Examining Solute-Solvent Interactions at the Electrode Double Layer by Impedance Measurements of Proteins: 1. Collagen

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Cyclic voltammograms of Type IV collagen from human placenta in the absence and presence of NaCl in aqueous solutions using a static mercury drop electrode indicate adsorption of the collagen at the electrode surface and strong interaction between collagen and NaCl. Impedance measurements, especially admittance, of the collagen in the absence and presence of NaCl suggest structural effects of water near the double layer. For collagen only, the admittance increases from 5000 Hz to about 50 Hz and then decreases. During this process the two admittance maxima shift to less negative potentials suggesting changes in the orientation of water. The first maximum seems related to the orientation effects near the peptide groups. The shift in the second maximum seems similar to the common electrolytes and is related to the double layer change when the potential of the mercury changes from negative to slightly positive. The admittance behavior is somewhat similar in the presence of NaCl with the admittance decrease happening at much higher frequencies than 50 Hz. The negative differential resistance observed at 0.4V, though not spectacular, suggests chaotic behavior, similar to many biological molecules, and is indicative of a tunnel diode or resonant tunneling. There was no clear-cut evidence for any saddle-node or hopf bifurcations in this system. Capacitance data show dispersion effects and there is a slight NaCl dependence on the potential of the capacitance maximum. Mott-schottky plots indicate both p-type and n-type semi conduction behavior. Phase microscopy shows self-assembly is dependent on the presence of sodium chloride.

1. INTRODUCTION

Our laboratory is involved in the search for organo-metallic redox complexes with natural organic ligands. Such complexes we believe have potential for medical use. Our therapeutic and electrochemical experiences with palladium-lipoic acid complex [1-2] suggest that molecules with self-assembly or liquid crystal solution behavior have catalytic electrochemical properties. This led us to search for natural biological molecules that possess the property of self-assembly, to evaluate

biological electronic signal behavior. Since electronic signaling processes in biological systems involve DNA, RNA, and proteins in a milieu of aqueous electrolytes, we direct our focus to these molecules and ions. In earlier presentations [3-5], we reported results with DNA.

Collagen, the most abundant mammalian protein, is the fibrous matrix component of skin and blood vessels, and is the major component of connective tissues such as bone and tendon. The triple helical cable structure of this protein provides its characteristic tensile strength [6]. Collagen is a family of at least nineteen proteins. Another ten proteins have collagen-like domains. Collagens have repeating –Gly-X-Y- sequences where Gly is glycine, with X and Y most frequently being proline and 4-hydroxyproline. The three polypeptide chains that are each coiled into a left-handed helix are then wrapped around each other into a right-handed super helix. With the action of vitamin C and the enzyme prolyl hydroxylase, proline residues are converted to hydroxyproline residues after the build up of the collagen chain polypeptides. It is also a pleasure to note that last year was the 50th anniversary of the discovery of the coiled-coil triple helical collagen structure [7-8] based on the postulates that only trans residues occur in protein structures, and the structure should contain one-third the total number of residues as glycine. The three polypeptide chains may be staggered with the restriction that the –Gly-X-Y- residues from the three chains occur at the same levels. Their orientation allows strong hydrogen bond formation of the N-H of each glycine, with the carbonyl oxygen of an X-residue on a neighboring chain. The rigidity of the entire assembly comes from the inflexible and bulky proline and hydroxyproline residues.

The collagen group of proteins is organized into different classes on the basis of their structures and structural features [9]. Some examples are: Types I, II, III, V, and XI that form fibrils, Types IV, VIII, and X that form network-like structures, Types IX, XII, XIV, XVI, and XIX that are known as fibril-associated collagens with interrupted triple helices (FACITs) and Type VI collagen that forms beaded filaments. Slight differences in the primary amino acid sequence establish differences between the different types. Collagen breaks down in the body to release N-telopeptide in urine and this is used as a diagnostic test for screening osteoporosis.

We have chosen Type IV collagen from human placenta for our studies because of its solubility in water at moderate pH. Type IV collagen is found in basement membranes. Basement membrane is the deepest layer and is fragile. It secures the overlying layers of stratified epithelium. This type of collagen contains about 1400 amino acids and is longer than the fibril forming collagens. Short noncollagenous sequences often interrupt the -Gly-X-Y- sequence. The N-terminus and C-terminus of the molecule have the short and major noncollagenous domains. When the molecule self-assembles into network-like structures, the monomers associate at the C-termini and N-termini to form dimers and tetramers respectively. The triple helical domains also intertwine to form supercoiled structures [9].

During the last four years we found that impedance is a powerful technique for understanding solute-solvent interactions. Admittance measurements, often neglected by a majority of electrochemists, provide powerful insights into the nature of solvent interactions with solute molecules and we have explored this technique quite extensively. We have also found that by venturing into both

the normal as well as the passivation region of mercury we can obtain a lot of information on biological surfaces that contain both positive and negative regions of charges. Also no other surface can provide such a clean reproducible surface as a fresh mercury drop.

2. EXPERIMENTAL PART

2.1 Materials

Collagen Type IV from human placenta, (Fluka BioChemika, Cat. No. 27663), molecular mass app. 125,000 was used. The NaCl used was Analytical grade. Distilled water was used for preparation of all solutions.

2.2. Methods

An EG & G PARC Model 303A SMDE tri electrode system (platinum counter electrode and Ag/AgCl (saturated KCl, reference electrode) along with Autolab eco chemie was used for cyclic voltammetric and electrochemical impedance measurements at 298 K. The solutions were carefully purged (due to foaming problems) with N_2 for about 10 minutes before the experiment. Impedance measurements were carried out using about 7 mL solutions in the frequency range 1,000 Hz to 5 mHz. The amplitude of the sinusoidal perturbation signal was 10 mV.

3. RESULTS AND DISCUSSION

3.1 Cyclic Voltammetry

These measurements were made for 0.5, 2.5, and 5 mg/mL collagen in the absence (pH 3.6, 3.6 and 3.5 respectively) and presence of 0.01 M NaCl (pH 6.2, 6.2 and 3.9 respectively), at a scan rate of 100 mV/s by scanning in three potential regions. The first region is in the range 0 to -1.0 V and back to 0V. This falls within the conventional range of the mercury working electrode. The second range is 0.3 to – 1.0 V and back to 0.3V so that the mercury is partly passivated and the chloride begins to interact. The third range is 1.0 to –1.0 V and back to 1.0V. This is the range where mercury is completely passivated and the chloride is known to interact strongly. The idea behind scanning through this wide region is to encompass natural biological surfaces with both positive and negative charges. We chose the mercury electrode because we understand its polarization range. Of course there is the ease with which we can get fresh electrode surfaces by using a new drop each time. Three scans were made for each potential region. The results for the third scan are shown in Figures 1 and 2. The results in Figure 1a for the scan from 1.0 to -1.0 and back to 1.0 V suggest adsorption of the collagen at the mercury surface and the adsorption increases with increasing concentration. The cathodic peak shifts from -0.37 V to -0.46 V when the concentration is increased from 0.5 to 2.5 and 5 mg/mL. We also observe a small hump on the cathodic side at 0.2 V. In the presence of 0.01 M NaCl, shown in Figure 1b, there is practically no collagen concentration dependence on the observed current. However the cathodic peak is shifted to about – 0.1 V. Also a broad anodic peak observed at 0.86V for collagen at the higher concentrations is shifted to 0.5 V in the presence of NaCl. The peak also becomes sharper in the presence of NaCl.
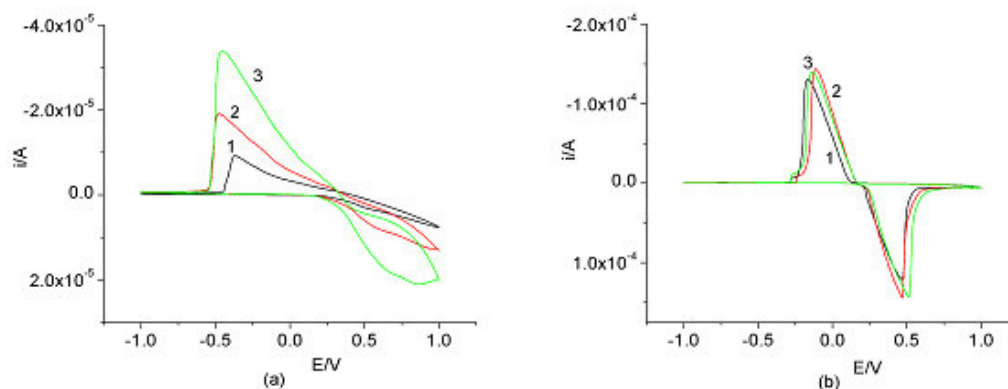
Figure 1. Cyclic voltammogram of Type IV collagen from human placenta. a) 1, 0.5mg/mL; 2, 2.5 mg/mL; 3, 5mg/mL; b) the same in the presence of 0.01M NaCl

However, we must point out that when the curve3 in Figure 1b is replotted in an expanded window using the region -1.0 to -0.29 V a peak at - 0.46 V appears. However the current is about 100 times
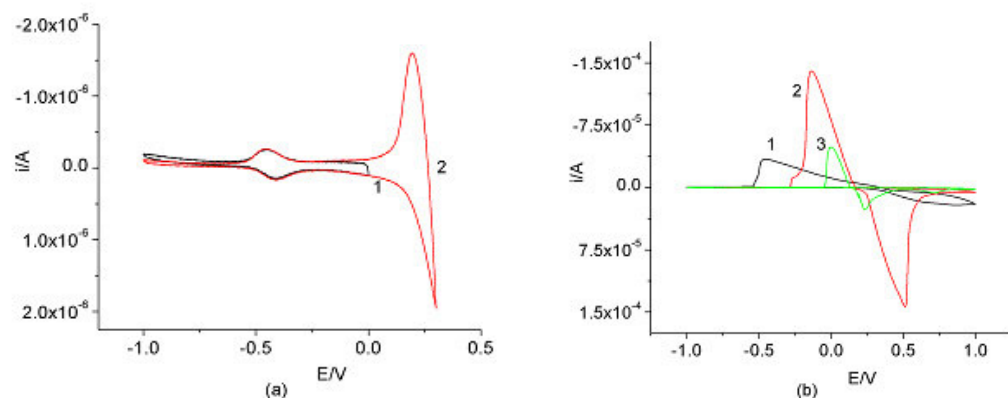

Figure 2. a) Cyclic voltammogram of 5 mg/mL Type IV collagen from human placenta in the scan range, 1, 0 to -1.0 and back to 0 V; 2, 0.3 to -1.0 and back to 0.3V; b) Influence of NaCl on the Cyclic voltammogram of Type IV collagen; 1, 5 mg/mL; 2, same in the presence of 0.01M NaCl; 3, 0.01 M NaCl only.

less than the curve 3 of Figure 1a. Figure 2a shows the results for the two scans starting at 0 and at 0.3 V. The current is very small when the scans are made in the potential region 0 to -1.0 and back to 0V. But a very small peak is observed at -0.46 V and this corresponds to the same peak observed in the scan range used in Figure 1a. The peak at 0.2 V corresponds to the hump seen in Figure 1a. That curve is not overlayed here because of the much higher current. Figure 2b compares the data of collagen in the absence and presence of NaCl and the NaCl blank. The strong interaction of NaCl with collagen is obvious from these curves as evidenced by the shifts in both the cathodic and anodic peak potentials and the much higher cathodic and anodic peak currents. The above observations are in accordance with the characteristics of adsorption process. When there is strong adsorption, a post wave or post peak is often observed [10]. This contributes to higher cathodic currents. In the case of weak adsorption no post peak is observed, but only an increase in cathodic current.

3.2. Admittance

The admittance data for 5mg/mL collagen only are shown in Figure 3. The data at 5000 Hz were very close to that of 1000 Hz but with more scatter. At negative potentials, the admittance increases with decreasing frequencies up to about 50Hz and then decreases at lower frequencies. The peak admittance at about -0.5 V or the minimum at about – 0.4 V stays at nearly the same potential in the high frequency range. However this peak (or minimum) starts shifting to less negative potentials at lower frequencies. When this shift remains steady at frequencies lower than 50 Hz, another shift towards still higher positive potentials takes place at low frequencies. Also a third peak around 0.38 V begins to appear with lower and lower frequencies. At lower frequencies the admittance increases with increasing potential. It is due to the apparent shift of the second maximum after its growth is complete. We attribute these changes in the admittance and shift in the second maximum to the change in the orientation of water molecules as the double layer re-configures during the potential changes from negative to positive.
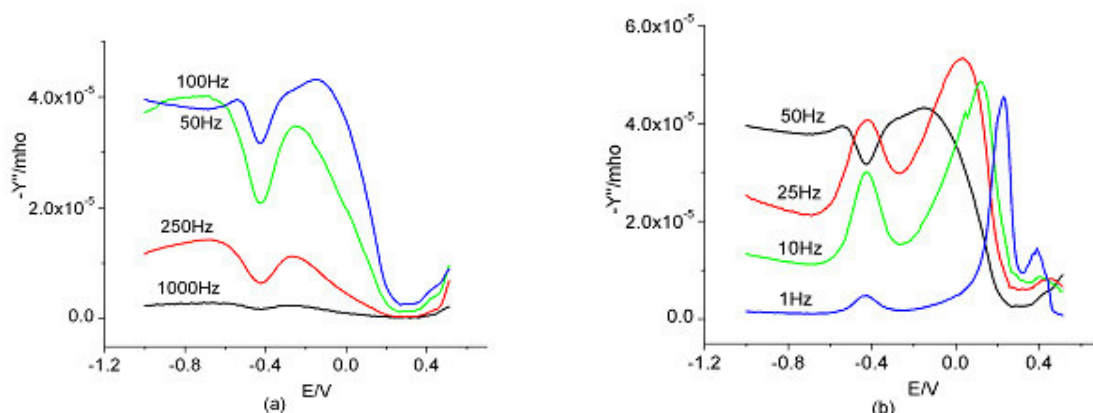

Figure 3. Admittance of 5 mg/mL Type IV collagen from human placenta a) Frequencies 1000, 250, 100 and 50 Hz; b) 50, 25, 10, and 1 Hz. The data for 50 Hz are included in Figure b for the purpose of continuity and comparison.

The unusual observation in Figure 3 is that the admittance increases with lower frequencies. In our past experience with simple electrolytes, the admittance increases from 10,000Hz to about 250 Hz and then decreases. However we have always observed an increasing trend in admittance values as the potential shifts to less negative values at lower frequencies. This is observed here also and is consistent with orientation changes of the water in the double layer and or the collagen surface.

The admittance data for collagen in the presence of 0.01 M NaCl are shown in Figure 4. In many ways the data are similar to that in Figure 3. However the data at 5000 Hz are much less than that at 1000Hz in the presence of 0.01M NaCl where they were nearly the same in the absence of NaCl. Also the decrease in admittance with decrease in frequency starts at much higher frequencies in the presence of NaCl and is consistent with the general behavior observed for background electrolytes. For the purpose of comparison the admittance data for the background electrolyte, 0.01M NaCl, are shown in Figure 5. The behavior observed is very similar to the data observed for other alkali halides [11]. As
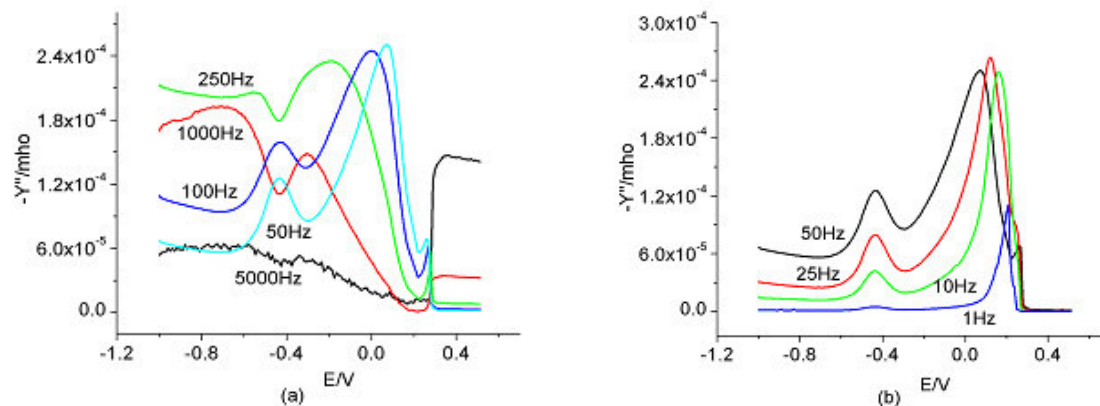
Figure 4. Admittance of 5 mg/mL Type IV collagen from human placenta in the presence of 0.01 M NaCl a) Frequencies 5000, 1000, 250, 100 and 50 Hz; b) 50, 25, 10, and 1 Hz. The data for 50 Hz are included in Figure b for the purpose of continuity and comparison.

We have suggested before for aqueous solutions of potassium halides [12], the continued shift of the admittance peak to less negative potentials with decreasing frequencies and the increase in admittance
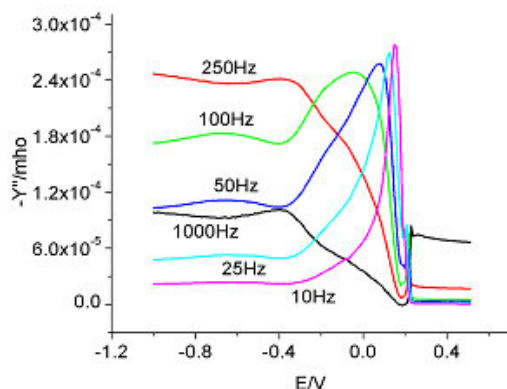

Figure 5. Admittance data for 0.01 M NaCl at 1000, 250, 100, 50, 25 and 10 Hz.

at the region of potentials close to zero can be attributed to the orientation changes of water. These parts of the curves are similar even for collagen.

Broad line NMR study of collagen fibers from tail tendon in water and heavy water [13,14] indicated that the water molecules do not rotate isotropically. Also the line broadening in the presence of salts and at high temperatures is attributed to the proton exchange between the water molecules. Two kinds of water, the adsorbed water on the collagen present in the form of chains parallel to the fiber axis, and water reorienting freely between the collagen macromolecules, have been postulated to explain the NMR spectra [14]. The adsorption of water restricts its rotation around one axis only and these water molecules reorient anisotropically. We plan to look at the impedance behavior of Type I collagen fibrils to gain information on this.

The peripheral amino-acid side chains of proteins display larger thermal vibrations. Therefore the water around them exhibits even more thermal vibrations and the hydrogen bonds formed are often

called "soft" hydrogen bonds [15]. In systems with a homodromic arrangement, where several O-H ….O-H ….O-H bonds all run in the same direction, cooperativity of water contributes to additional stability. The movement of water along the surface of macromolecules seems to favor entropy preferred three center (bifurcated) hydrogen bonds with "flip-flop" dynamics [16]. This facilitates configuration changes smoothly. Macromolecules also seem to favor pentagonal motifs of water molecules. Collagen, having water molecules oriented in the fiber direction, is likely to exhibit this "flip-flop" dynamics and may contribute to the observations of the shift in the first admittance maximum. In a protein, in general, 42% of the peptide carbonyl oxygen atoms are hydrated compared to the 14% hydration of the peptide N-H groups. The side chain oxygen and nitrogens are about 44% hydrated. Thus there must be a dominant contribution from the hydration of the carbonyl oxygens to the observed admittance behavior.

3.3. Nyquist and Bode Plots

We have carried out the impedance measurements at -1.0, -0.8, -0.5, -0.25, 0.0, 0.1, 0.2, 0.3, and 0.4 V for 2.5 and 5 mg/mL collagen in the absence and presence of 0.01 M NaCl. In the case of collagen, the data could be fitted with R1(R2C1)(R3C2)(R4C3)(R5C4) for all the potentials up to 0.3 V. For all normal electrolytes including NaCl the data could be fitted for potentials from -1.0 to 0.0 V with R1(R2C1) as expected. The data could be similarly fitted with 4 RC units for collagen in the
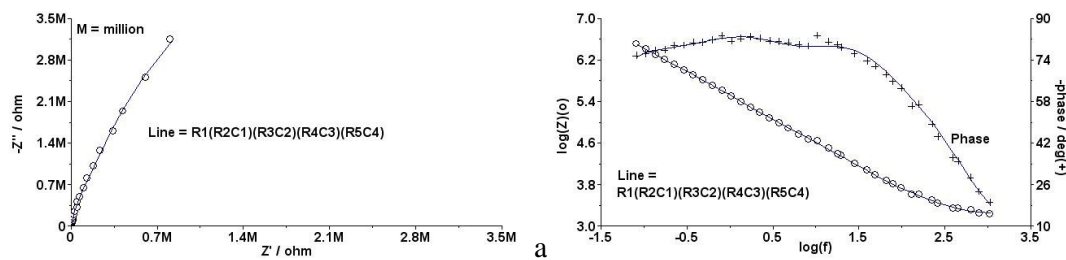

Figure 6. Type IV collagen from human placenta, 5mg/mL, 0.01 M NaCl, -0.5 V; a) Nyquist plot, b) Bode plot. The circuit that fits the data is shown in the figure.

presence of NaCl up to 0.2 V. A typical data set is shown in Figure 6 for collagen in the presence of 0.01 M NaCl at -0.5V. This kind of fit fails at potentials greater than 0.1V. A more pronounced capacitive impedance is observed for 0.2 and 0.3 V for collagen in the presence of NaCl. Figure 7 gives the results for two sets of measurements for pure collagen at 0.4V. Though not spectacular, the results do seem to suggest a definite trend for negative differential resistance. A similar kind of plot with negative differential resistance is observed for 2.5 mg/mL collagen in the presence of 0.01 M NaCl at 0.34 V. The presence of the salt seems to shift the observation of this phenomenon to less anodic potentials. This kind of impedance behavior with negative differential resistance has been seen in a few systems with passivation of electrodes, and it has been suggested it is tunnel diode behavior or resonant tunneling. We have also observed such a behavior for DNA-H_2O_2-sodium acetate buffer
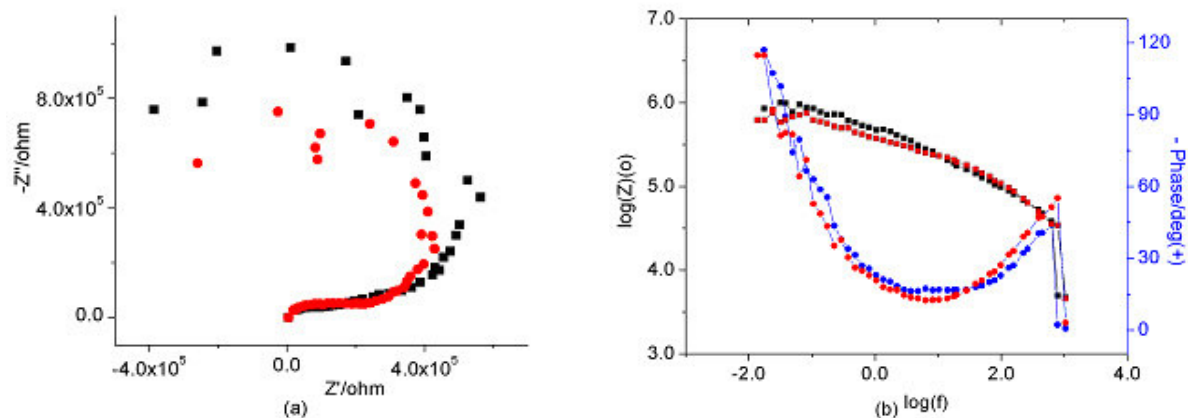
Figure 7. Type IV collagen from human placenta, 5mg/mL at 0.4V; two sets of data a) Nyquist plot b) Bode plot

system[3]. Systems with impedance loci in four quadrants have also been observed [17-23] in electrode passivation studies. Nonequilibrium thermodynamics with saddle-node and hopf bifurcations have been utilized to explain these observations. Such passivation systems have been thought to explain dynamical spatio temporal phenomena and oscillations in biological systems. We have observed several examples of biological systems, for the first time, to have impedance loci in four quadrants [24]. Our present system, though not spectacular, is indicative of chaos, the ultimate fate of the oscillations. The network-like structure of this particular Type IV collagen, under the present experimental conditions, seems inadequate to demonstrate impedance loci in four quadrants but favors the chaotic state. To know more about this we plan to investigate soluble Type I collagens in acidic solutions. We want to emphasize here that Type I forms fibrils compared to the Type IV studied here that forms network-like structures.

3.4. Passivation of mercury

It is well known that mercury begins to oxidize at 0.1 V. In the presence of chloride, it can also form a film of Hg_2Cl_2. Our admittance data shown in Figure 5 for 0.01M NaCl confirm this. Thus applications of positive potentials to mercury electrodes not only changes the charge on the surface but also results in the modification of the metal surface. We had studied this aspect in detail earlier [12] for potassium halides and found that admittance measurements can definitely pinpoint the region of this interaction. We have also found that by increasing the concentration of the halide, this interaction region can be extended to less anodic potentials.

By careful observations of admittance, Nyquist, and Bode plots one can still gain a lot of information regarding solute- solvent interactions. Thus the natural question that arises about the current observation of negative differential resistance for collagen even in the absence of NaCl is whether it is due to the mercury oxide formation, or does it reflect to some extent on the properties of collagen? To answer this question, the results of the NaCl impedance at these potentials are shown in Figure 8. Clearly the results indicate the importance and dominance of collagen interactions to produce

the results in Figure 7. Both the nature of impedance and phase behavior are different for both the systems.
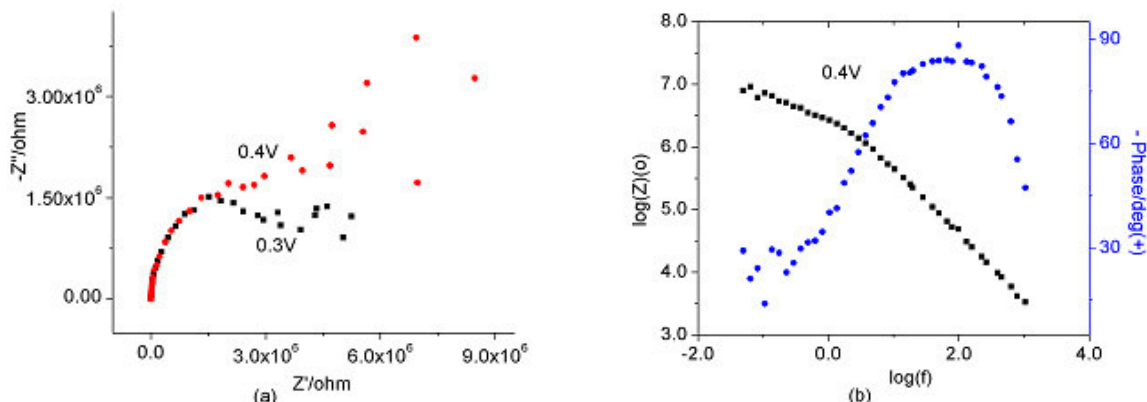

Figure 8. Sodium chloride, 0.01M, pH 6.02 a) Nyquist plot at 0.3 and 0.4 V; b) Bode plot at 0.4 V

We wish to point out that the phenomenon of negative differential resistance and the impedance loci occurring in two, three or four quadrants is not dictated by the passivation of mercury alone. For example we have observed impedance loci for the molybdate-peroxide system at about -1.0V in basic solutions, at about -0.4 V in slightly acidic solutions, and at about 0.2 V at pH values less than 2 [25]. We have also observed negative differential resistance for the molybdate-flavin adenine dinucleotide system at about -0.8 V [26].

3.5. Differential Capacitance

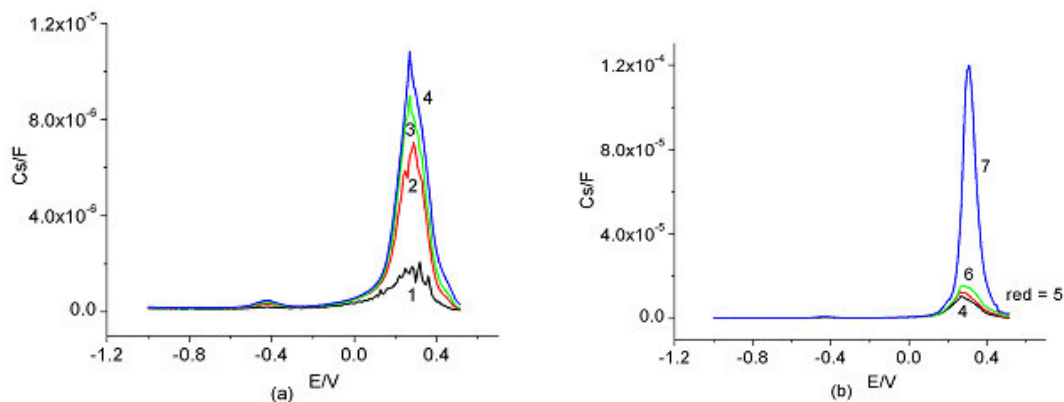When ohmic resistance is compensated, differential capacitance measurements yield double layer

Figure 9. Differential capacitance data for 5 mg/mL Type IV collagen from human placenta. a) 1, 1000 Hz; 2, 250 Hz; 3, 100 Hz; 4, 50 Hz b) 4, 50 Hz; 5, 25 Hz; 6, 10 Hz; 7, 1Hz

capacitance, C_{dl} or C_s. In such a case the C_s should be independent of frequency. The differential capacitance data shown in Figure 9 indicate some frequency dependent dispersion. We have observed

similar dependence of differential capacitance for aqueous solutions of potassium halides [12]. Dispersion in differential capacitance observed for Ag(111) in 0.01 M NaCl [27] has been attributed

the potential is clearly cathodic at this peak. Similar results are obtained for 5 mg/ mL collagen in 0.01M NaCl. Figure 10b shows minor variations in potentials where the peak capacitance is observed. The potential for the peak capacitance of collagen-NaCl mixture is in between that of NaCl and pure collagen. The capacitance values also follow the same trend. to the real surface with fractal character instead of an ideal homogenous electrode surface. It is clear from figure 10a that the observed dispersion is not connected with any passivation of mercury because the potential is clearly cathodic at this peak. Similar results are obtained for 5 mg/ mL collagen in 0.01M NaCl. Figure 10b shows minor variations in potentials where the peak capacitance is observed. The potential for the peak capacitance of collagen-NaCl mixture is in between that of NaCl and pure collagen. The capacitance values also follow the same trend.
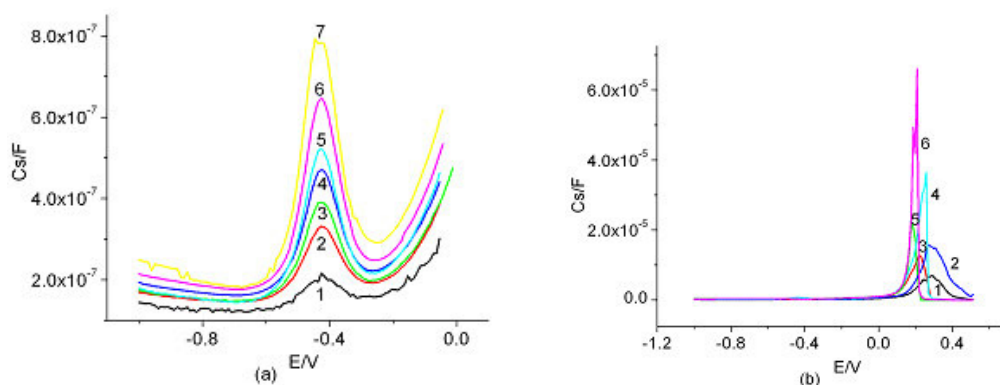

Figure 10. a) Differential capacitance for 5 g/mL Type IV collagen from human placenta, expanded window to highlight the small peak in Figure 9a. 1, 1000Hz; 2, 250Hz; 3, 100Hz; 4, 50Hz; 5, 25Hz; 6, 10Hz; 7, 1Hz; b) a comparison of the differential capacity at 250 Hz for 5 mg/mL collagen only, collagen with 0.01M NaCl, and 0.01 M NaCl blank (1, 3, and 5 respectively) and corresponding data at 10 Hz (2, 4, and 6 respectively).

3.6. Semiconduction

Semiconduction behavior is observed on passivation of many metals and alloys. An outer n-type hydroxide layer and an inner p-type oxide layer with a p-n heterojunction have been suggested before [28-29]. The characteristic capacitance of these passive films obeys the Mott-Schottky relationship. The flat band potential is obtained from the intercept on the potential axis. The doping concentration can be obtained from the slope provided we know the dielectric constant of the passive film.

The Mott-Schottky plots are shown in Figure 11. This is indicative of semiconduction that facilitates collagen signaling with other molecules and is consistent with our electronic signal drug discovery model. However we want to caution that similar semiconduction behavior at the mercury electrode has been observed by us with simple electrolytes such as potassium halides and we have discussed the role of mercury(I) halides on passivation [11-12]. It is amazing to note the convergence

of the data at all frequecies well before the beginnning of the passivation of mercury. Similar results are also obtained for collagen in the presence of 0.01 M NaCl.
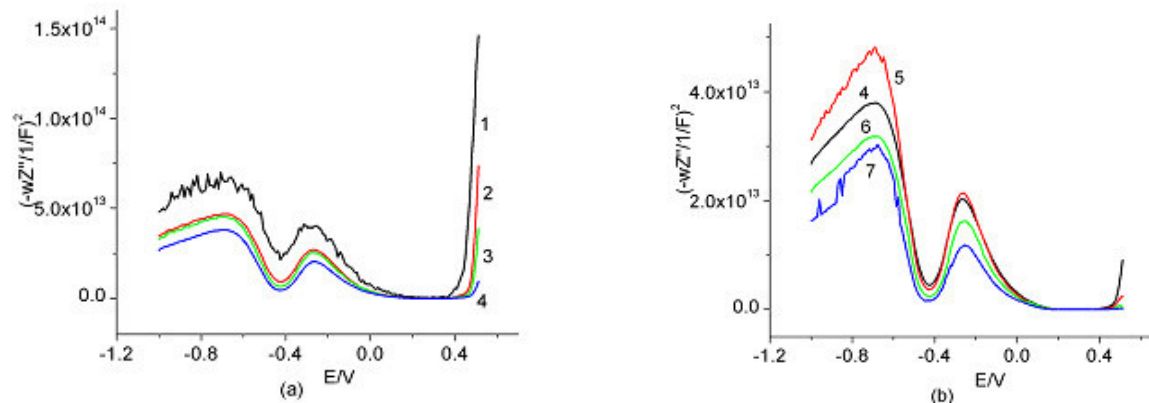

Figure 11. Mott-Schottky plots for 5 mg/mL Type IV collagen from human placenta. a) 1, 1000 Hz; 2, 250 Hz; 3, 100 Hz; 4, 50 Hz b) 4, 50 Hz; 5, 25 Hz; 6, 10 Hz; 7, 1 Hz

4. LIQUID CRYSTAL BEHAVIOR OR SELF-ASSEMBLY IN SOLUTIONS

The phase microscopy pictures (300X) of collagen in the absence and presence of sodium chloride are shown in Figure 12 . The differences are quite spectacular and significant. For the collagen triple helical structure, glycine is essential in every third position because a larger amino acid will not fit in the center of the triple helix when the three chains come together. The proline and 4-hydroxyproline
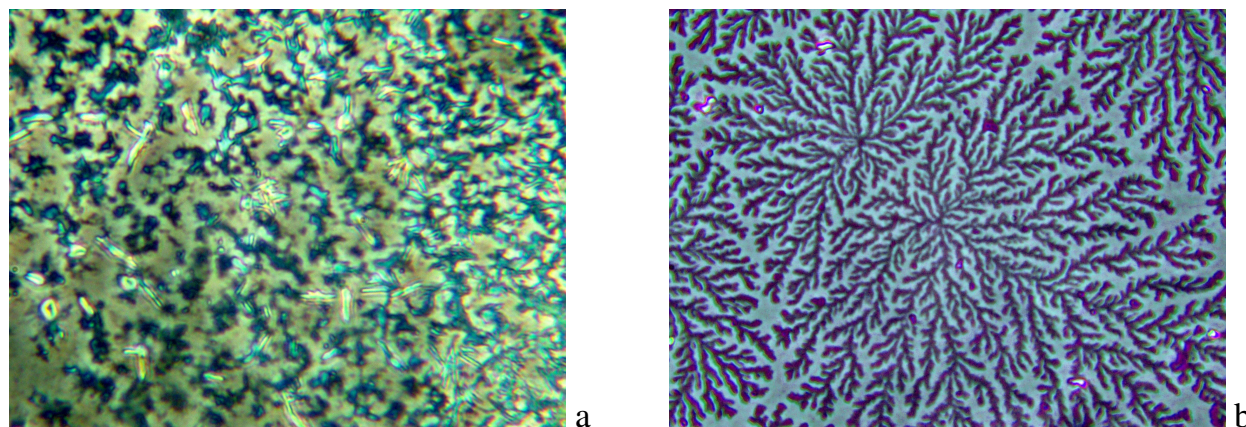

Figure 12. Phase microscopy 300X. (a) 5 mg/mL Type IV collagen from human placenta at pH 3.5 (b) 5mg/mL Type IV collagen in the presence of 0.01 M NaCl at pH 3.9

limit or restrict the rotation of the polypeptide chains. The three separate helical chains forming a triple helical structure are stabilized by inter-chain hydrogen bonds. On the other hand in the α–helix, the hydrogen bonds are intra chain. The side chains of the amino acids X and Y are placed on the surface of the molecule. The multiple clusters of hydrophobic and charged side chains direct self-assembly into precisely ordered structures. It must be pointed out that for liquid crystal behavior, a smectic A → B transition, has been suggested for the one dimensionally ordered and three- dimensionally ordered

phases of collagen fibrils [30]. As far as we know the influence of such self- assembled molecules near the double layer has not been investigated for formation of spatiotemporal periodicities. In homogenous systems, autocatalytic reactions and diffusion resulting from chemical instabilities lead to the formation of spiral waves and other concentration patterns of spatiotemporal phenomena. Our data suggest the electrical phenomena in the double layer in the presence of such self-assembled systems propagate among the packing units and extend into the bulk. Thus the self-assembly of molecules, especially biological molecules, seems to facilitate local disturbances to be felt at long distances by global coupling. The influence of the sodium chloride on the self-assembly is quite remarkable and we call attention to the connection between the sodium chloride contribution to the collagen configuration, and the normal electrolyte requirements for physical activity.

5. CONCLUSIONS

Impedance measurements offer a technique of investigating the role of inorganic electrolytes in controlling the electronic signaling processes in biological molecules. Depending on the system, admittance and impedance data provide a variety of information on the role of water on biological surfaces at low frequencies. For collagen, the admittance data suggest reorientation effects of water near the peptide group. Water with NaCl introduces symmetric packing to Type IV collagen, and a shift to slightly less positive potential for producing negative differential resistance.

References

1. C.V. Krishnan, M. Garnett, 1st Spring Meeting of the International Society of Electrochemistry, Spain, Abstract No. P06 (2003)
2. C.V. Krishnan, M. Garnett, 203rd Meeting of Electrochemical Society, Paris, Abstract No. 2703(2003)
3. M. Garnett, C.V. Krishnan, 204th Meeting of Electrochemical Society, Orlando, Florida, Abstract No. 1379 (2003)
4. C.V. Krishnan, M. Garnett, 204th Meeting of Electrochemical Society, Orlando, Abstract No. 1378 (2003)
5. M. Garnett, C.V. Krishnan, 204th Meeting of Electrochemical Society, Orlando, Abstract No. 1377 (2003)
6. D. Voet, J.G. Voet, Biochemistry, 2nd Edition, Wiley, p.158 (1995)
7. G.N. Ramachandran, G. Kartha, *Nature* 174 (1954) 269; 175 (593) 1955; *Proc. Indian Acad. Sci.*, A42 (215 (1955)
8. G.N. Ramachandran in "Collagen", Edited by N. Ramanathan, Interscience Publishers, a division of John Wiley & Sons, New York, 1962
9. D.J. Prockop, K.J. Kivirikko, *Annu. Rev. Biochem.*, 64 (1995) 403
10. A.J. Bard, L.R. Faulkner, Electrochemical Methods, John Wiley & Sons, Inc., 2nd Edition (2001) 599
11. C.V. Krishnan, M. Garnett, 226th American Chemical Society National Meeting, Abstract No. Inor. 0028(2003)
12. C.V. Krishnan, M. Garnett, *Electrochimica Acta*, 51 (2006) 1541
13. R.E. Dehl, C.A.J. Hoeve, *J. Chem. Physics*, 30 (1969) 3245
14. H.J.C. Berendsen, *J. Chem. Physics,* 36 (1962) 3297
15. W. Saenger, *Ann. Rev. Biophys. Biophys. Chem.*, 16 (1987) 93

16. W. Saenger, Ch. Betzel, B.E. Hingerty, G.M. Brown*, Nature*, 296 (1982) 581
17. P. Strasser, *The Electrochemical Society Interface*, Winter (2000) 46
18. M.T.M. Koper, *J. Chem.Soc. Faraday Trans.*, 94 (1998) 1369
19. A. Sadkowski, M. Dolata, J.P. Diard, *J. Electrochem. Soc*., 151 (2004) E-20
20. A. Sadkowski, *J. Electroanal. Chemistry*, 573 (2004) 241
21. B. Miller, A. Chen, *Electrochim. Acta*, 50 (2005) 2203
22. M. Keddam, H. Takenouti, N. Yu, *J. Electrochem. Soc.*, 132 (1985) 2561
23. D.D. Macdonald, *Electrochim. Acta*, 35 (1990) 1509
24. C. V. Krishnan, Merrill Garnett, in *"Passivation of Metals and Semiconductors, and Properties of Thin Oxide Layers"*, P. Marcus and V. Maurice (Editors), Elsevier, Amsterdam, (2006) 389
25. C. V. Krishnan, Merrill Garnett, B. Chu, 4th Spring Meeting of the International Society of Electrochemistry, Singapore, Abstract No. HC-O-23 (2006)
26. C. V. Krishnan, Merrill Garnett, B. Chu, paper accepted, to be presented at the 210th Electrochemical Society Meeting, Cancun, Mexico (2006)
27. V. D. Jovic, B.M. Jovic, *J. Electroanal. Chem.*, 54 (2003) 1
28. H. Tsuchiya, S. Fujimoto, T. Shibata, *J. Electrochem. Soc.*, 151(2004) B39 and references therein
29. D.S. Kong, S.H. Chen, C. Wang, W. Wang, *Corrosion Science*, 45 (2003) 747 and references therein
30. D.J.S. Hulmes, J.C. Jesior, A. Miller, C.B. Colominas, C. Wolff, *Proc. Natl. Acad. Sci. USA*, 78 (1981) 3567