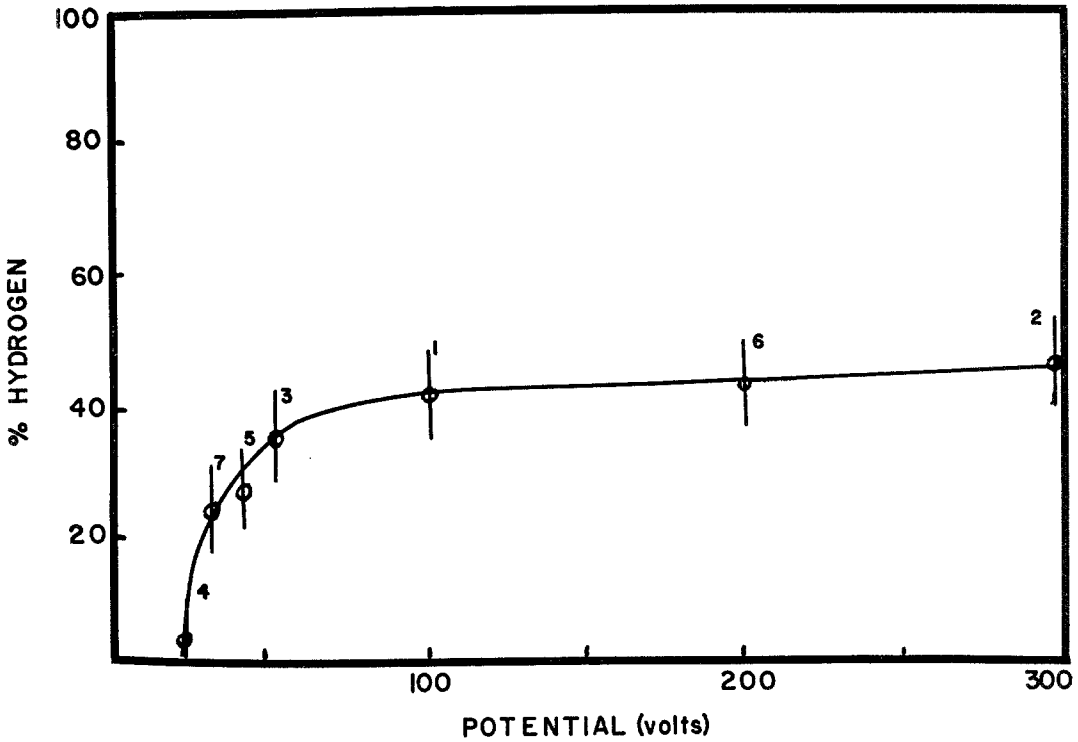

Figure. 14. The yield of hydrogen as a function of voltage for one hydration state (28%) of hemoglobin.

The yields of evolved hydrogen are expressed as percent of the theoretical yield if all charge carriers discharge hydrogen at the electrode. If electronic charge carriers could be injected by the electrodes, this would contribute to the current but would not evolve hydrogen. Therefore, since the electrodes used could not inject protons, any departure from 100% hydrogen is evidence of the electronic nature of some fraction of the current carriers. 100% hydrogen indicates a purely protonic (or ionic) charge carrier: 0% hydrogen indicates a purely electronic charge carrier; and intermediate values indicate the relative contributions of the two carrier types.

In no case was there found to be an abrupt change in the electronic/protonic charge carrier ratio in the range of two to four BET monolayers. This was previously suspected as a possible occurrence when water molecules formed a continuous coverage over the

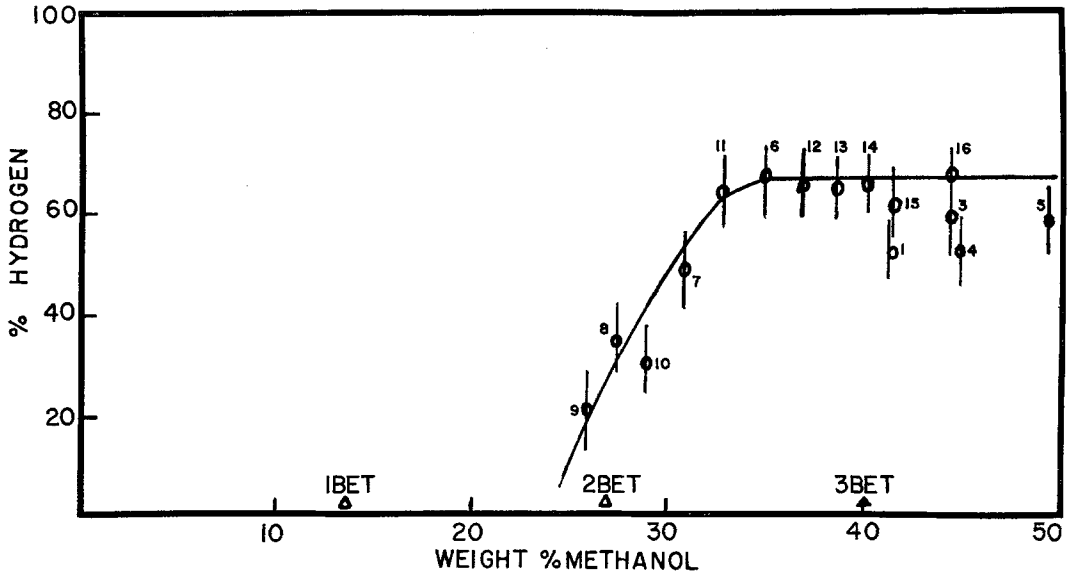

Figure 15. The yield of hydrogen as a function of solvation (methanol) for hemoglobin.

surface of the molecule. On the contrary, the change in carrier ratio was found to be approximately linear with hydration.

Further electrolysis studies were made on hemoglobin. It was found in earlier work that potentials on the order of 400 volts would produce very large amounts of hydrogen evolution in the dryer samples (this could often be greater than the predicted amount). We have therefore tested the effects of varying the applied voltage. One hydration state was maintained in a hemoglobin sample (28%, or $4\frac{1}{2}$ BET monolayers) and the applied voltage was varied. The sample was electrolysed from 25 volts applied (field strength = 250v/cm) to 300 volts applied (3000 V/cm). The results are shown in Fig. 14. At potentials of less than 50 volts applied, the hydrogen yield drops precipitously to a point where the hydrogen was undetectable. Above about 100 volts applied, the hydrogen yield was almost constant. There was also found to be a large deviation from Ohm's law in the hydrated states of all five compounds tested at less than 30 volts applied. Deviations were also found for hemoglobin solvated with methanol and ethanol.

This difference in hydrogen yield could be the result of a change in the number of protonic carriers at high potential (cf. the Wien effect) or a difference in the efficiency of hydrogen evolution at the sample-metal electrode interface. Further work along this line is presently in progress.

The solvents which are capable of effecting a conformation change in a macromolecule can produce a non-linear change in the charge carrier ratio with solvation. This is believed to be true for the water-DNA system.[9] Water on DNA produces reversible changes in the conformation of the bases. Methanol adsorbed on hemoglobin can cause irreversible effects. Electrolysis of this latter system shows an abrupt change in the charge carrier ratio at about the 2 BET monolayer coverage. This is distinctly different from the linear relation and is shown in Fig. 15. In addition, to the extent that we were able to determine it, the methanol-hemoglobin system does not seem to exhibit the sharp (within one order of magnitude) saturation of current as is exhibited by most materials with adsorbed water. The rise is a slower continuous one, similar to that seen in Fig. 4 for DNA and water.

IV. *Discussion*

It is seen from Fig. 4 that the current increase with increased hydration for the lower hydration states can be given by the equation

$$\sigma(m) = \sigma_{\text{dry}} \exp(\alpha m) \tag{1}$$

where α is a constant and m is the amount of adsorbate. Rosenberg [19] has proposed a theory to explain this large increase in the conductivity with increasing solvation. This phenomenological theory is based upon the concept that adsorption of high dielectric solvents (or indeed any substance that increases the effective dielectric constant by whatever means) in a low dielectric substrate would serve to increase the effective dielectric constant of the material. This would increase the polarizability of the substance and therefore increase the charge stabilization energy. This would decrease the work necessary to separate charge carriers, and therefore exhibit itself as a decrease in the activation energy for semiconduction. This theory leads to the following equation for semiconduction:

$$\sigma(T,K') = \sigma_0 \exp[-E_D/2kT] \exp[e^2/2kTR(1/\kappa - 1/\kappa')] \tag{2}$$

where E_D is the dry state activation energy of the substrate; κ the low frequency dielectric constant in the dry state; κ' the low frequency dielectric constant after solvation; and R is a cavity radius (3–5 Å) and is the one arbitrary parameter in the system. This theory has recently been verified in quantitative detail by Postow and Rosenberg.[20] The saturation point in the current vs. hydration curves comes at the same point where there is a large change in the capacitance for the three compounds tested, viz., lecithin, hemoglobin, and cytochrome-*c*. According to Equation (2), the current is a maximum when $1/\kappa'$ is small compared to $1/\kappa$.

The theory, however, does not specify the details of the conduction mechanism, and therefore cannot be used to determine the nature of the charge carriers in the substance. This must be experimentally determined for each substance and for all solvation states.

In principle, all charge carrier types, electrons, holes, protons and ions, will make a contribution to the current depending on the product of the particular charge carrier density and its mobility. The experiments described above are an attempt to determine this product for electronic and protonic carriers in various biomolecules as a function of solvation. Separate measurements of either the carrier densities or the mobilities are not yet feasible, and much further work is necessary in this area.

It had been anticipated that as the hydration state increases to the point where the solvent molecules form a continuous pathway over the surfaces of the molecules (at about 2 to 4 BET monolayers), a protonic conduction mechanism through the water would dominate over intrinsic conduction processes within the substrate molecules.

As can be seen in Figs. 9 through 13, there is no large change in the charge carrier ratio in the region from two to four BET monolayers even though the water must be more loosely bound as indicated by the increase in capacitance. There appears to be no evidence to indicate that adsorbate-adsorbate interactions take place in the two to four BET monolayer region to effect a large protonic conductance mode.

We wish at this time to suggest that the protonic mode of conduction may be intrinsic to the semiconductor in the same manner that electronic conduction is intrinsic. Of the compounds tested, it is known that σ_0 is a constant during hydration for hemoglobin,[19] melanin,[17] and collagen.[17] This means that the density of available states for charge carriers are not changed during the hydration process. The adsorbate, through the dielectric constant effect, merely promotes the production of free charge carriers by decreasing the activation energy for semiconduction of the adsorbent.

The experimental results reported here allow us to conclude the following:

1. All biomolecules tested here appear to be mixed semiconductors (i.e. both electronic and protonic charge carriers make significant contributions to the currents).
2. While the conductivity of each increases exponentially with solvation, the ratio of protonic to electronic conductivity increases linearly with hydration for the globular proteins, hemoglobin and cytochrome *c*.
3. The fibrous protein collagen may be a protonic semiconductor in the dry state (dry, as defined by no further weight change in vacuum at 40° C, but it may still have water of hydration in the internal helical spaces), and the electronic component increases linearly with hydration.
4. Melanin is a mixed semiconductor whose protonic to electronic ratio (65:35) does not change over a hydration range of 10 to 35%.
5. The DNA-water and hemoglobin-methanol systems both show electronic conductivity below a threshold solvation state, and a fairly sharp transition to a dominantly protonic mode above this threshold.
6. The protonic to electronic ratio may be a function of the applied dc voltage—being mainly electronic below a threshold, and mixed above this threshold for the hemoglobin-water system.

Acknowledgement

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