FINAL REPORT

ON CONTRACT N00014-85-K-0572

PROTONIC AND ELECTRONIC CHARGE CARRIERS IN SOLVATED BIOMACROMOLECULES

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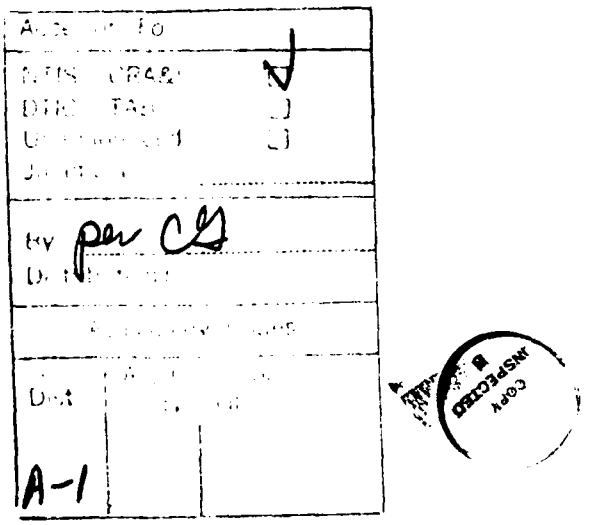

SUMMARY

1. Evidence for intrinsic protonic conduction in solvated biomacromolecules was sought during vectorial charge transport.

2. The methodology involved the adsorption of water and other organic liquids on biomacromolecules and subsequent solid-state electrolysis.

3. The electrical conductivity changed upon solvation in a manner which was suggestive of the bulk dielectric theory first proposed by Rosenberg (1962 b).

4. The electrical conductance changes were related to the bulk dielectric constant of the adsorbed liquid but not related to its ionization potential.

5. Solid-state electrolysis indicated that protons were part of the vectorial conductance process, as evidenced by the production of hydrogen. Oxygen, in contrast, was <u>not</u> found in an electrolysis product even when water was the adsorbate.

6. The conclusion is that protonic conductance in biomacromolecules is intrinsic and remains so in the solvated state (1-3 BET monolayers). Current is not being carried on the "bulk" adsorbed liquid.

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I. INTRODUCTION

Charge transport in biological systems concerned itself initially with electron transfer, in redox reactions in enzymes. Emphasis on protonic mechanisms has been steadily increasing since the phenomenolical concepts of Peter Mitchell (1961) and R.J.P. Williams (1961) focussed on membrane processes. Here we encounter the "protein pump" of bacteriorhodopsin (Stoeckenius et al, 1979) and ATP synthetase (Papa, 1982). Phenomenological concepts may require mechanisms which do not, however, exist in nature.

Proton movement in ATP-driven "pumps" has been implicated in kidney (Mego, 1975), chromaffin granules (Apps and Schatz, 1979), turtle bladder (Al-Aquati and Dixon, 1982), liver (Reeves, 1981), E. coli and S. lactis (Maloney, 1982). Proton transport is postulated in the enzymes transhydrogenese (Ernster and Schatz, 1981; Pennington and Fisher, 1981) cytochrome oxidase and the bc_1 loop of the mitochondrial respiratory chains (Fillingame, 1980; Papa, 1982; and Wilkstrom et al, 1981).

A. Electrical Conductivity and Hydration

The history of the study of electrical conductivity increase in hydrated biomacromolecules is rather extensive, and it can be traced back to the investigations of Evershed (1914); he studied the effect of moisture on materials being employed at that time to insulate electrical wires (e.g., cotton). He reached the conclusion that virtually all the current increase was effected by water condensation on the internal and external surfaces of the fiber.

Murphy and Walker (1928) studied the d.c. conductivity increase of cotton, silk, and wool with adsorbed water and found that the resistance for cotton was given by

$$\log R = -9.3 \log M + B \qquad [1]$$

It was conjectured that bulk water flowed in channels, and the fiber resistance depended upon the number of restrictions in these channels; the number of restrictions was reduced with the increase of adsorbed water.

Murphy (1929 a) studied the a.c. conductivity of cotton and silk and found that the parallel conductance increased with frequency, but it became frequency independent at high humidities. He found that the a.c. conductivity could be represented by

$$G = G_o f^n \qquad [2]$$

where G_o and n are constants, and f is the frequency; n was found to be an approximately linear function of the humidity.

Marsh and Earp (1933), working with single wool fibers, found a decrease of resistance with increasing hydration, but the currents were found to be ohmic with no evidence of polarization. It was their opinion also that the current was carried by water in water-filled pores in the fiber.

Baxter (1934), however, concluded that the water in the pores could not be of the same form as water in the liquid state in that the activation energy for conduction was different in water (an ionization mechanism) than in hydrated wool. He obtained the following equation for the conductance:

$$\sigma = A \exp (-E/ kT) \quad [3]$$

where σ is the conductivity, and A is a constant. He proposed that conduction was the result of electron tunneling between the adsorbed water molecules. The resistance would then vary exponentially with the water-water molecular spacing in that the probability of electron tunneling from one water molecule to another is proportional to r, the width of the barrier (assuming a constant barrier shape and height), and this probability is given by

$$P = B \exp (hr) \quad [4]$$

where B and h are constants.

Fuoss proposed an ionic conduction mechanism based upon the idea that plasticizing agents facilitate ion migration. This idea was tested by King and Medley (1949 a) with the conclusion that substances such as water and formic acid not only lower the diffusion resistance of ions in the polymer matrix, but that increased dissociation of the ions also occurs. They electrolyzed a keratin-water sample and found that, at 18% adsorbed water, the yield of hydrogen was 92% of the theoretical amount. From this, King and Medley (1949 b) concluded that electrolysis was taking place as was predicted by the ionic theory, albeit, the amount of oxygen found was only 18% of the theoretical amount.

E.J. Murphy (1960) used a variation of the theory proposed earlier concerning the completion of water channels. In the revised picture, the water was adsorbed on specific sites, and these water adsorption sites were proposed to lie between "ion-generating sites", loci where the dissociation

emergy for the formation of H^+ was less than for the generation of H_3O^+ and OH^- in liquid water.

The water molecules were generally envisioned to connect the easily ionized protons and thus provide a conduction pathway. This is distinct from current concepts where the biomacromolecule is primarily involved in charge transfer. If the amount of water adsorbed at saturation is α_o, and the amount of water present at any given hydration state is αm then the probability of any site being occupied is (α/α_o). If there are n sites for water adsorption between any two ion-generating sites, then the probability that all of the sites will be filled is given by $(\alpha/\alpha_o)^n$.

The conductivity is then calculated empirically from this probability. The conductivity at saturation is given by σ_s. Thus, for any state less than saturation, the conductivity is given by

$$\sigma = \sigma_s(\alpha/\alpha_o)^n. \qquad [5]$$

The theory is applicable only to protonic conductors insofar as the idea of ion-generating sites is utilized. However, in the case of cellulose, to which Murphy directed his theory, the primary conductors were found by him to, indeed, be protons (Murphy 1963, 1965).

Studies by Eley (Eley and Spivey, 1960) have led him to the early conclusion that adsorbed water increases the conductivity of proteins by <u>injecting</u> either electrons or holes into the conduction bands of the material. If the water is distributed over the surface of the spherical molecules, the exponent in Equation 1 would be ½, and the equation of the energy gap would then be

$$E_D = E_{Do} - \alpha n_D^{\frac{1}{3}} \qquad [6]$$

According to Eley's injection mechanism, the total equation for the conductivity then becomes

$$\sigma = e\mu(2n_D)^{\frac{1}{3}} \left[\frac{(2\pi mkT)^{3/4}}{h^{3/2}}\right] \exp - \left[\frac{(E_{DO} - \alpha n_D^{x})}{2kT}\right] \qquad [7]$$

or

$$\log \sigma = \text{Constant} + \log n_D^{\frac{1}{3}} + \beta n_D^{x} \qquad [8]$$

where x is a function of the distribution on the physically adsorbed water on the molecule and of the forces between the adsorbate molecules; in the case of inorganic compounds, x is equal to 1/3.

Another theory proposed by Rosenberg (1962 b), in which intrinsic conduction was proposed, was based upon the change of the bulk dielectric constant of the conducting medium. This will be discussed later.

B. Organic Electronic and Protonic Semiconductors

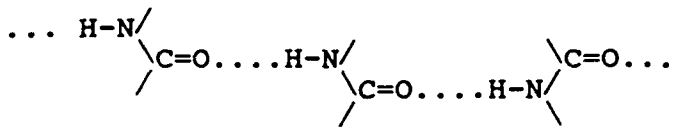
Albert Szent-Györgyi (1941), responding to an idea expressed to him by one of his graduate students regarding the similarity in order between organic and inorganic crystals, proposed that conduction "energy bands" could be found in biological compounds. This concept of similarity was based upon the periodic array found among the amino acids in the hydrogen-bonded crystals. (This periodicity is not as short-ranged as found in metals and metalloids.)

A method of energy transfer and charge transport became possible which did not involve the diffusion of molecules,

the customary method in solution chemistry. This was particularly important for energy transfer in solid structures such as the cytochromes rigidly fixed within the walls of mitochrondria.

The war interrupted the testing of this valuable idea for several years. It was not until later that combinations of proteins and dyes showed that energy migration could take place in these photoactivated systems. Some processes, however, were later explained as resonance transfer (Förster mechanism).

Not until 1949, when Evans and Gergley (1949) published their calculations, was anything known about the possible existence of the electronic energy bands in organic solids and the possibility of intrinsic charge conduction. The calculation was based upon the hydrogen-bonded repeat structure



It yielded an electronic band gap of 3 e.v. between the highest filled and lowest empty band.

Since the early sixties, an extensive study of organic semiconducting compounds has been made; much of the initial work on biomacromolecules was conducted in the laboratory of D.D. Eley in Britain. Pressed powders were studied by Eley et al. (1953), and they observed that the resistances and activation energies decreased with increasing compression; more importantly they found that the presence of adsorbed water lowered the activation energy. They did not note a direct correlation between the number of π electrons and the conductivity, but they did conclude that "linear polyenes are

less effective semiconductors than polynuclear systems with a similar number of π electrons"; they associated the electrical conductivity with the π electrons of the aromatic molecules.

Seanor (1968) demonstrated that nylon 66 possessed a large protonic conduction component. The percent of protonic conduction was found to greatly increase about 90°C. Below this, electrons were the primary carrier as evidenced by the low amount of evolved hydrogen.

In the 1980s, interest has become focussed upon both electronic (Lewis, 1982) and protonic processes in biological systems (Morowitz, 1978; Cha et al, 1989). Quantum-mechanical tunneling of electrons in proteins has been extensively studied (DeVault, 1984); these are now joined by studies of hydrogen tunneling (Bell, 1980), especially in enzyme systems (Cha et al, 1989).

Much of the current interest concerns the movement of protons across biological membranes (Morowitz, 1978; Nagle and Tristram-Nagle, 1983; Fruend, 1982). Generally all of these authors favor proton jumping along linear aggregates of water molecules along side the macromolecules or in channels. Indeed, this is also possible with adsorbed water-protein systems although Powell and Rosenberg (1970 a,b) and Rosenberg and Postow (1969) showed that electrical conductivity did not demonstrate sudden increases (non-linearity) associated with formation of BET monolayers as their mechanism should suggest. What was not demonstrated in the earlier studies was whether oxygen was being evolved; the presence of oxygen would, of course, imply that simple electrolysis of water was occurring.

Properties of those proteins which had relatively extensive previous investigation by others of their electronic/protonic conduction properties follows.

C. Earlier Conductivity Studies On Biomacromolecules

Over the past several decades, several macromolecular systems have been investigated with respect to electrical conductivity in the hydrated state. Some of these will now be reviewed. Early work was primarily connected to concepts of electron conductance.

1. Hemoglobin

The early studies of hemoglobin by Cardew and Eley (1959) showed an activation energy of 2.75 e.v. for dry hemoglobin and 2.97 e.v. for dry globin (some dependence on pressure was noted for both compounds). It was postulated that the charge carriers were electronic rather than protonic in the dry state because: (i) steady state values of the current were reached within one minute, (ii) the effect of decomposition was to give a lower conductivity (higher conductivities would be expected if the carriers were ionic), and (iii) the conductivities were 10^3 to 10^5 times smaller than polyamides--suspected protonic conductors. They postulated that adsorbed water would induce a protonic conductivity in solid proteins. Since the mid-1970's, it has been protonic conduction which has attracted the greatest amount of theoretical interest, particularly with regards to proton movement through biological membranes.

Studies of water adsorption on hemoglobin by Cardew and Eley (1958) gave values for the Brunauer, Emmett, and Teller (1938) (BET) monolayer which differed for the freeze-dried and alcohol-denatured hemoglobin and illustrates the

variability of this parameter, most likely a result of conformational changes. For native material, they calculated v_m the BET monolayer, as 5.76 g/100 g. protein (30°C,) and 5.48 g./100g. (40°C,). These values supported their idea that the water was adsorbed by the polar side chains on the surface of the molecule; about 73% of the side chains were involved in the adsorption process.

Adsorbed water was first found by Eley and Spivey (1960 a) to greatly increase the conductivity of hemoglobin. They postulated that water donated electrons into the conduction band of the hemoglobin rather than enabling an intrinsic protonic conduction mechanism. Eley and Spivey (1960 b) found that alcohol denaturation changed the value of E from 2.36 e.v. for native hemoglobin to 2.88 e.v. for denatured material; there was only a small change in the value of σ_o.

The possibility of electronic carriers in slightly hydrated hemoglobin (7.5% water) being dominant over protonic carriers was first tested by Rosenberg (1962 a) in an experiment where a current decrease with time was examined. The absence of this decrease indicated that the water was not being electrolyzed, and Rosenberg therefore postulated that charge transport by the dissociation and ionization of water was negligible. This concept assumed that any protons were being donated by the water itself. [This author (M.R.P) noted a rather large conductant, as mentioned in the original paper, and believes that the study actually measured an artifactual leakage current.]

A separate study by Rosenberg (1962 b) of the water/hemoglobin system indicated that the water served only to reduce the activation energy for intrinsic charge carrier generation (either electronic or protonic); it did not affect the value of σ_o.

On the basis of the few early measurements which had been made, Rosenberg (1964 b) suggested that the charge carriers were:

(1) electronic in the range where the current increases exponentially with hydration,

(2) in the region where saturation of current occurs, the carriers could be mixed ionic and electronic, and

(3) in the range where the amount of adsorbed water is high, the carriers could be primarily protonic.

It was not stated whether this protonic conduction utilized the protons of the organic conductor or whether those of the ionized water with simple electrolysis occurring. In the later case, we would expect a concomitant evolution of oxygen. We analyzed for this latter gas in this present study.

Eley and Leslie (1964) found that there was not a saturation in the current at the first BET monolayer as they had earlier found; this finding was in agreement with the finding of Rosenberg (1962 b). The former proposed that the energy gap E_d decreases with hydration with the form

$$E_d = E_d^{\circ} - a\, n_d^{1/3} \qquad [9]$$

here n_d is the amount adsorbed, and the exponent 1/3 is derived on the basis that the interaction energy between impurity molecules is inversely proportional to the distance between them. By plotting log conductivity vs. (water adsorbed)$^{1/3}$, they obtained a straight line which showed current saturation at high adsorption.

Maričić, Pifat and Pravdić (1964) were the first to attempt a solid state electrolysis of hemoglobin at various hydration levels. Their procedure was to measure the increase of volume of the electrolysis cell during the release of hydrogen (this was accomplished by noting the movement of a slug of mercury in a capillary). Their data indicated protonic conductivity only at 18% hydration or above, but the scatter in their data on the amount of protonic conductivity (20% to 94%) made interpretation very difficult indeed.

Measurements of the dielectric constant of hemoglobin with adsorbed water, ethanol, methanol, and ammonia by Postow (1968) showed that the increase in conductivity could be roughly explained by the increase in the dielectric constant according to the equation of Rosenberg (1962 b).

Similar effects of capacitance increase with hydration have been noted by Rosen (1963) on bovine serum albumin. He noted a large change in the capacitance at about 20% adsorbed water. Takashima and Schwan (1965) found similar effects by water on the capacitance of ovalbumin.

2. DNA

Observations of DNA were undertaken from a theoretical point of view of solid state biophysics; there is not any definite implication in living systems, although there may be some relation between conduction and carcinogenesis or radiation damage. Löwdin has also produced a theory to explain mutations based upon the movement of protons from one base to another; these movements are generally of short distance, however. These studies here were extensions of the original work of Powell and Rosenberg (1970 a).

Eley and Leslie (1963) studied the conduction activation energies of nucleic acid components and found the following values for E: (a) nucleoside (base + ribose) 4.5 - 5.2 e.v., (b) nucleotide (base +ribose + phosphate) 2.0 - 2.2 e.v. This last value is comparable to DNA and RNA, each of which have a conduction activation energy of about 2.4 e.v. They suggested that the conduction state would be electronic and correspond to a π - π^* excitation (or n-π^*), using a band theory approach; for the nucleoside, a π-π^{**} excitation would be necessary. Measurements suggest electron conduction, but that most charge is carried by protons in the hydrated samples, (Powell and Rosenberg, 1970a).

Solid-state electrolysis experiments were made on Na-DNA by Maričić and Pifat (1966); they found that hydrations of 37% and higher gave very large values for the amount of protonic conductivity. Unfortunately, the scatter in the data was not amenable to a good interpretation of their results. It was their contention, however, that at hydrations of less than 37%, the amount of protonic conductivity was small or even non-existent. These results were expanded in the study by Powell and Rosenberg (1970a).

Employing proton-injecting electrodes (hydrogen-saturated palladium), Thomas et al. (1969) found large current increases with the pyrimidine base isocytosine. The difference between the current in the presence and the absence of hydrogen was greater than 10^{10}. This would likewise argue for a protonic conduction mechanism in mucleotides.

Studies of the d.c. conductivity of Na-DNA by O'Konski, Moser and Shirai (1964) showed regions of non-ohmic behavior

in a hydrated sample (p/p_o = 0.31) with potentials of from 0 to 200 v. cm. $^{-1}$ at 25°C.; at larger potentials the current was ohmic. From various observations (the effect of O_2, photoconduction, and space-charge effects), they concluded that dry DNA was an electronic conductor, and that hydrated samples likely possessed a large complement of ionic (protonic) conduction.

3. Cytochrome

Eley and Spivey (1960) measured the semiconduction activation and parameters for dry cytochrome-c and found an E = 2.60 e.v. and a log σ_o = 4.8; these values being slightly smaller than those which they found for hemoglobin (cf. E = 2.66 e.v. and log σ_o = 5.0). In a manner similar to hemoglobin, cytochrome-c displayed ohmic behavior up to field strengths of 2,000 v. cm.$^{-1}$ at 115°C. in the dry state.

Cytochrome-c is of interest to the biophysicist and biochemist by virtue of its role in the oxidative phosphorylation chain. Furthermore, this material appears to be rigidly bound to the cristae of the mitochondria making charge transfer by diffusion of the cytochrome to the substrate a rather improbable event.

Two possible mechanisms exist for this mitochrondrial electron transfer, the first being semiconduction and the second being quantum-mechanical tunneling, although in cases where inter-molecular tunneling is the rate limiting step in semiconduction, there is little difference between the two. At present, there remains a difference of opinion amongst workers in the field as to which is the correct method (if there is indeed a difference), and no irrefragable evidence exists for either side; since 1980, the tunneling mechanism involving protons has gained favor (Cha, et al., 1989).

Work under the current contract was not undertaken to resolve the controversy, but rather to show that, at high degrees of solution and consequently lower resistances, the component of protonic conductivity was large, could conceivable be used to explain charge transfer, and did not involve simple electrolysis of adsorbed water.

At present, there is evidence that the movement of both electrons and protons can proceed by long range tunneling process (about 60 Å). DeVault, Parkes, and Chance (1967) showed that the oxidation of cytochrome-c in the purple sulphur bacterium could occur at liquid helium temperatures with a time constant of 2.3 ms and no indication of an activation energy at temperatures of less than 120° K.

Electron tunneling between metal sites in proteins has been extensively modeled on a theory first presented by Marcus (1968). The electron motion is proposed to be fully quantum mechanical; the temperature dependencies arise from ligand reorganizations to account for the effective transfer of electrons. Current investigations (DeVault, 1984) have demonstrated effective electron tunneling between donor and acceptor sites.

Proton tunneling is related to the de Broglie wave length, and transfer is the result of wave-particle duality of matter. For a particle with an energy of 20 kT/mole, for an electron λ = 2Å and for a proton, λ = 0.6Å (Cha et al. 1989). The rate of tunneling is a function of the barrier shape which is generally unknown.

II. THEORY

A. Charge Transport

Rosenberg (1962 b) proposed a phenomenological process, what was essentially an electrostatic continuum theory of conductivity increase upon hydration using the premise that the effective dielectric constant of the adsorbent/adsorbate system will be increased. This would lead to a reduction in the energy needed to separate the positive and negative charges whose movement constitute the current. Individual characteristics of the proteins (e.g., conformation angles, bond lengths, energy band gaps, etc.) were not utilized. It is a phenomenological concept and useful for envisioning the global aspects of the problem and is not specific for protonic mechanisms utilizing either pumps, channels, or conformational changes. It, thus, cannot be effectively used to differentiate one from the other. In any mechanism of charge transfer, electrostatic shielding would be energetically favorable.

Many biomacromolecules have been found to follow the operational definition of a semiconductor, that is, they follow the equation

$$\sigma = \sigma_o \exp(-E/kT) \qquad [10]$$

where $$\sigma_o = \Sigma_i \eta_i e_i \mu_i$$

and η_i is the number of the i^{th} charge carriers of charge e_i and drift mobility μi. Their conductivity increases with increasing temperature. This is contrasted with metallic conductors which show a resistance increase with increasing temperature. Representative macromolecules of the four categories of biomacromolecules, viz., carbohydrates, proteins, lipids, and nucleic acids have been demonstrated to

be operational semiconductors. Equation 10 does not specify either (i) the mechanism of conduction, or (ii) the nature of the carrier(s).

Of interest in biological systems is the fact that organic semiconductors change their conductivity upon hydration; this change can be represented by the equation

$$\sigma_m = \sigma_{Dry} \exp(\alpha m) \qquad [11]$$

where m is the amount of water adsorbed, and α is a constant. Rosenberg (1962 b) has shown that σ_o does not change upon hydration (implying an intrinsic mechanism), but instead it is E which is a function of hydration. It is therefore possible to combine Equations 10 and 11 and obtain

$$\sigma(T,m) = \sigma_o \exp(-E_D/2kT) + \alpha m \qquad [12]$$

where E_D is the dry state activation energy. The activation energy E is then

$$E = E_D - 2kT\alpha m \qquad [13]$$

If one desires to move free changes in the presence of an applied field, the first prerequisite is the generation of the charges themselves. To Rosenberg, this process was the one requiring the greatest energy and could be thought of as simply removing a proton or electron from the molecule and placing at a point where the coulombic attraction is now less than kT. The energy for this process would correspond to the ionization potential of the molecule. If the charge carrier is placed on another molecule, extra energy, the "electron affinity" or "band energy" depending on electron or proton, is returned. In addition, the lattice will relax in such a fashion that the dipoles will orient themselves to further lower the energy of the system; this energy is termed the

"polarization energy". The polarization energy is multiplied by two in that both the negative and positive charges are included. The energy for the total process in the dry state would be

$$E_D = I_g - A_g - 2P \qquad [14]$$

where the "ionization potential", I, and the "electron affinity", A, are taken as the gas phase values. This polarization energy is comparable to the orientation of solvent molecules around a dissolved ionic compound.

The amount of the polarization energy for a molecule possessing spherical symmetry is given by

$$P = (e^2/2R)\ (1 - 1/K) \qquad [15]$$

where e is the electron charge, R is the radius of polarization, and K is the dielectric constant of the medium. The actual value of K could not be calculated without a knowledge of the positions of all of the dipoles involved, so Rosenberg employed the bulk dielectric constant as a first approximation. If Equations 14 and 15 are combined, the following equation results:

$$E_D = I_g - A_g - (e^2/2R)\ (1 - 1/K) \qquad [16]$$

For the hydrated systems, the value of K will change; this new value is represented by K'. The gas phase "ionization energy" and "electron affinity" are not functions of hydration. The new energy for hydrated systems is then

$$E = I_g - A_g - (e^2/R)(1 - 1/K') \qquad [17]$$

Combining Equations 16 and 17 yields

$$E = E_D - (e^2/R)\left[(1/K) - (1/K')\right] \qquad [18]$$

and from Equations 13 and 18 we have

$$2kt\alpha m = (e^2/R)\left[(1/K) - (1/K')\right], \qquad [19]$$

Substituting (19) into (13) gives Rosenberg's final result:

$$\sigma(T,K') = \sigma_o \exp(-E_D/2kT) \cdot \exp\left\{(e^2/2kTR)\left[(1/K) - (1/K')\right]\right\} \qquad [20]$$

Continuum models experienced difficulties in that amorphous samples are more conductive than crystalline ones; long-range order is not especially beneficial. Ionic conduction would benefit from short-range disorder while electronic conduction is favored in crystals but the carrier is trapped in amorphous regions (Maxwell-Wagner interfacial polarization).

Because the continuum model of Rosenberg is phenomenolical, it cannot easily explain much of the "fine structure" which is found in studying electrical conductivity; this is especially true at low mole percents when water or organic liquids are adsorbed. When saturation is reached, the current can be more easily related to the bulk dielectric constant (c.f. Figures 19 and 20), but this is not always true of the region of exponential rise. With water as the adsorbate (or possibly even with organics), some degree of "plasticization" occurs. This would produce conformational changes which could favor increases in mobility, especially for ionic carriers. Macroscopic mobility was found by Lewis and Toomer (1981) to increase according to the equation.

$$\mu = \mu_{dry} \exp(\beta m) \qquad [21]$$

where μ is the mobility, m is the % adsorbate and μ is a constant. Water appears to aid in the transport of charges as well as modify the local structure.

It is known, however, that ionic conduction in polymers does not admit to simple theoretical models. Thus, Waldren's rule that relates mobility to viscosity is not followed. Waldren's rule relates the viscous drag on an ion to the accelerating force of the applied electric field. At equilibrium, the viscous force f_v on an ion of radius R is given by Stoke's law:

$$f_v = 6\pi\eta Rv \qquad [22]$$

where η is the viscosity and v is the effective velocity ($=\mu E$). The force (fe) of the electric field is given by

$$f_c = zqE \qquad [23]$$

where z is the charge. Naturally, at equilibrium,

$$fe = fv \qquad [24]$$

and $zqE = 6\pi\eta R\mu E.$

If we substitute

$$\sigma = qn\mu,$$

we then find

$$\sigma\mu = z(q^2 n/6)\pi R. \qquad [25]$$

Since the drift mobility μ is related to the diffusion constant D by the Nernst-Einstein equation

$$\mu/q = D/kT, \qquad [26]$$

Finally we have

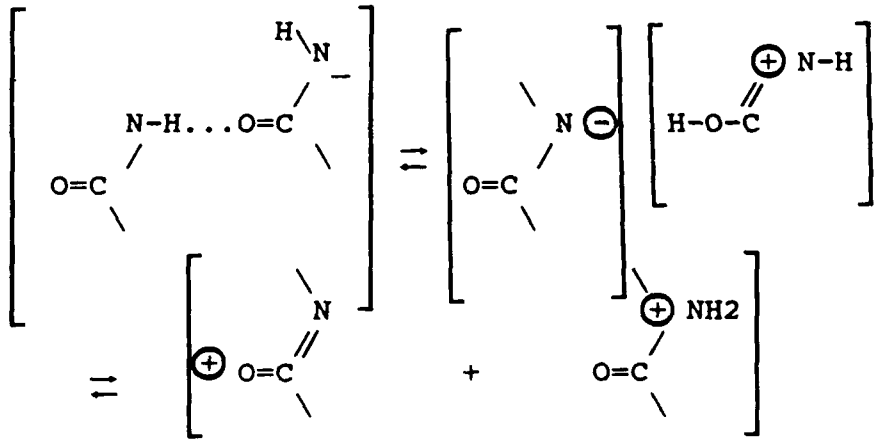
$$\sigma/D = \eta q^2/kT. \qquad [27]$$

Available evidence does not agree with Equation 27. In general, the product $\sigma\eta$ is not constant over a large range of σ and μ.

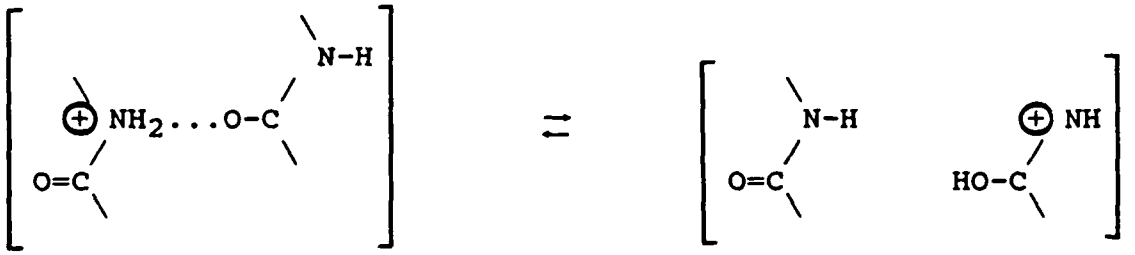
The viscosity required in the Stoke's law approximation is that of the local environments. In solid state polymers, this is known to be a poor assumption and local variations can strongly dominate the ionic motion. Thus macroscopic viscosity will not reflect the micro environments.

Seanor (1982) has proposed several protonic mechanisms as follows:

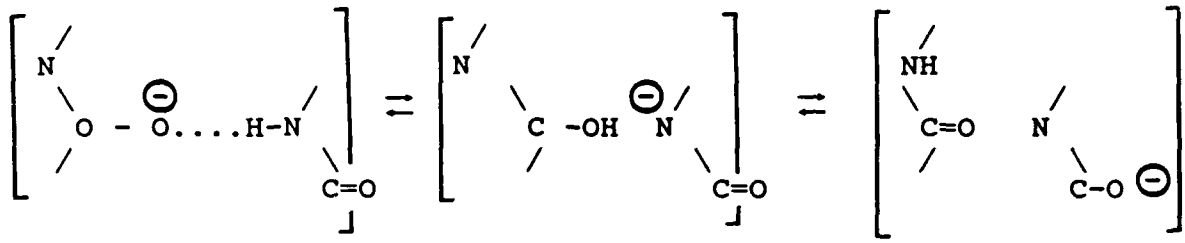
(1) Self ionization



(2) From (1) would follow proton transfer:



(3) Transfer of a proton and an electron:



B. Adsorption Isotherms

These were used to determine the monolayer coverage. The system used to analyze the data was the Brunauer, Emmett, and Teller (1938) (BET) isotherm.

In the BET theory, it is assumed that a specific heat of adsorption exists for the first monolayer; all subsequent layers will yield a heat equal to the heat of vaporization of the compound being adsorbed.

The BET model assumes that there exist particular adsorption sites, and, at any one time, S_o of these will be free, S_1 will have one molecule adsorbed, S_2 will have two molecules adsorbed and so on. When the system is in a state of equilibrium, a certain probability of coverage will exist on each site. For the first monolayer, the ratio of covered to uncovered sites will be

$$S_o/S_1 = (b_1/a_1p) \exp (-E_1/ RT) \qquad [28]$$

where a_1 is a constant from kinetic theory, p is the vapor pressure, b_1 is a constant (a function of the frequency of molecular oscillation), E_1 is the heat of adsorption of the first monolayer, nd R and T are the gas law constant and the absolute temperature, respectively.

For each subsequent layer, the adsorption is

$$S_{i-1}/S_i = (b_i/a_i p) \exp(-E_i/RT) \qquad [29]$$

If x represents the total amount of vapor adsorbed for any given pressure p, and x_m is the monolayer coverage, it is possible to show that

$$x/x_m = \frac{Cy}{(1 - y)(1 - y + Cy)} \qquad [30]$$

where C is given by

$$C = \exp\left[(E_1 - L)/RT\right] \qquad [31]$$

in which L is the heat of vaporization, and y is p/p_o. This yields the familiar expression for the BET isotherm

$$\frac{p}{x(p_o - p)} = \frac{1}{x_m C} + \left[\frac{C - 1}{x_m C}\right] \frac{p}{p_o} \qquad [32]$$

Thus a plot of $p/[x(p_o - p)]$ vs. p/p_o yields a straight line whose slope is $(C - 1)/x_m C$ and whose intercept is $1/x_m C$. From this the monolayer coverage x_m may be determined in that there are two equations for the two unknowns (x_m and C).

The isotherm developed by Bradley (1936) has its main use in cases where the adsorbate possesses a permanent dipole moment. The Bradley equation is mainly empirical. In its general form, the isotherm is described by the equation

$$T \log_{10}(p_o/p) = K_1 K_3^A \qquad [33]$$

where T, p, and p_o have their usual significance in adsorption theory, and K_1 and K_3^A are constants for each adsorbent and adsorbate. The exponent A in the K_3 term is

the amount of adsorbed gas. The term K_3 is equal to K_2^b, where b is a constant. For use, Equation 33 is put into a log form and A is plotted against log log (p_o/p); the result is a straight line.

III. EXPERIMENTAL

A. Samples

All biomacromolecules used in these studies were obtained from Sigma Chemical Company, St. Louis, Missouri; they were all of reagent grade. Those materials studied were:

1. casein
2. casein - methylglyoxal
3. phenylalanine
4. polyphenylalanine
5. lysozyme
6. bovine serum albumin
7. hemoglobin
8. cytochrome c
9. collagen
10. DNA

The adsorbates were:

1. water
2. methanol
3. acetone
4. dimethyl sulfoxide (DMSO)
5. chloroform
6. dioxane
7. ethanol

B. Adsorption Isotherms

Powder samples were placed in the Cahn Vacuum Microbalance (Model RH). The hang-down tube of the balance was thermostated by circulating water in an outer jacket. The

jacket temperature was 26.0° ± 0.1°C, maintained by a Haake water bath. The diagram of the equipment within the vacuum manifold is shown in Figure 1.

All samples were heated prior to the start of the experiments with a heating tape to determine the dry weight. The temperature was about 75°C. At this temperature, the sample could be brought to constant weight (less than a change of 0.02%) within two hours. On some samples, pyrolysis would occur if heated to higher temperatures - this was very true of collagen samples.

While the samples were being heated, the system was evacuated to a pressure of less than 5 millitorr as measured on a thermocouple gauge. While the system was being pumped, the traps were always cooled with liquid nitrogen to prevent back streaming of oil which could coat the samples and affect the adsorption.

Adsorbate vapor was introduced from side tubes containing vacuum-degassed water. The vapor pressure was determined by means of a Dubrovin ("cartesian diver") gauge which had a least count of 0.1 torr and a range of from 0.0 to 10 torr. Above this limit, the temperature of the water in the side tube was varied to control the pressure. The water temperature could be measured to ± 1.0 torr.

The Cahn RH was connected to a strip-chart recorder to determine more easily the adsorption equilibrium and the equilibrium weight. The balance pans were counter-weighted so that the full-scale measurement was 10.0 mg. The sensitivity of the balance at this setting was such that a weight change of five micrograms could be detected; this was 0.01% of the sample weight.

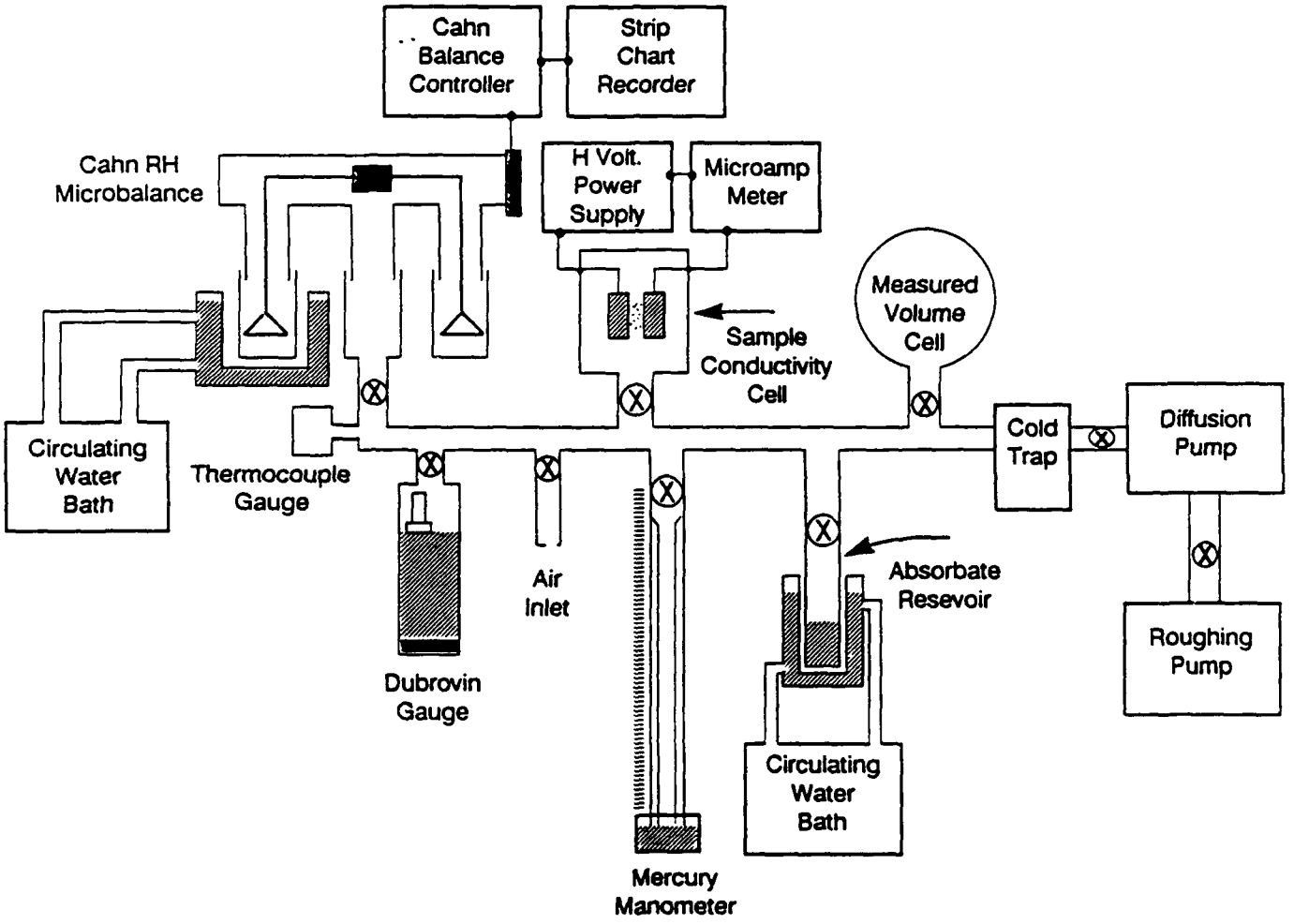


Figure 1. Diagram of the apparatus employed to measure the adsorption isotherms and the electrical conductivity as a function of adsorbed vapor. Its use is explained in the text.

Buoyancy effects could be calculated from the equation

$$\beta = P M W / \rho R T \qquad [38]$$

where P is the gas pressure (atm), M is the molecular weight of the gas, W is the weight of the sample, ρ is the density of the sample, and R and T are the gas constant and the absolute temperature, respectively. It was found, however, that even at saturation vapor pressure, the buoyancy correction (for water) was only one microgram. In this work, no buoyancy corrections were used.

Equilibrium was generally reached in less than six hours for a p/p_o of from 0.0 to 0.4; equilibrium was defined as no detectable weight change after three hours (note that weight changes of 0.01% were detectable). The higher values of p/p_o always required longer periods to reach equilibrium, and the measurements were made after a period of 24 hours. The time to reach equilibrium was less in the instances where powder samples were used as compare to pressed pellets. This is, no doubt, the result of easier diffusion of the water vapor. In that equilibrium weights were not affected by the sample form, power samples were used to reduce the time needed for each weight determination.

C. Electrical Conductivity with Adsorbates

Electrical conductivity measurements were made on samples pressed into thin pellets. The die (Thomas Scientific) was pressed with the palm of the hand so that the pressure would not be great enough to denature the sample. These small pressures were found to be sufficient to provide samples with sufficient mechanical strength. The pellets were approximately one millimeter thick and were placed between two platinium foil electrodes. These were held by

stainless steel plates with Teflon spacers. Before assembling the cell, the block was washed sequentially with ethanol, water, and again with ethanol; it was then only handled with forceps. The area of the electrodes was about 1 cm^2. This sandwich cell was placed in a glass tube in the vacuum line. Electrical connections to the outside were made with feed-throughs sealed with epoxy to minimize electrical leakage.

The potentials were varied from 1 volt applied (10 volts/cm.) to 300 volts applied (3,000 volts/cm.). All measurements were d.c. Voltage was maintained by means of a Fluke 408B (John Fluke Instruments, Everett, Washington). Conductance, at equilibrium, was measured with a Keithley 610 BR Electrometer (Keithley Instruments, Cleveland, Ohio).

Samples were solvated at varying partial pressures of adsorbent. These were obtained by thermostating the conductivity cell by means of a circulating water bath Haake FE (Haake Instruments, Saddle Brook, New Jersey) coupled with an immersion cooler (Model KR-70, Polyscience Corporation, Niles, Illinois).

Conductivity measurements were made after sufficient time for equilibrium.

D. Hydrogen and Oxygen Electrolysis

The evolution of hydrogen and oxygen was calculated from Faraday's law of electrolysis, i.e., one Faraday of charge produces one equivalent of compound; diatomic hydrogen contains two equivalents so two Faradays are needed, oxygen requires four equivalents thusfour Faradays. From the fact that one ampere equals one coulomb/second, and that one Faraday is equal to 96,500 coulombs, one can calculate the

theoretical gas production as 5.18 x 10^{-12} [moles H_2/sec. -micro amp.] and 2.59 X 10^{-12} [moles O_2/sec.-micro amp.]

Using the equation PV = nRT, one can now calculate the pressure change since n, in [moles gas/sec.-micro amp.], is now known from Faraday's law. The system must be calibrated prior to use so that the volume V is known.

The glass and metal system used here had a volume of 152 $cm.^3$ so that the generation of hydrogen corresponded to 6.36 x 10^{-4} millitorr/μamp-sec; for oxygen it was one-half that value. The apparatus, when well out-gassed, had a sensitivity of approximately 10^{-9} moles of hydrogen and approximately 10^{-11} moles of oxygen; the sensitivity was dependent upon the background pressure for each gas species.

E. Electrolysis Studies

The pressed pellet samples for electrolysis were prepared and mounted in a manner similar to that described under "Conductivity Measurements". The samples were suspended in glass sample chambers, care being taken to avoid contact with the walls. This was easily accomplished when the chambers were attached to the vacuum line, Figure 2, by simply tilting them. The platinium electrodes were blocking for protons so all protonic carriers were released as hydrogen gas.

The glass sample chambers were connected with glass joints sealed with Apiezon W wax. The system was pumped with a fore-pump, glass three-stage oil diffusion pump, and an ion pump (Varian). The total system pressure was less than 5 x 10^{-6} torr. The principal contaminants were hydrogen, water vapor, and argon. These resulted from off gassing of the non-bakeable components, i.e., the organic samples. All

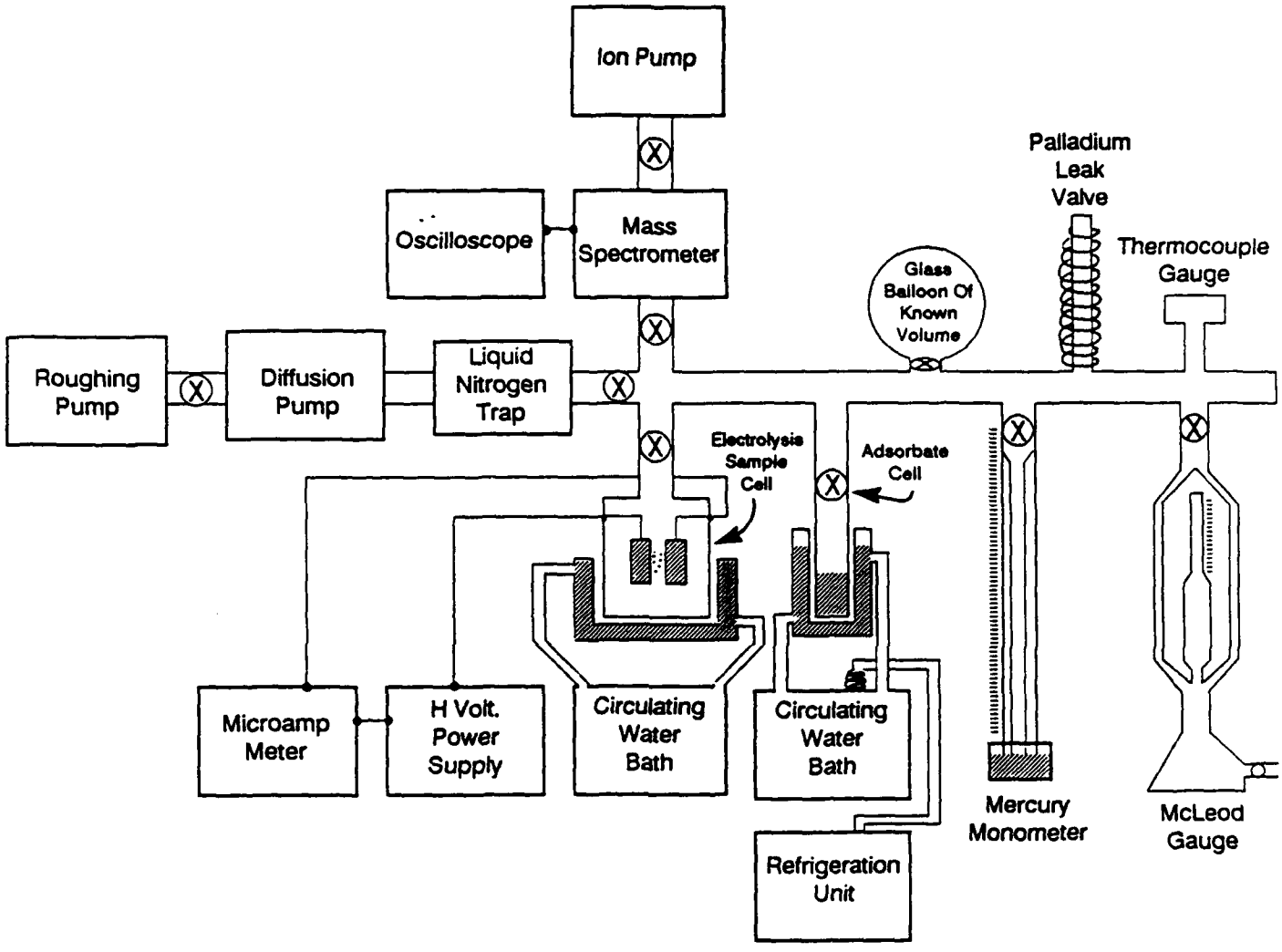


Figure 2. Diagram of apparatus employed to analyze small quantities of hydrogen and oxygen and varying BET monolayer averages. Its use is explained more fully in the text.

samples were pumped continuously (but unheated) from the time they were sealed to the line except for the time of electrolysis and measurement.

The volume of the electrolysis manifold was measured using a mercury manometer, a calibrated volume, and Boyle's law. The volume was determined to 1%.

The number of moles of gas generated could be determined both with a mass spectrometer and, in some cases, from a measurement of the pressure with the McLeod gauge, the palladium leak valve, and a knowledge of the manifold volume and the equation PV = nRT.

The sample chambers were immersed in a circulating water bath held at the same temperature at which the adsorption isotherms were made (26.0°C.) (Haake FE). The samples were solvated for about three hours (sufficient for electrical measurements) by opening the stopcock to the water reservoir. The vapor pressure was changed by adjusting the temperature of the water in the reservoir; this was done with a separate circulating water bath (Haake FE). The solvation state was then determined from the adsorption isotherm. The samples used in the electrolysis were from the same bottle as those used in determining the adsorption isotherm.

Potential differences for the electrolysis experiments were kept between 50 and 200 volts (Fluke 408B), as it was found that erroneous results could arise from the application of higher applied potentials, especially with the drier samples. The currents passed with these potentials varied from 1×10^{-10} amperes to 5×10^{-4} amperes (Keithley 610 BR) depending on the sample used and its solvation state. Background gas, the result of out-gassing of the walls of the sample chamber and the sample itself, determined the minimal

sensitivity of the apparatus. The "background" pressure was on the order of 10^{-5} torr for hydrogen and 10^{-9} torr for oxygen as determined with the mass spectrometer gauge (VG-ARGA, Varian Instruments). In the "Scan" mode, a typical spectrum of the residual gas is shown in Figure 3. The individual gas species and their pressures could be determined by setting the mass number on the ARGA control panel.

The number of coulombs passed was determined with an electrometer (microampmeter; Keithley 610 BR), sometimes in conjunction with a strip-chart recorder. The number of coulombs passed could be determined to about 2%.

With a knowledge of the number of coulombs passed, the theoretical yield of hydrogen and oxygen could be determined, assuming 100% ionic conduction, from the laws of electrolysis. Two equivalents of charge are needed to produce one mole of diatomic hydrogen and 1/2 mole of oxygen.

The amount of hydrogen and oxygen evolved during electrolysis was measured by means of a VG-ARGA 160 (VG Quadrapoles, Cheshire, England). This was employed almost exclusively in this contract as the primary interest was low solvation levels and oxygen as an electrolysis product. In a few cases, hydrogen was analyzed by means of a heated palladium tube which allowed the hydrogen to leak out. Palladium is specifically permeable to hydrogen. The hydrogen removal generally took about 20 to 30 minutes. The second pressure measurement with the McLeod gauge then determined the remaining residual gas. The hydrogen pressure was the difference between the first and second measurement.

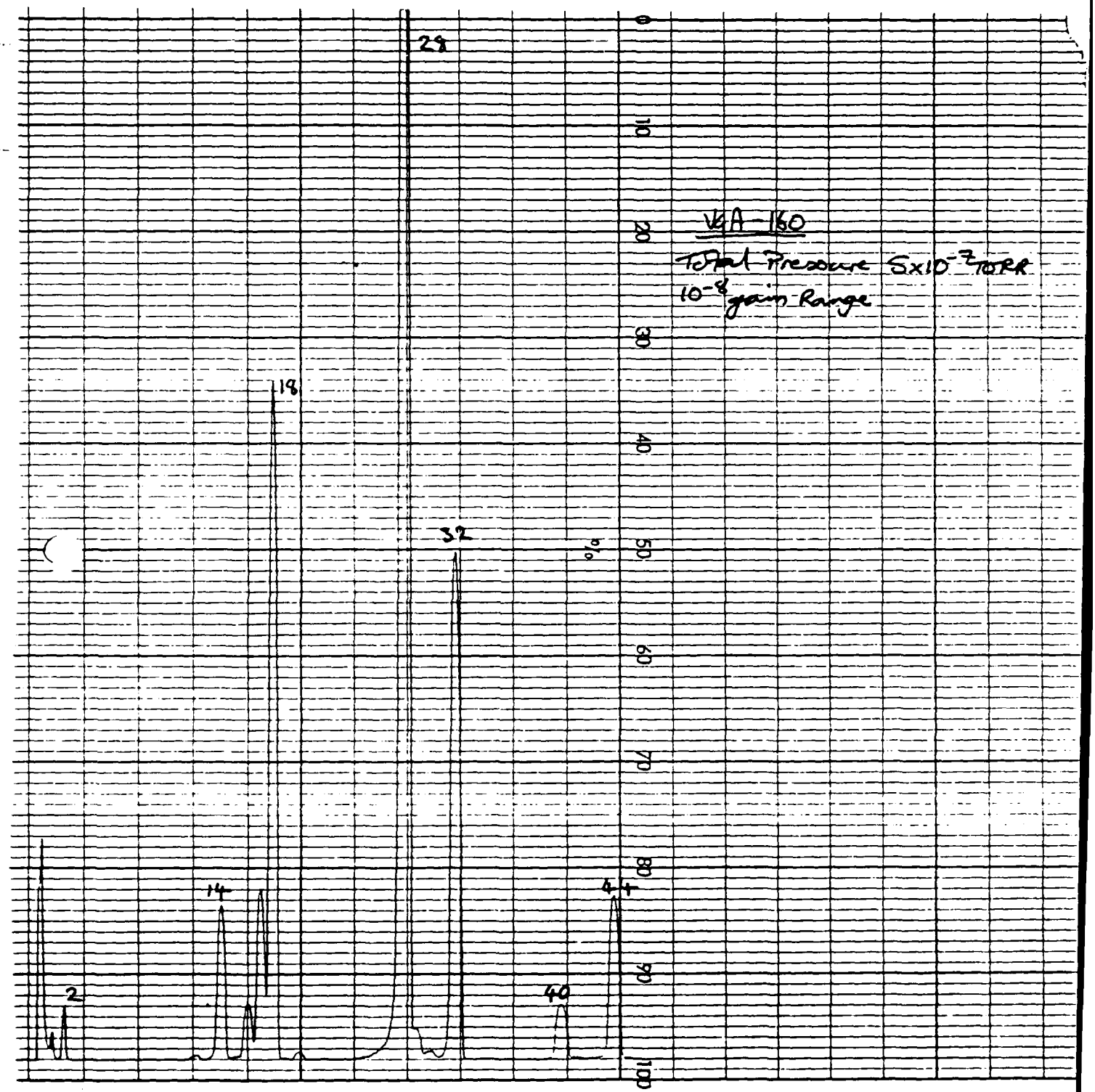


Figure 3. Partial pressure vs. atomic mass number for the species commonly encountered in a vacuum system such as that employed in these studies.

To insure that there was no loss of hydrogen from the system, several tests were made using oxalic acid dihydrate. This compound was pressed into pellets and mounted in a similar manner to the biological samples and hydrated. It is known that these crystals are protonic conductors from the work of Pollack and Ubbelohde (1956) on the activation energy and conductivity.

A sheet of Teflon was also placed into the sandwich cell, and the chamber was fully hydrated ($p/p_o = 1.0$). The leakage current was 10^{-10} amperes at 500 volts; this was 10^{-6} of the current which would pass through a protein sample at this same hydration and potential. Thus leakage currents were negligible.

IV. RESULTS

A. Adsorption

Of the materials selected for study, some were found upon testing on the absorption balance to possess very weak absorption properties. The low degree of adsorbance resulted, in turn, in no measurable current, even at applied voltages of greater than 500 volts. Thus testing of hydrogen/oxygen evolution by these systems was impossible; these were:

1. phenylalanine water,
2. polyphenylalanine with water,
3. casein-methylglyoxal with water,
4. lysozyme with water,
5. bovine serum albumin with water.

Some liquids (dimethyl sulfoxide, for example) were tested but, having such a low vapor pressure at room temperature, there was but negligible adsorption on all adsorbents tried. As would be expected, the resultant current increases were negligible. Other combinations gave both measurable adsorptions and current increases. These are listed in Table I with the BET monolyer coverages.

In Figures 4 to 9, it can be seen that adsorption follows the Bradley isotherm over a considerable range. This is indicative of polar molecules binding to polar sites on the macromolecule. (Note isotherms were not shown here for all combinations which were tested.)

B.E.T. monolayer coverage has been calculated for several of the combinations and these are given in Table 1.

TABLE I

Adsorbent/Adsorbate	B.E.T. Monolayer(at 26°C)
Collagen/water	7.8%
DNA/Water.	12.2%
Casein/water	6.0%
Casein/acetone	2.1%
Casein/methanol.	5.2%
Hemoglobin/water	5.9%
Hemoglobin/methanol.	11.6%
Hemoglobin/ethanol	6.9%
Cytochrome/water	6.2%

These materials possessed sufficient adsorption properties such that electrical conductance could be measured.

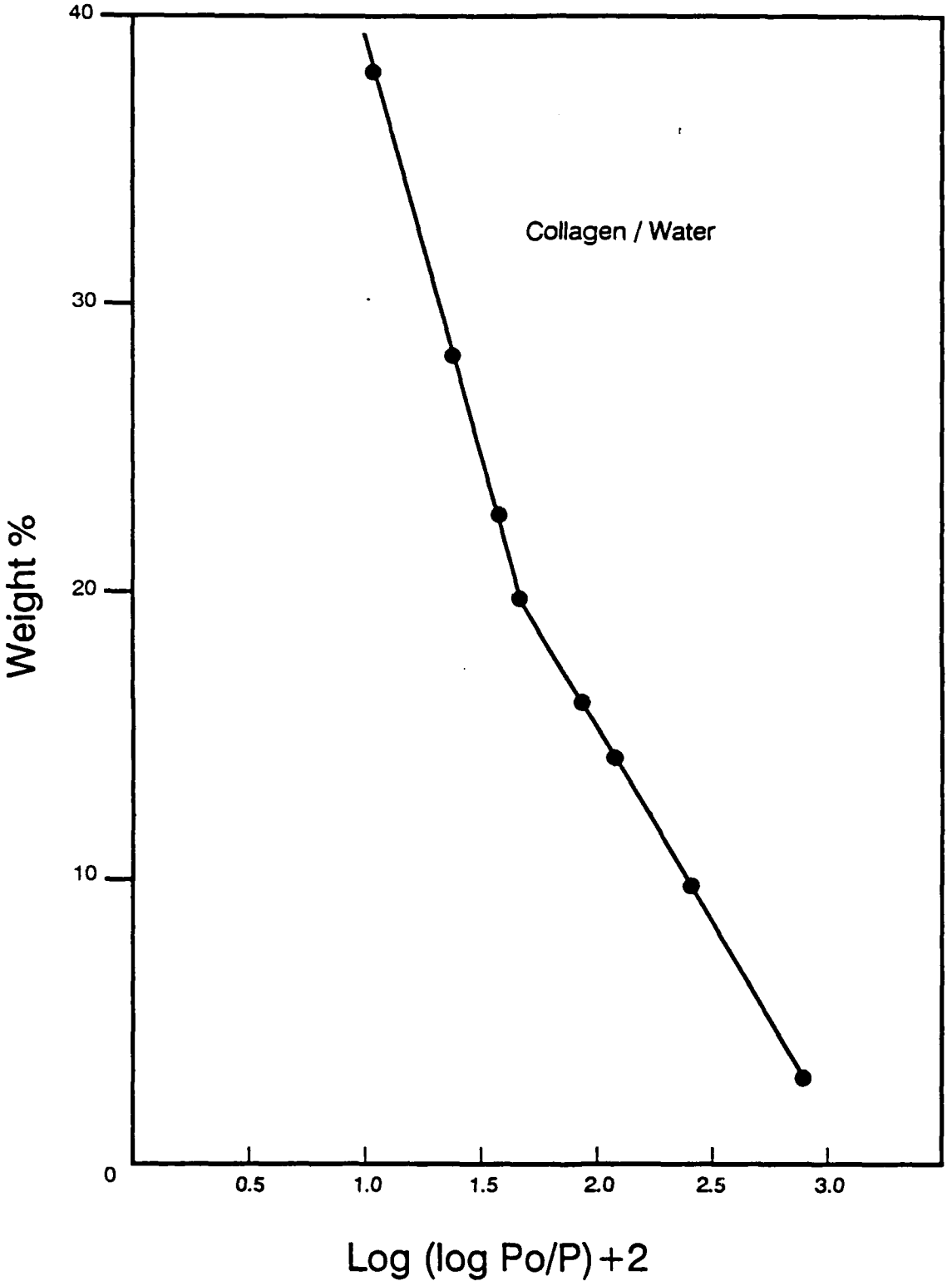


Figure 4. Bradley isotherm of water on collagen (26.0°C).

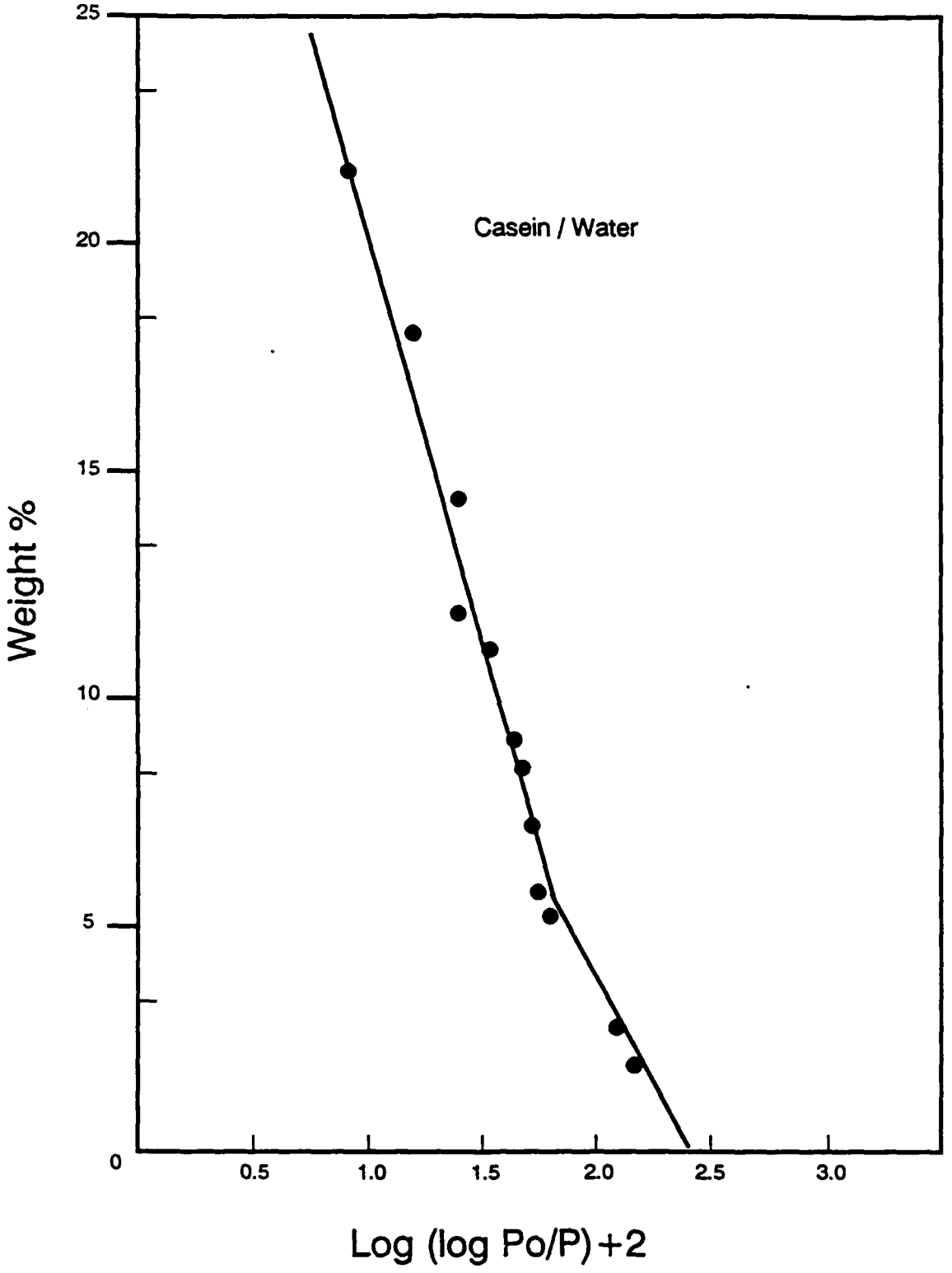


Figure 5. Bradley isotherm for casein and water. (26.0°C).

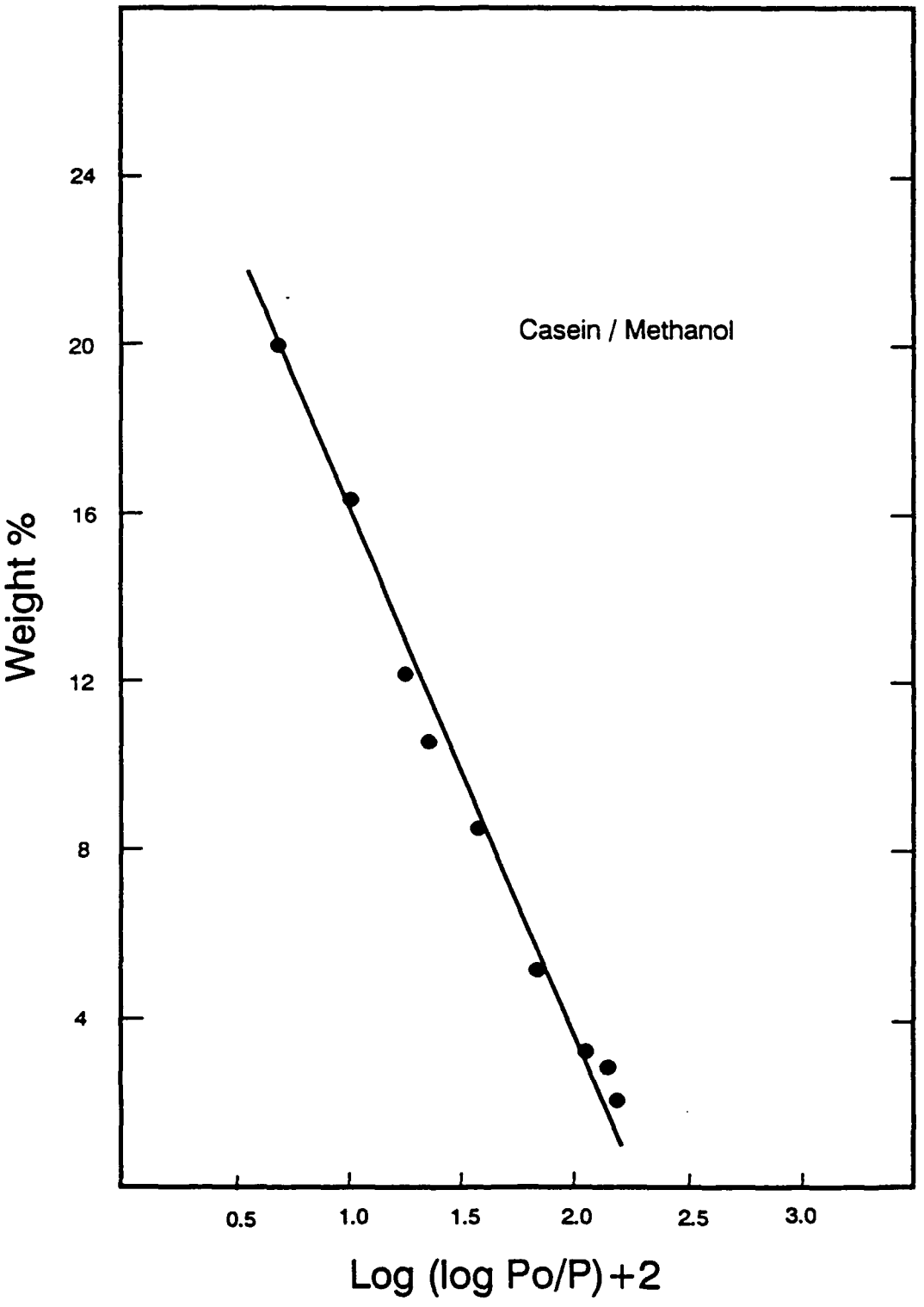


Figure 6. Bradley isotherm for methanol on casein (26.0°C).

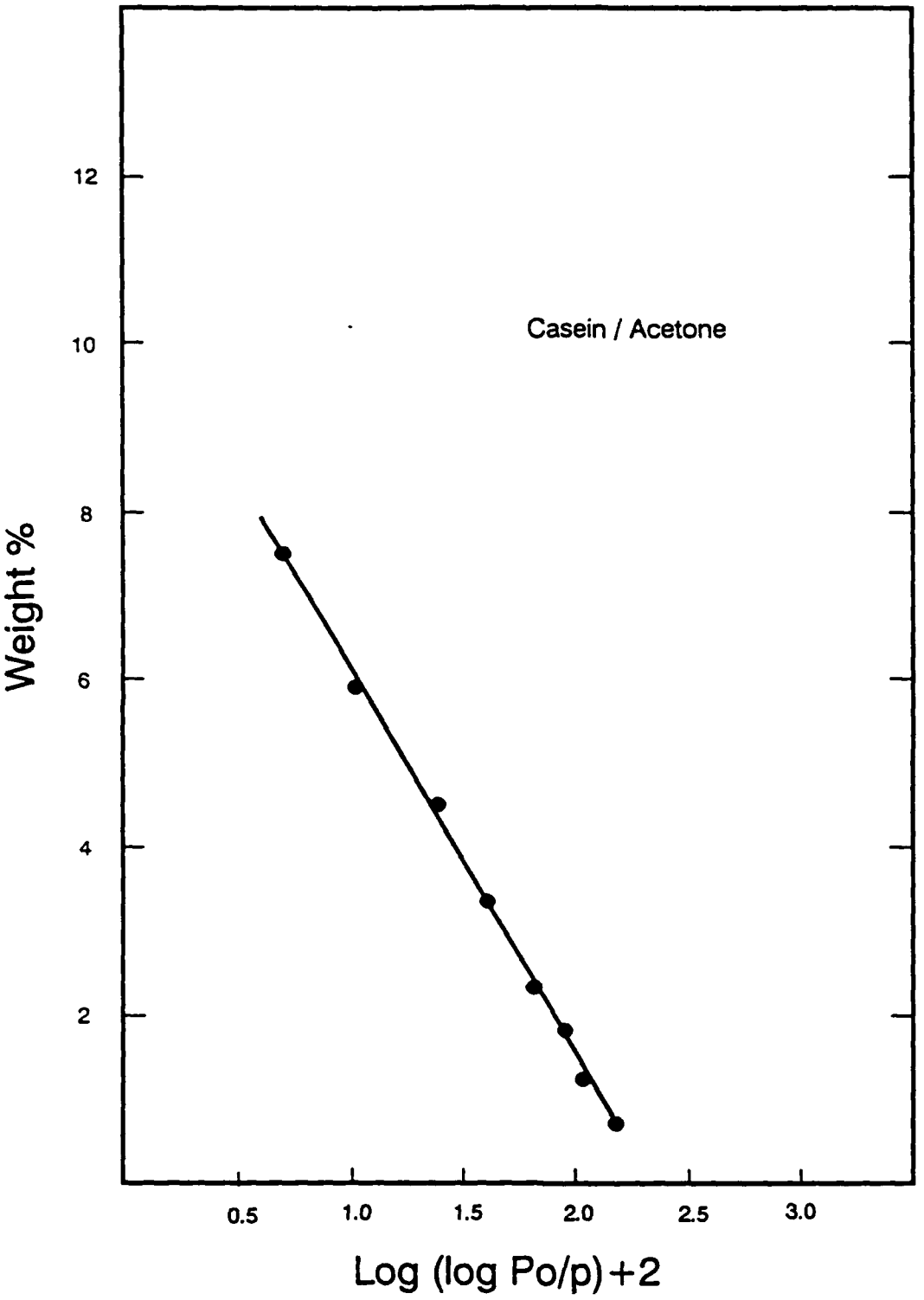


Figure 7. Bradley isotherm for acetone on casein (26.0°C).

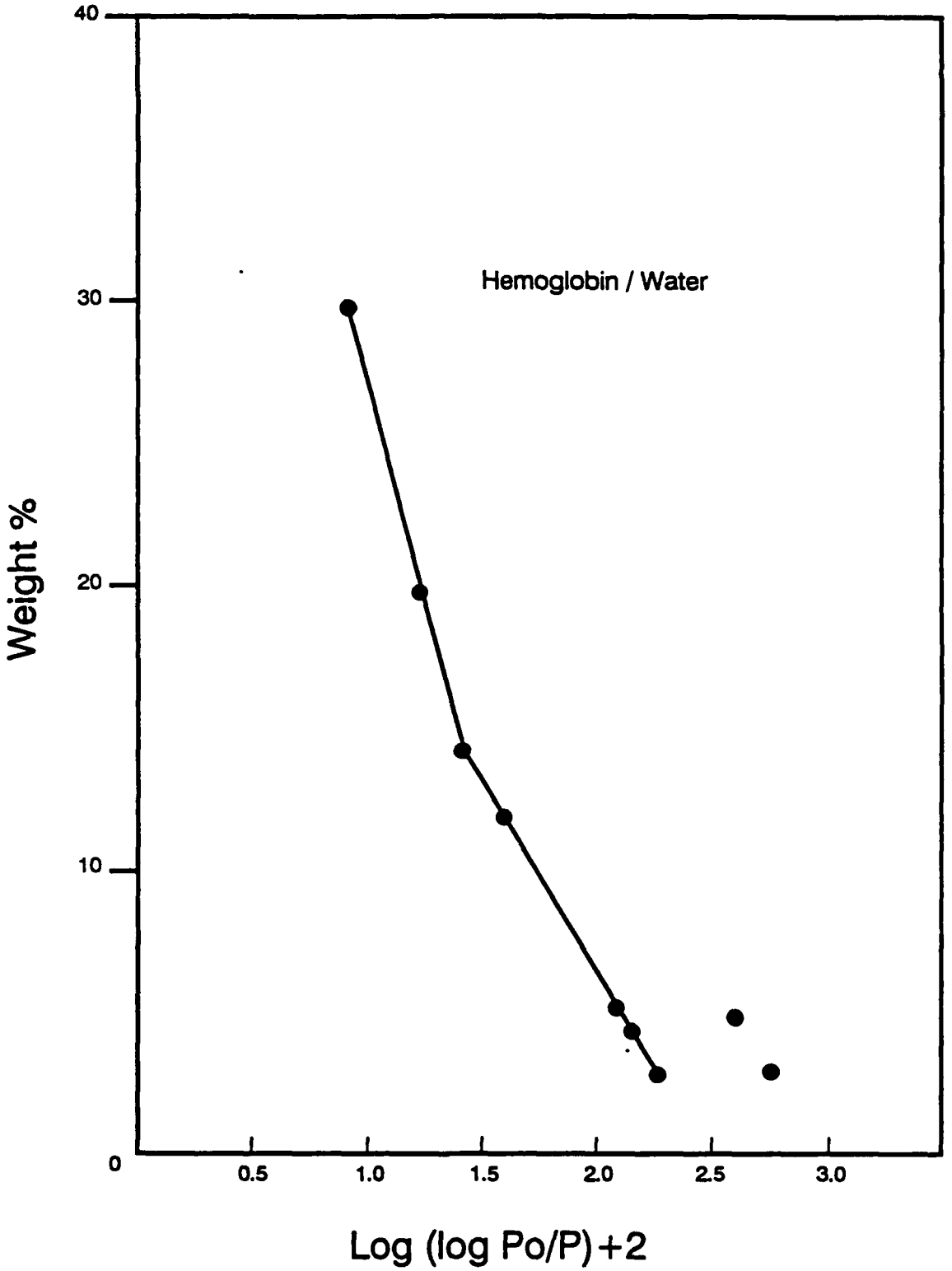


Figure 8. Bradley isotherm for water on hemoglobin (26.0°C).

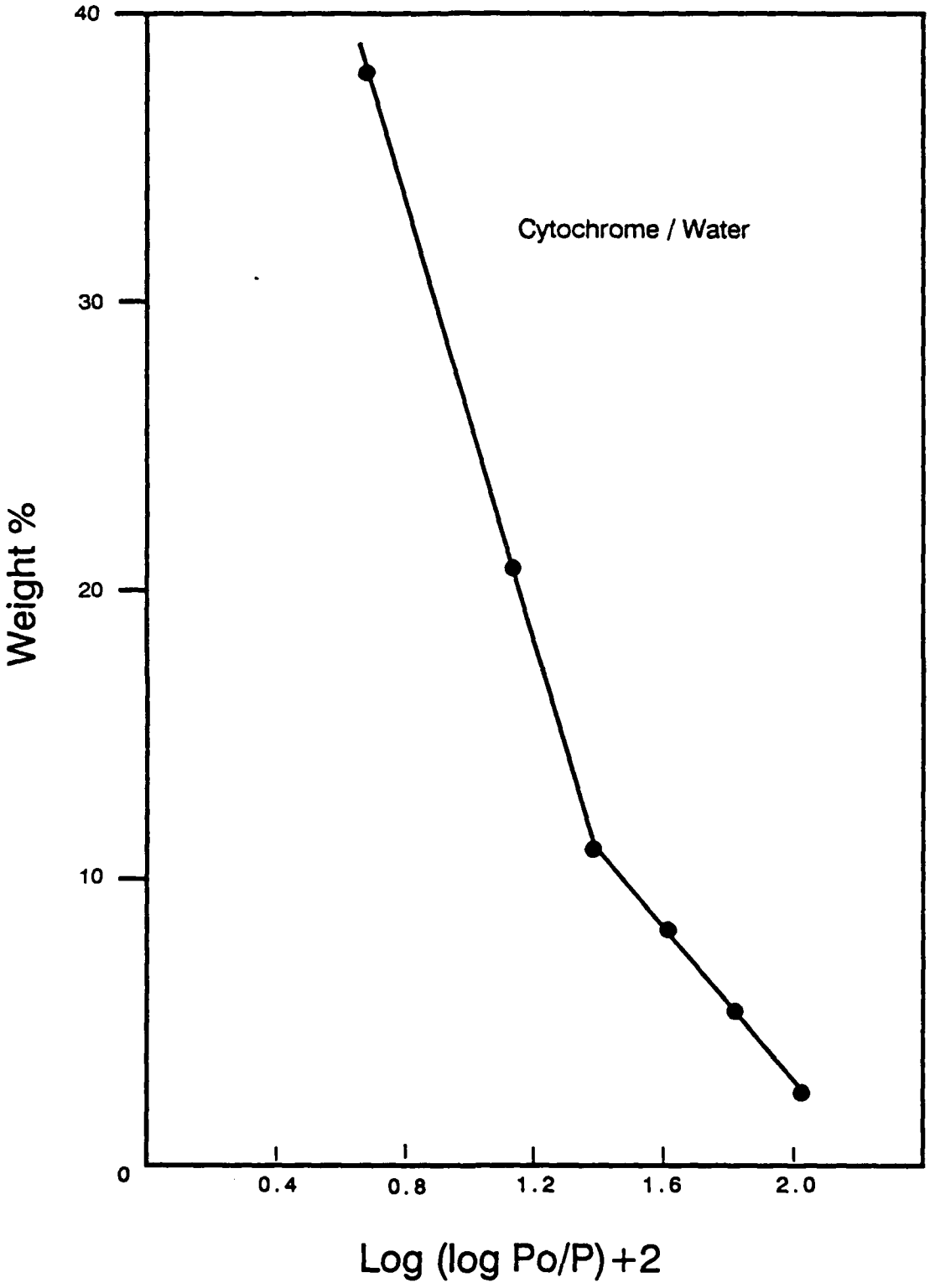


Figure 9. Bradley isotherm for water on cytochrome (26.0°C.)

B. Electrical Conductance

The conductance of several biomacromolecules as a function of amount of adsorbate is given in Figures 10 to 14. It is easily seen that the general shape of the curves is similar; i.e., a sharp rise (on semi-log paper) with a subsequent saturation of current with increased adsorption. The level of saturation is different for each macromolecular adsorbate and differs also for each adsorbate. As can be seen from Figures 13 and 14, there is a general relationship between the dielectric constant of the adsorbate and the peak electrical conductivity.

The increased conductance with organic (or non-ionizable) liquids is quite important. It indicates that electrical conductivity, with these adsorbates, could not be an extrinsic phenomenon such as these agents cannot donate protons (and there is poor correlation of conductivity and ionization potential, see "Discussion"). These organic liquids are insulators in the bulk phase.

The possibility exists that some of these organic liquids aid in the conductivity increase both (i) through the effect of the bulk dielectric constant and (ii) as "plasticising" agents, they aid in effecting conformational changes as described by Seanor (1982). The protonic conductance is intrinsic as shown in the case of hemoglobin/methanol and hemoglobin/ethanol (vide infra).

C. Electrolysis

While reported in 1970 by Powell and Rosenberg that protonic conduction could be determined by the production of hydrogen, it was nonetheless an inference that this protonic

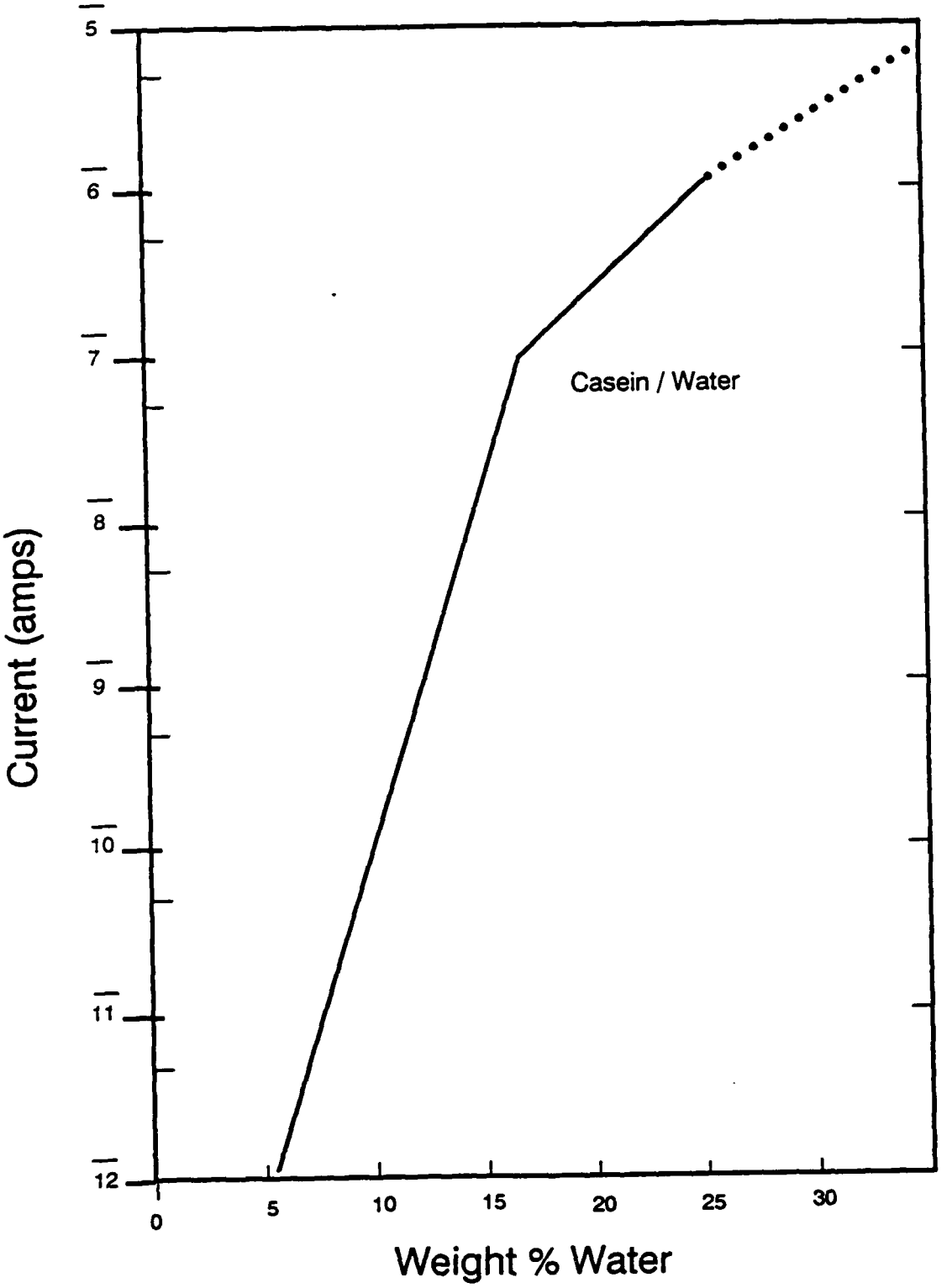


Figure 10. Electrical conductance in casein as a function of adsorbed water.

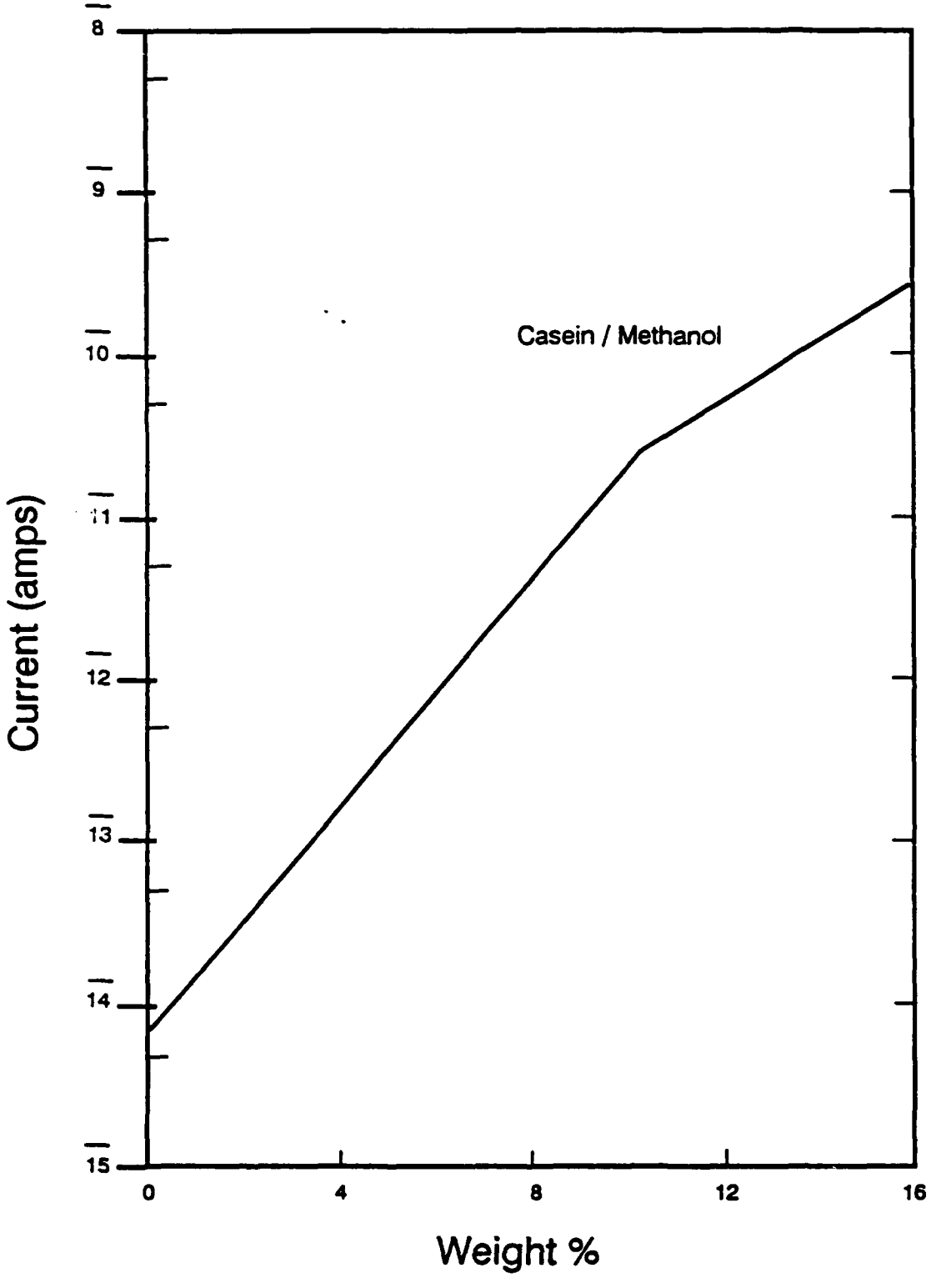


Figure 11. Electrical conductance in casein as a function of adsorbed methanol.

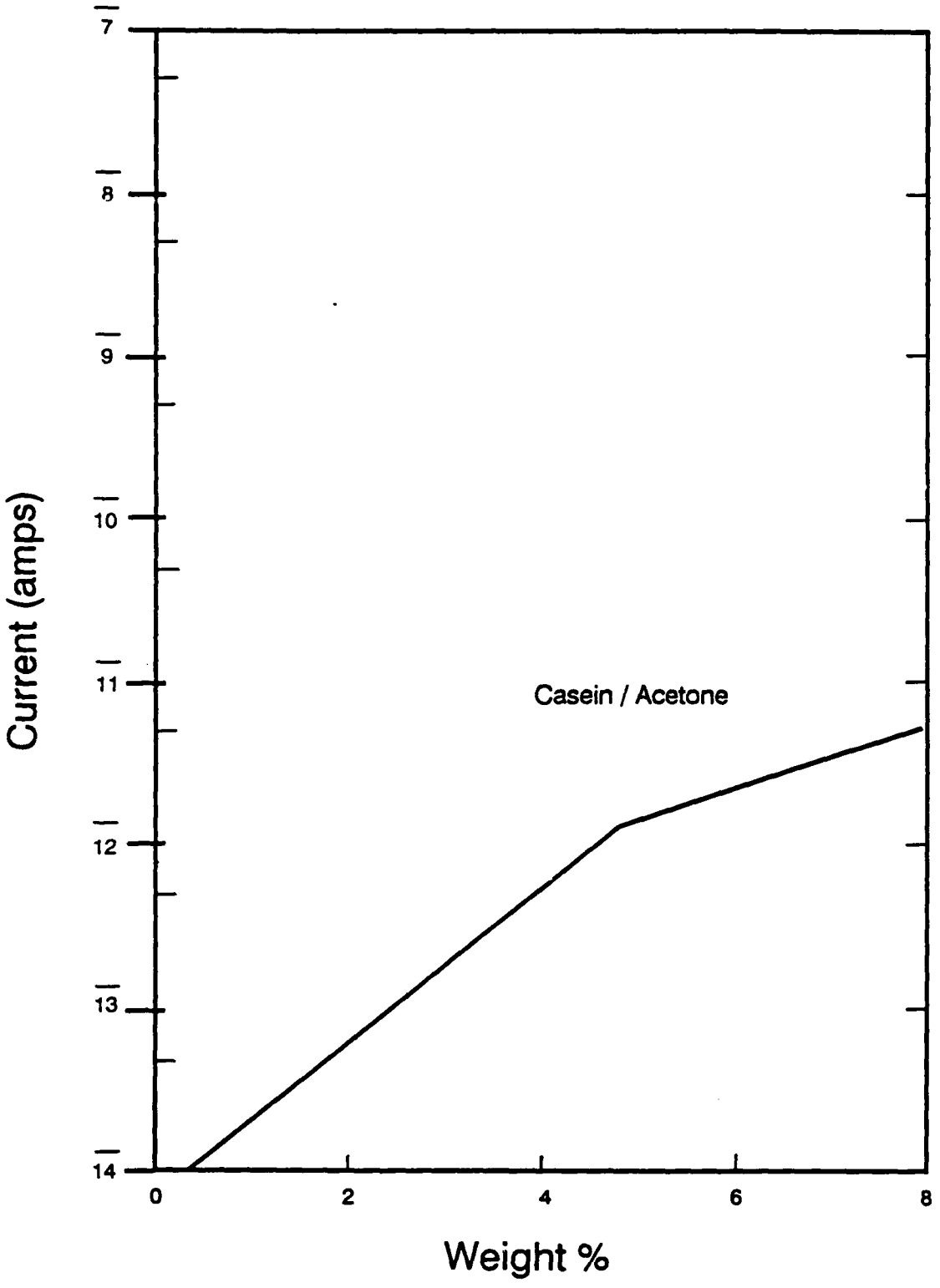


Figure 12. Electrical conductance in casein as a function of adsorbed acetone.

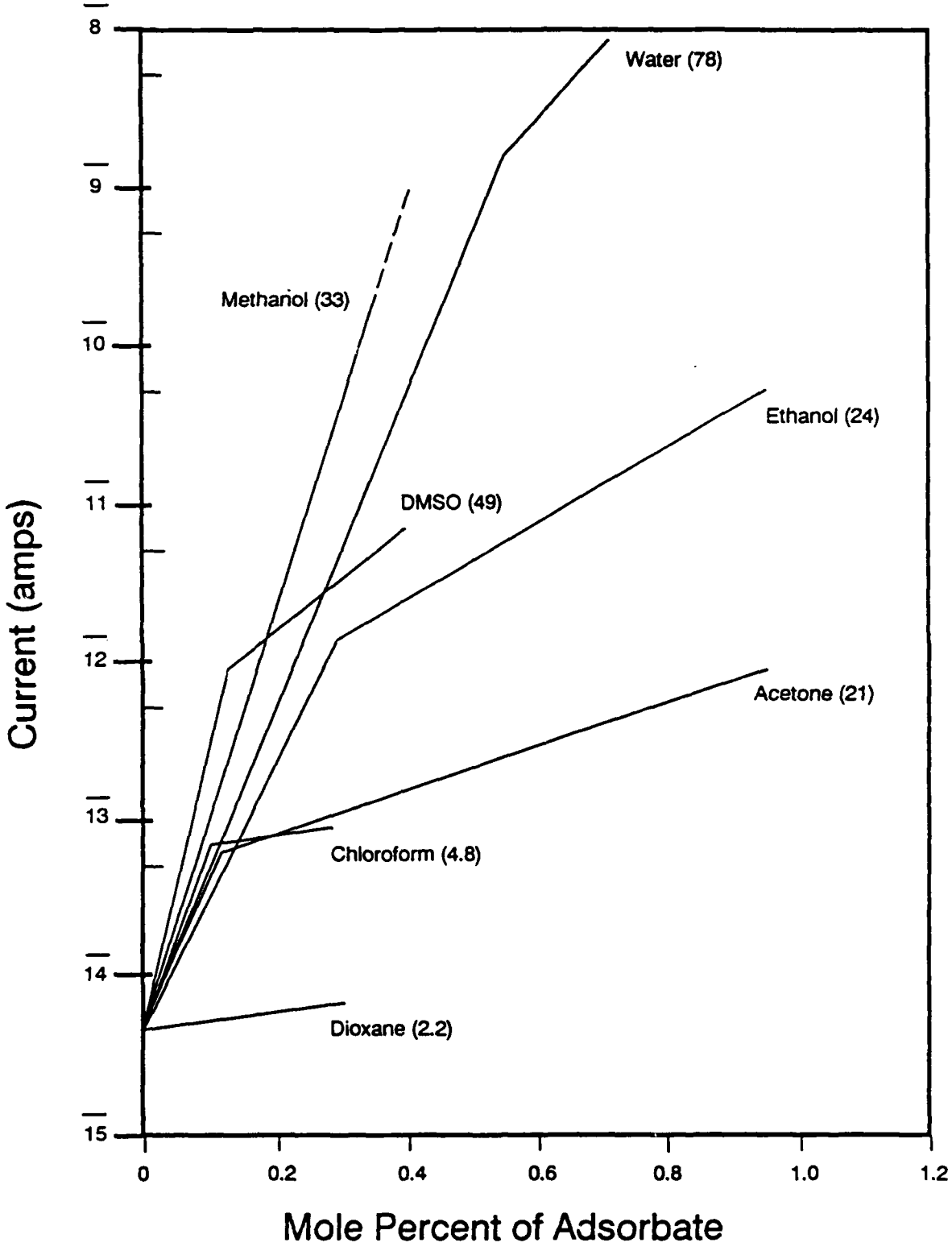


Figure 13. Electrical conductance in collagen as a function of various adsorbates. Bulk dielectric constant is in parentheses.

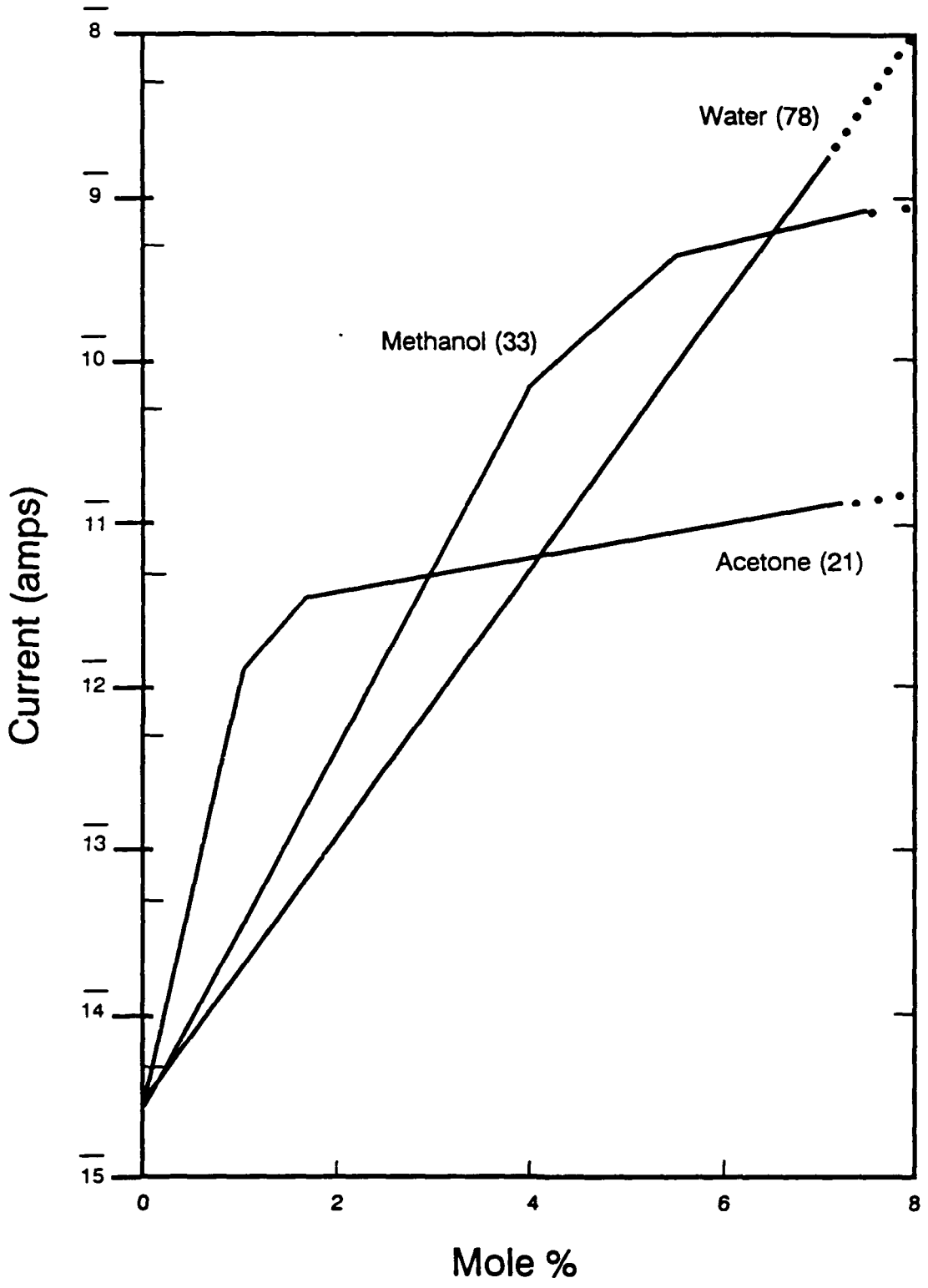


Figure 14. Electrical conductance in casein as a function of various adsorbates. Bulk dielectric constant is in parentheses.

conduction was intrinsic to the adsorbent biomacromolecule and not a simple electrolysis of the adsorbed water. The inference was based upon the empirical evidence that the degree of protonic conductivity (or hydrogen generation) was unrelated to the BET monolayer coverage. One would assume that proteins would not exhibit protonic conduction until at least one, if not two or three, BET monolayers were formed. Protonic conduction in an approximate form to bulk water conductivity would then ensue. As reported by them, this was not found.

Historically, the protonic conduction concept, either intrinsically or in the concept of the "hydrogen bonded chains" (HBC) of Nagel, has become of greater interest than that of electronic conduction. Thus, if this mechanism is to be invoked, and the work of Powell and Rosenberg cited as evidence, it is necessary to demonstrate that these original studies did not measure simple "bulk water" electrolysis. That is, hydrogen would be generated but not oxygen.

Such studies in the detection of oxygen were the focus of this work, for, if oxygen was detected in quantities commensurate with Couloub's law, then the implication must be that simple electrolysis of bulk water was occurring. The early studies of Powell and Rosenberg would therefore be valid only for the electronic component and irrelevent with respect to intrinsic protonic conduction.

The results for the electrolysis of compounds with varying amounts of adsorbed water and organic solvents are as expected, that is, (i) increasing solvation results in increased electrical (either electronic or protonic) conduction but, (ii) not in the production of oxygen. This was true for all samples tested.

1. Collagen, as determined earlier, (Powell and Rosenberg, 1970) in the dry state was a protonic conductor and became more of an electronic conductor with hydration. The bound water and the hydrogen bonding may be the cause of this large protonic mode in the "dry" state. With increased hydration, there may result small conformational changes which allow the increase of electronic conduction. [It should be noted that the total amount of protonic conduction does not decrease with hydration, only the ratio of protonic to electronic carriers.] At high hydrations (greater than 2 BET monolayers) oxygen could not be detected [The rate was less than 1% of that expected from Couloub's law].

When dryer samples (less than 5% water), were electrolysed, the amount of oxygen could only be qualitatively determined since the system was approaching the background limit for this gas; none was found.

Since less than 1% simple bulk-water electrolysis was found in more hydrated samples, we feel it is safe to conclude that even dry conductance is by an intrinsic mechanism. Dry state conductance with the evolution of oxygen, furthermore, would be difficult to envisage mechanistically in a vectorial, steady-state mode.

2. Cytochrome-c was the only compound found by Powell and Rosenberg (1970) to change from electronic conduction as the majority mode to protonic as the main form while varying linearly with hydration. The hydration varied from two BET monolayers to about six.

In this contract study, both dry and hydrated samples were tested for oxygen evolution. Qualitatively, none was found in dry samples, and, in wet (<3 BET monolayers), oxygen formation was less than 1% of the theoretical yield.

3. Hemoglobin exhibited a pattern of change of charge carrier with hydration which was very similar to cytochrome-c. This similarity was also seen in their adsorption isotherms and BET monolayers (6.1% for hemoglobin and 6.3% for cytochrome-c). Water (>2 BET monolayers) adsorbed produced amounts of oxygen less than 2% of theoretical following electrolysis; hydrogen yields were similar to the earlier findings of Powell and Rosenberg (1970).

When hemoglobin adsorbs methanol instead of water, the change of carriers is not linear with solvation as found by Powell and Rosenberg (1970). The cause for this is not known, but it may be the result of conformational changes accompanying "plastization". These Hb/meOH systems, when electrolyzed, yielded hydrogen in amounts indicating approximately 20-30% of the stoichiometric yield; oxygen was present in less than 2% of the theoretical yield.

4. Casein when electrolyzed yielded hydrogen in amounts which were 30-40% of the theoretical prediction in samples hydrated in the ranges of 1 to 3 BET monolayers. Oxygen was not detected above the system's off-gassing background; this would indicate that its appearance was less than 2% of theoretical.

5. DNA when electrolyzed demonstrated the production of hydrogen when hydrated above the 3 BET monolayer state; this is in accordance with the earlier findings of Powell and Rosenberg (1970). There was no detectable oxygen above background which would place the upper limit on its production at less than 2% of the stoichiometric amount.

Since these higher hydration states are in the range where original studies indicated a marked change of carrier

types, it was important to note that this change of entirely electronic to mixed protonic/electronic (Powell and Rosenberg, 1970) could not be the result of "bulk water" conductivity. We must, instead, attribute some other processes such as conformational changes and/or more favorable hydrogen bonding patterns conducive to vectorial proton movement.

In the dry state, there was no detectable oxygen when sufficient amounts of current were passed. The upper limits would be approximately 20% of the theoretical oxygen yield.

V. DISCUSSION

A. Intrinsic Protonic Charge Carriers

The charge carrier change with solvation was found earlier by Powell and Rosenberg (1970a,b) to follow what had been suspected by earlier workers in the field; that is, the amount of protonic conductivity would increase with increasing amounts of adsorbed water. However, it was generally thought that it was not until two to three BET monolayers had formed that protonic conduction would become appreciable and that the protonic conduction would be extrinsic. This would imply the mechanism first suggested by Murphy (1960).

On the basis of this contract study, the question of intrinsic protonic conductivity is more secure since in all of the compounds that were tested, oxygen formation was not detected thus indicating that simple electrolysis of the adsorbed water was not occurring. For some of the samples tested, the current was of a sufficient level that it was possible to extend the electrolysis measurements down to the one BET monolayer hydration level. If these molecules were to be called "charge injection (or transfer) conductors" in the dry state, the transition from mixed protonic and electric to pure electronic conduction would have had to occur in a very small hydration range. There does not seem to be any theoretical justification for believing that less than one BET monolayer could alter the conduction properties to such a degree.

Indeed, in the case of collagen, the material has a negative slope (Powell and Rosenberg, 1970,b) of percent protonic conduction vs. hydration, and thus when dry would be a pure protonic conductor. (Collagen, though, is never

completely dry; high temperatures used for a thorough drying produce a change in the material as evidenced by a different adsorption isotherm.) During these experiments, collagen would contain some residual water in the intersticies of the triple helix which could contribute to its observed protonic conductivity in the "dry" state.

The basic premise in the interpretation of electrolysis experiments is that steady-state protonic conductivity must result in the evolution of hydrogen (and simple bulk electrolysis will result in the release of oxygen). This is a consequence of the fact that metals are "blocking electrodes" with respect to ionic flow. The hydrogen evolution, for example, would not have occurred if another type of electrode (potassium chloride in agar) had been used. Indeed, this latter type is "blocking" for electrons.

Here we have determined that the evolution of hydrogen reflects intrinsic protonic flow, and that this is the only ionic species since oxygen was not found within the limits of the device and must be less than 1% of the stoichiometric yield.

The models proposed in the past have utilized either "pumps", confirmational changes in the macromolecules, or "carriers" in the case of membranes. (Figure 15). The "hydrogen bonded chains" of Nagel are illustrated in Figure 16.

B. Carrier Type Charge with Solvation

It is found for hydrated proteins that the current as a function of hydration can be given by Equation 11. For many molecules the current is a function of two intrinsic components, viz., protonic (ionic) and electronic. It is

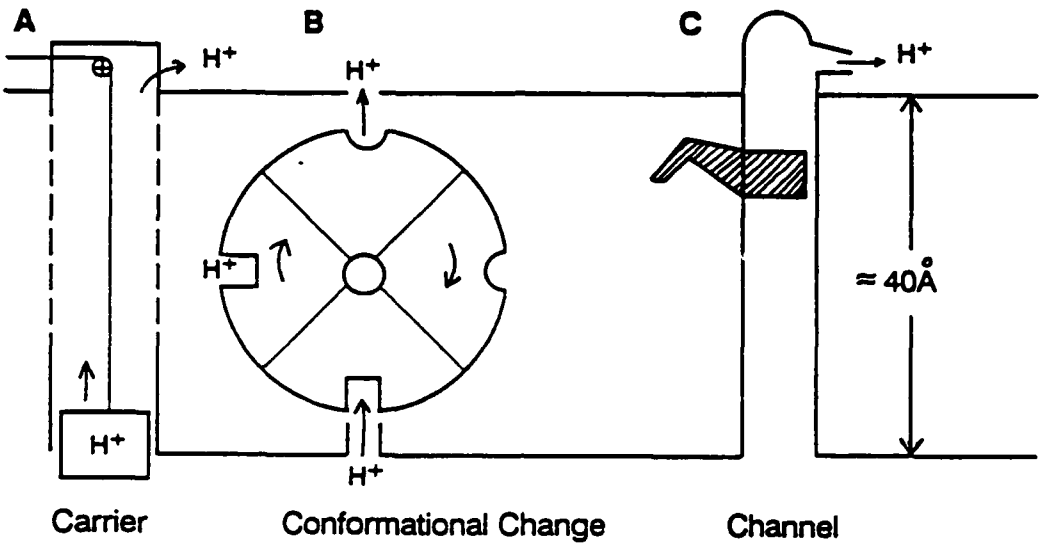


Figure 15. Schematic representation of three types of vectorial protonic conductance mechanisms. Type A suggests a carrier mechanism. Type B is a conformational change. Type C is a channel with active site regulator. From Nagle and Tristram-Nagle (1983).

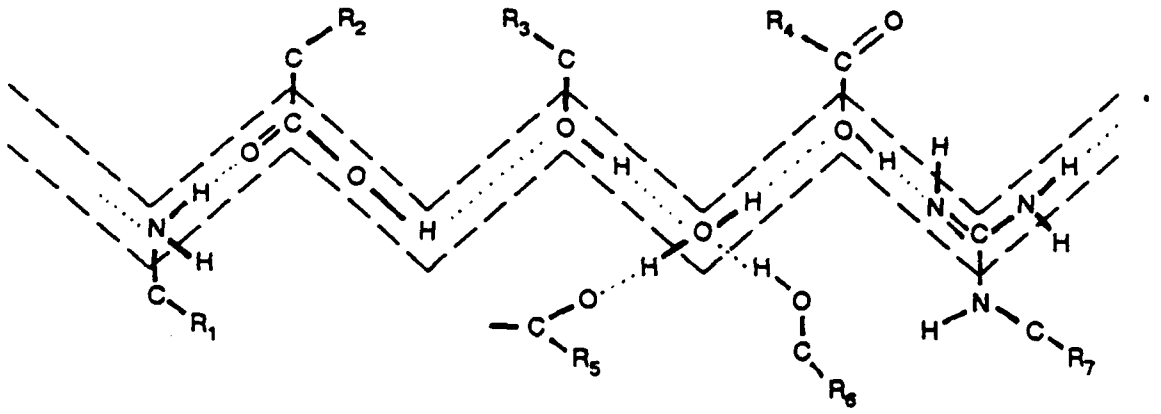


Figure 16. Amino acid side chains in membrane proteins and bound water molecules form a protonic conductance mechanism, the "hydrogen bonded chain" (indicated by dashel lines). From Nagle and Tristram-Nagle (1983).

possible to resolve the conductance into the respective intrinsic protonic and electronic currents as a function of hydration. This is shown for the case of cytochrome-c in Figure 17; this substance even shows a crossover point at 2½ BET monolayers. As can be seen, both the electronic and the ionic components follow Equation 39 (protonic).

$$\sigma^{(+)} = \sigma_{dry}^{(+)} \exp(\beta m) \qquad [39]$$

and Equation 40 (electronic)

$$\sigma^{(-)} = \sigma_{dry}^{(-)} \exp(\gamma m) \qquad [40]$$

where β and γ are constants, m is the amount of adsorbed water, and $\sigma^{(+)}$ represents the protonic current and $\sigma^{(-)}$ represents the electronic current. The total current is then the sum of these two, or

$$\sigma = \sigma^{(+)} + \sigma^{(-)} = \sigma_{dry}^{(+)} \exp(\beta m) + \sigma_{dry}^{(-)} \exp(\gamma m) \qquad [41]$$

These equations are applicable in the region from zero to about 2½ BET monolayers, that is, before current saturation occurs.

It has been shown (Postow, 1968) for the systems of hemoglobin (with water, methanol, and ethanol), melanin (with water) and collagen (with water) that the activation energy changes upon solvation, and that the value of σ_o in Equation 10 is a constant. This indicates that charge carriers are <u>intrinsic</u>, i.e., <u>not</u> added in the solvation process, and that it is only the value of E which changes. (This is not true in the hemoglobin/methanol and DNA/water system where probably both η_i and μ_i change.)

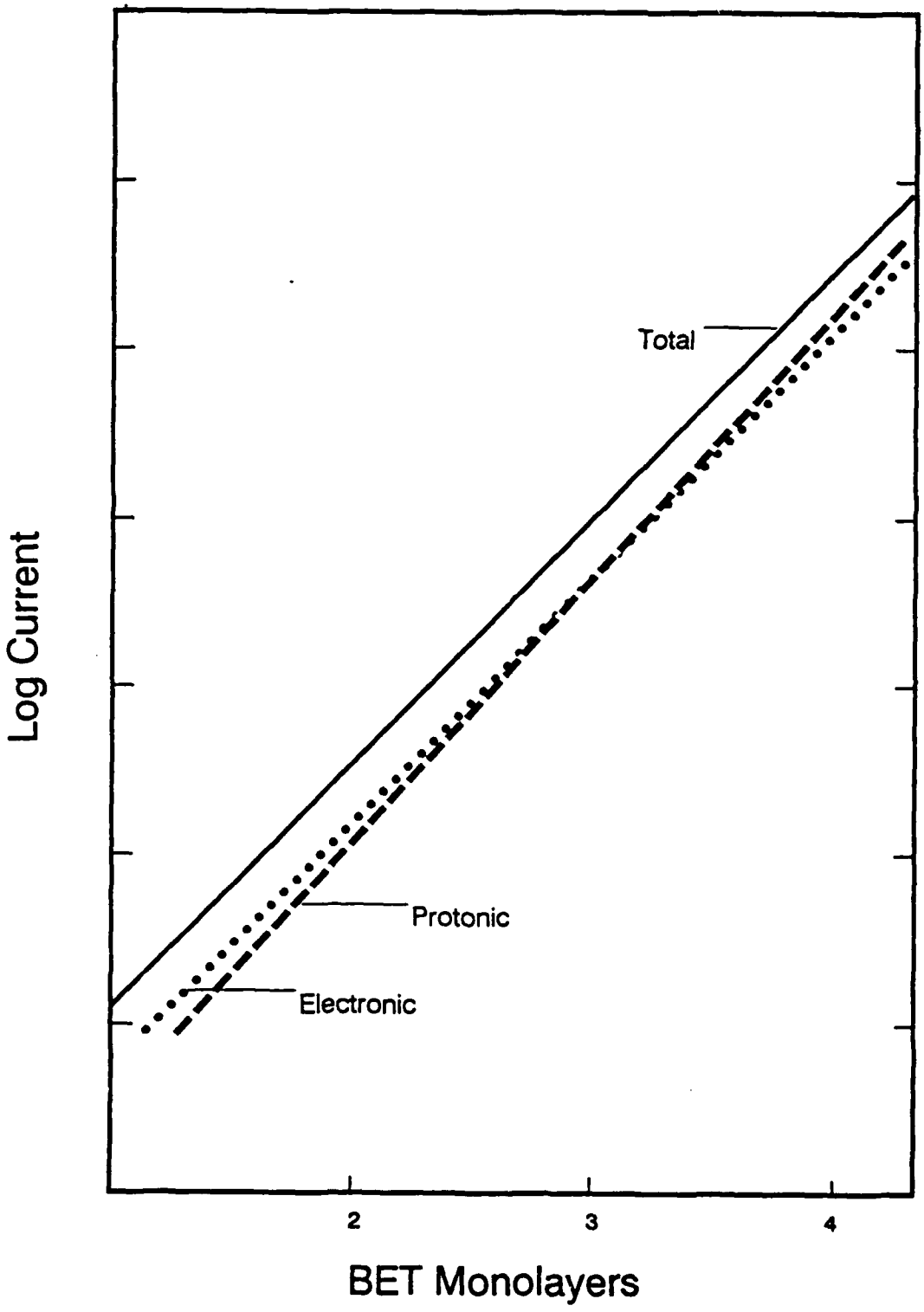


Figure 17. Resolution of Conductance Date into the Protonic and Electronic Components.

Rosenberg and Postow (1969), showed that the activation energy changes upon hydration in a manner which is similar in all materials investigated by them. Thus, one cannot associate the change of activation energy with a shift from one carrier type to another with each possessing a different value of E. The energy needed to overcome the coulombic attraction of the charge carriers and their "parent" molecule appears to be the principal energy barrier and is equivalent for protons and electrons.

One can then take Equations 39 and 40 and from them proceed in a manner similar to Equation 12.

$$\sigma_{(T,m)}{}^{(+)} = \sigma_o{}^{(+)} \exp\left[(-E_D/2kT) + \beta m\right] \qquad [42]$$

and

$$\sigma_{(T,m)}{}^{(-)} = \sigma_o{}^{(-)} \exp\left[(-E_D/2kT) + \gamma m\right] \qquad [43]$$

so that one gets for each species of charge carrier

$$\sigma_{(Tk')}{}^{(+)} = \sigma_o{}^{(+)} \exp(-E_D/2kT) \exp\left[(e^2/2kTR_1)(\tfrac{1}{k} - \tfrac{1}{k'})\right] \qquad [44]$$

and mutatis mutandi for the electronic carriers.

The total current is then given by the equation

$$\sigma_{(T,k')} = \left\{ \sigma_o{}^{(+)} \exp\left[(e^2/2kTR_1)(\tfrac{1}{k} - \tfrac{1}{k'})\right] + \sigma_o{}^{(-)} \right.$$

$$\left. \exp\left[(e^2/2kTR_2)(\tfrac{1}{k} = \tfrac{1}{k'})\right] \right\} \exp(-E_D/2kT) \qquad [45]$$

If the values of the radii of polarization R_i are about the same, the equation will reduce to

$$\sigma(T,k') = (\sigma_o^{(+)}+\sigma_o^{(-)}) \exp(-E_D/2kT) \exp\left\{(e^2/2kTR)(\frac{1}{k} - \frac{1}{k'})\right\} \quad [46]$$

the form usually found.

In some materials (1970b), such as hemoglobin/methanol and DNA/water (Powell and Rosenberg, 1970a), the current does not exhibit a saturation with large amounts of adsorbate (about two BET monolayers). It can be seen in Figure 18 (for hemoglobin water) that saturation usually occurs within one order of magnitude from a deviation from the linear region in a log current vs. solvation plot. For the DNA/water and

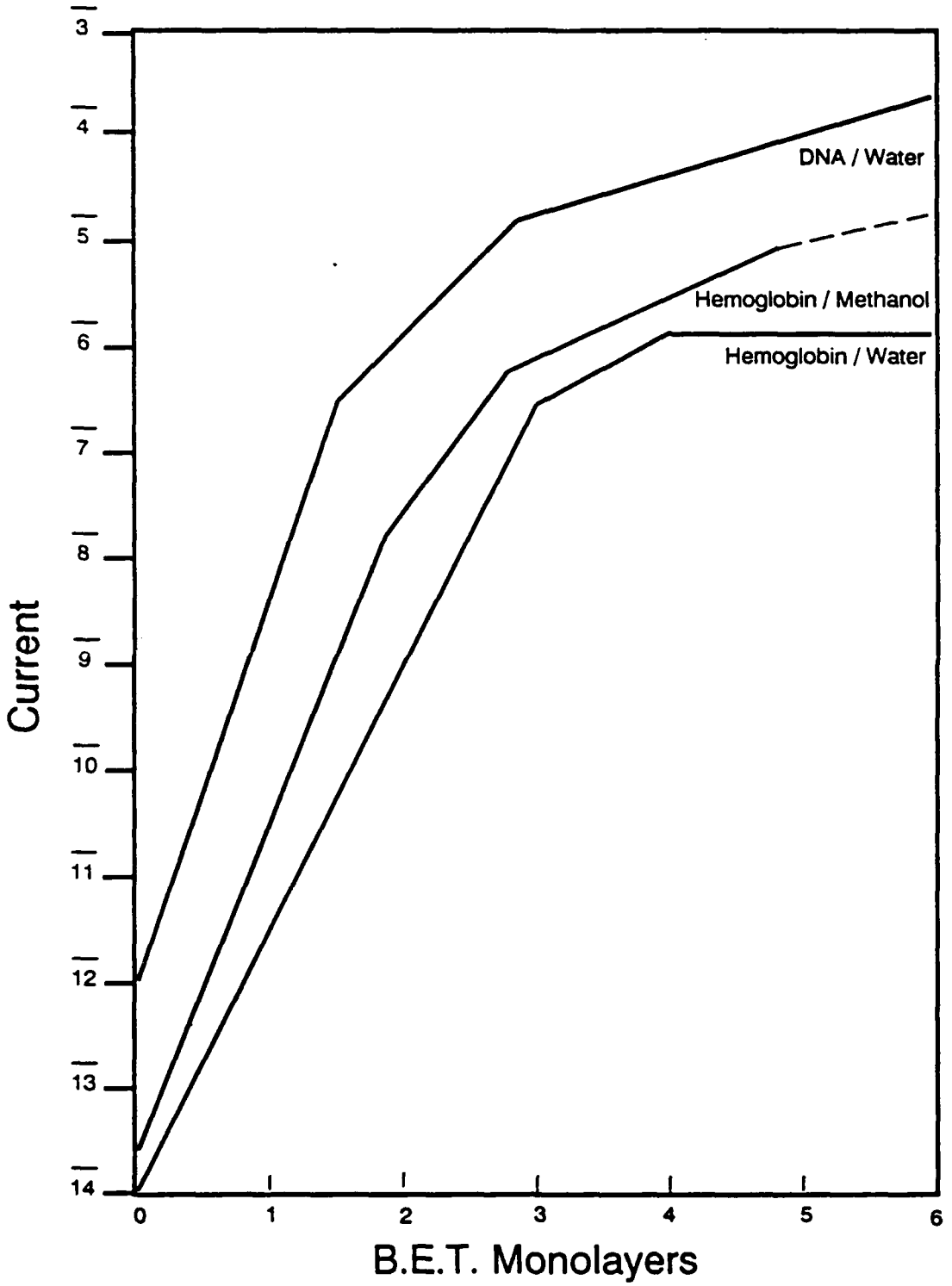


Figure 18. "Saturation currents" do not always occur; this is observed when systems show an onset of protonic conduction within a narrow absorbance range.

hemoglobin/methanol systems, the change of charge carrier species is not linear with increasing solvation, but instead shows a large change at the point where saturation would be expected. This is postulated here to be a conformation change as discussed in earlier sections of this report. This is the "disorder to A-form" transition in DNA/water which occurs at 75% relative humidity (Franklin and Gosling, 1953; Falk et al., 1963a, 1963b). While in these systems the protonic conduction may not be intrinsic (that is, the protons from the biomacromolecule), nevertheless, "bulk water" conduction is not occurring.

The saturation of current which occurs from two to three BET monolayers (Powell and Rosenberg, 1970b) is the result of the rapid change in the bulk dielectric constant (Rosenberg and Pastow, 1969) of the material which makes 1/k' very small and reduces the exponent in Equation 46 to a constant.

The adsorbed water is most likely very polarized with regard to its orientation on the macromolecule in that the adsorption process is found to obey the Bradley isotherm. That is to say, the theory is based on the polarization of one adsorbed layer by the substrate beneath it. This theory has been criticized by Brunauer et al. (1938), but they do state that "...if the adsorbed gas has a large permanent dipole, it is possible that many layers may be successively polarized..." It may be that after two to three BET monolayers, the rotation of the molecule may increase to allow the large increase in the bulk dielectric constant which is observed.

The difference found earlier (Powell and Rosenberg, 1970b) in the observed ratios of the electronic to protonic charge carriers in the different materials tested illustrates the large diversity of types in the conduction

process. The range was one of from pure protonic in the dry state (collagen) to an almost even contribution (melanin) to pure electronic (DNA). These protonic processes are furthermore generally intrinsic.

C. Dielectric Changes

In a combined grouping of several adsorbates, it was found that the current increasing capability of adsorbates was roughly a function of the dielectric constant of the adsorbate. (Figure 19 & 20) Thus compounds such as dimethyl sulfoxide (DMSO) or methanol which have no ionizable hydrogens, but which have a large dielectric constant could effect a large current increase when adsorbed on collagen.

If the protonic current is significant, as has been found for the hemoglobin + methanol system, then these protons must assuredly come from the biopolymer itself. It is obvious, however, from the data on the hemoglobin + methanol system, that not all adsorbates are equally effective in promoting protonic conduction at low adsorbate levels even if it is intrinsic to the adsorbent. Since protonic conduction, when it does occur for the aforementioned system and for the DNA + water system (Powell and Rosenberg, 1970a), sets in sharply, and there is no evidence of current saturation with increasing solvation.

Figures 19 and 20 also demonstrate the poor correlation of current increase by adsorbed compound vs the electron ionization potential. The connection with the reciprocal of the dielectric constance is superior.

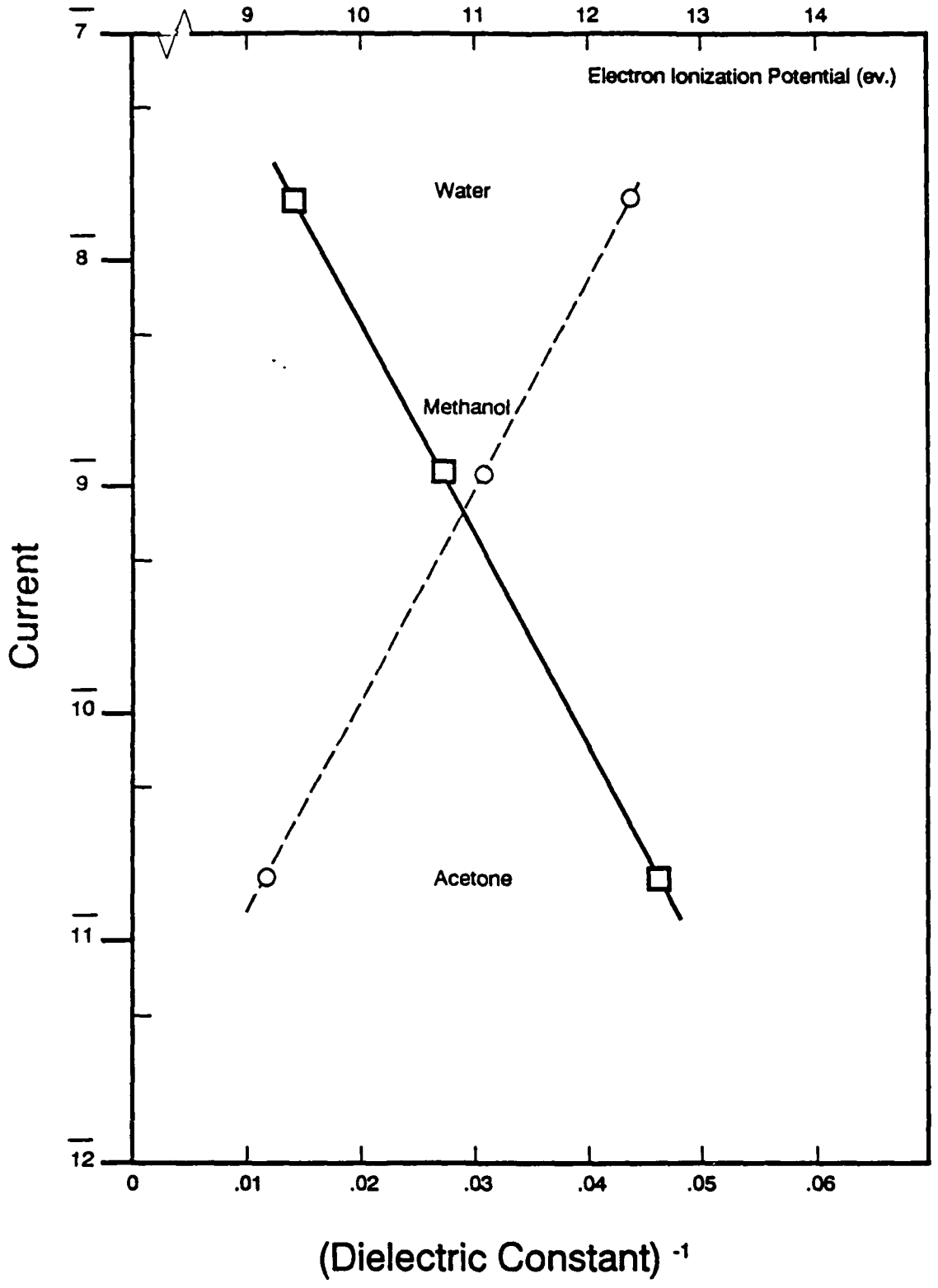


Figure 19. "Saturation currents" of adsorbed liquids on casein vs. reciprocal of the bulk dielectric constant (squares. below) and ionization potential (circles, above).

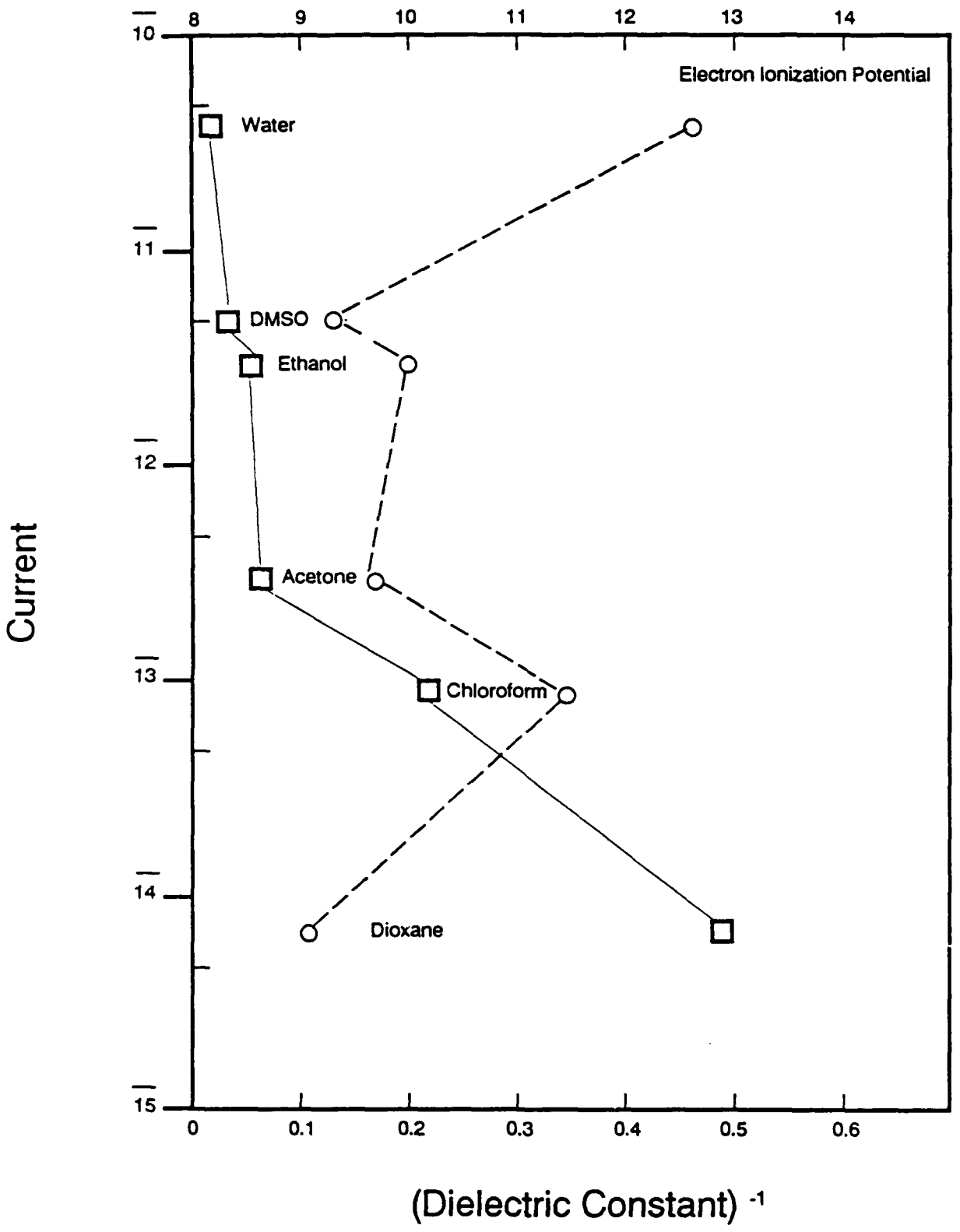


Figure 20. "Saturation currents" of adsorbed liquids on collagen vs. bulk dielectric constant (squares, below) and ionization potential (circles, above).

VI. References

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