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Histidine-Water Interactions at the Double Layer

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Ion and/or molecule-water interactions at the double layer for the systems, L-histidine-water and L-histidine-molybdate-water, were investigated using admittance measurements. At biological pH L-histidine has significant amounts of its protonoted form, which can resonate in the imidazole ring. This resonance and the presence of the nonprotonated form at the double layer were responsible for the complex admittance spectra near the biological pH. The admittance of histidine increased with decreasing frequencies from 1000 to 25 Hz and then decreased. Five admittance peaks and a shoulder were observed in the admittance spectra. With decreasing frequencies, the observed shift in potentials corresponding to the admittance maximum was attributed to cathodic and anodic shifts arising from the interaction of water with the δ_+ and δ_- of the dipoles of the imidazole ring in histidine. The interaction of molybdate with histidine reduced the number of admittance peaks from five to three suggesting less histidine-histidine interactions and more histidine-molybdate interactions at the double layer.

Keywords: L-histidine, molybdate, admittance, ion-solvent interaction, resonance.

1. INTRODUCTION

Electrochemical impedance measurement is a powerful technique used for characterizing passivation of electrode materials and elucidating semiconducting characteristics of metal oxides and hydroxides [1-4]. In an electrochemical system, the double layer interactions are quite important in determining the characteristics of the electrode reactions and electrode materials. Admittance is a powerful tool for understanding ion-solvent, solvent-electrode, and ion-electrode reactions [5-8].

All living systems exhibit dynamical spatiotemporal periodicities [9]. Impedance and admittance measurements are often used to understand solute-solvent interactions at the double layer and spatiotemporal phenomena in biological and inorganic systems. In the past, we have investigated molecules that are most relevant for the present investigation. These include L-cysteine, lidocaine

hydrochloride and aqueous NaCl-hydrogen peroxide [5-8]. We had introduced two new concepts, "potential induced and water structure-enforced ion pair formation" [6] and "potential induced and peroxide-mediated ion pair formation" [10], to explain the admittance data.

In the present investigation we have chosen another simple molecule, L-histidine. Its structure is shown in Figure 1 [11]. As the histidine structure is mostly protonated in the imidazole ring, it is common to represent the general structure of histidine as protonated in the nitrogen atom. This ion, however, is stabilized by resonance. We wanted to investigate the influence of this resonant charged structure on its interactions with water at the double layer. It is also not clear of the influence of the doubly charged molybdate when it reacts with the protonated histidine and the consequent interactions with the solvent at the double layer.

The interrelationship between admittance (Y) and impedance (Z) is given by the equation [5]:

$$Z'/Y' = Z''/Y'' = (Z')^2 + (Z'')^2 = 1/[(Y')^2 + (Y'')^2] \quad (1)$$

We have effectively used admittance data for L-cysteine as a function of pH to identify the water orientation effects and interactions between -α-NH_3^+ group and –COO^- group as well as Na^+ and -S^- group [7]. The lidocaine data allowed us to identify cathodic and anodic shifts in the interactions between two dipoles, the amino and carbonyl groups, by changing frequencies and applied potentials [5]. Molybdate was found to interact strongly with lysine and cysteine and the combined systems were very useful in understanding spatiotemporal phenomena in biological systems [1, 7, 12]. We have used molybdate also in our present investigations. In addition to the influence of charged resonant structures on ion solvent interactions at the double layer, the current investigation is intended to confirm and to highlight some of the salient features that can be obtained from admittance measurements, an often ignored parameter in electrochemistry.

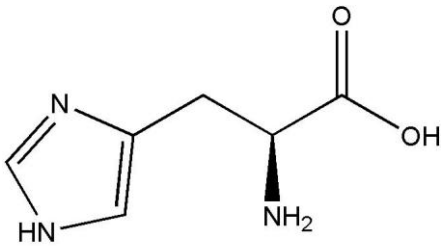

Figure 1. Histidine

2. EXPERIMENTAL PART

An EG & G PARC Model 303A SMDE tri-electrode system (mercury working electrode, platinum counter electrode and Ag/AgCl (3.5M KCl) reference electrode) along with Autolab eco chemie was used for cyclic voltammetric measurements at 298 K. The L-histidine and sodium molybdate dihydrate used were from Sigma. Distilled water was used for preparation of all solutions. Electrochemical measurements were carried out with no background electrolyte. The solutions were purged with N_2 for about 10 minutes before the experiment. Admittance measurements were carried out in the potential range -1.0 to +0.3 V. The frequencies used were in the range of 1000 to 1 Hz.

3. RESULTS AND DISCUSSION

In the past, we had suggested to avoid the usual practice of using background electrolytes, whenever possible. In admittance measurements, the background electrolytes mask the effect of the solutes under investigation and prevent from obtaining meaningful information on ion-solvent interactions. In the present case, the admittance of histidine was very low and we had to use fairly high concentrations. The admittance data for 0.19 M histidine in the potential range -1.0 to +0.3V at different frequencies are shown in Figure 2. The vast information on ion-water and dipole-water interactions embedded in these admittance measurements is obvious from the complexity of the admittance spectra. The admittance increased with decreasing frequencies from 1000 to 25 Hz and then decreased with further decrease in frequencies. We have observed similar patterns for many molecules in the absence of background electrolytes [12-14].

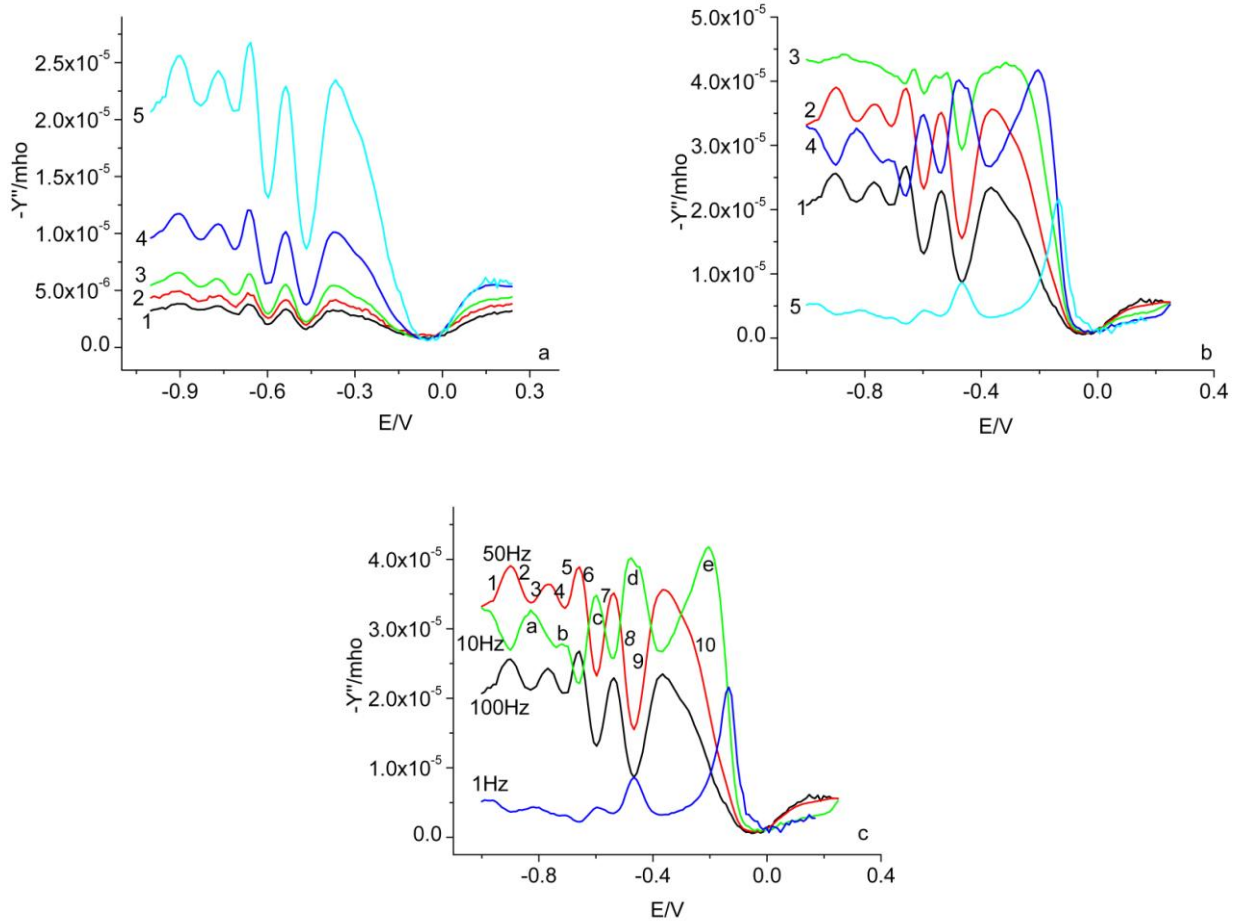

Figure 2. Admittance of 0.19 M L-histidine, pH 7.4 at a) 1) 1000 Hz, 2) 750 Hz, 3) 500 Hz, 4) 250 Hz, 5) 100 Hz; b) 1) 100 Hz, 2) 50 Hz, 3) 25 Hz, 4) 10 Hz, 5) 1 Hz; c) same as b) without the data for 25 Hz to avoid clutter

An interesting feature of the admittance spectra was that the data exhibited five maxima and a shoulder. In Figure 2c the data of Figure 2b are repeated without the data for 25 Hz to avoid clutter. At

higher frequencies there was no significant shift in the potential at which the admittance maximum occurred. However, the potentials at which the admittance maximum occurred are significantly shifted at frequencies less than 25 Hz. The peaks a, b, c, d, and e at 10 Hz may be explained by the anodic shifts of the sides 1, 3, 5, 7, and 9 and the cathodic shifts of the sides 2, 4, 6, 8, and 10 of the peaks shown at 50 Hz (Figure 2c). These shifts correspond to the complex interaction with water and the dipoles in the imidazole side chain as well as the terminal amino and carboxylate groups in histidine. The presence of these 5 peaks and a shoulder suggest that both the protonated and nonprotonated forms of histidine are active at the double layer and are involved in ion/molecule-water (dipole) interactions. In the past we have observed admittance peaks shifting anodic for positive ions (or δ_+ of dipole) and cathodic for negative ions (or δ_- of dipole) with decreasing frequencies [5, 12].

In the case of lidocaine hydrochloride, for example, the data were challenging because of the presence of polar carbonyl and amino groups, a phenyl group, and hydrophobic groups near the charge at the diethyl amino group (Figure 3a). By comparing with similar data we had interpreted the anodic shift in the admittance maximum at about -0.1 V to the charged diethylamino group, to the cathodic shift at about -0.8 V to the carbonyl group with the highly electronegative oxygen and the anodic shift at about -1.2 V to the amide group [5].

Similar cathodic and anodic shifts in admittance maximum were observed in cysteine (Figure 3b) [7, 12], and more complicated molecules such as collagen [13] and prothrombin [14].

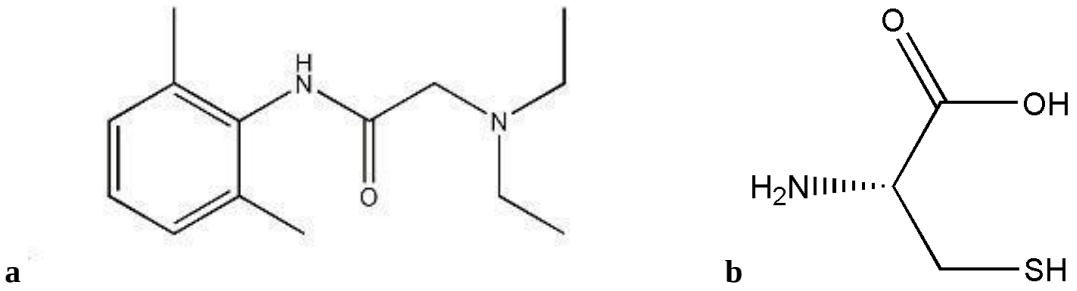

Figure 3. Structure of nonprotonated a) Lidocaine and b) Cysteine

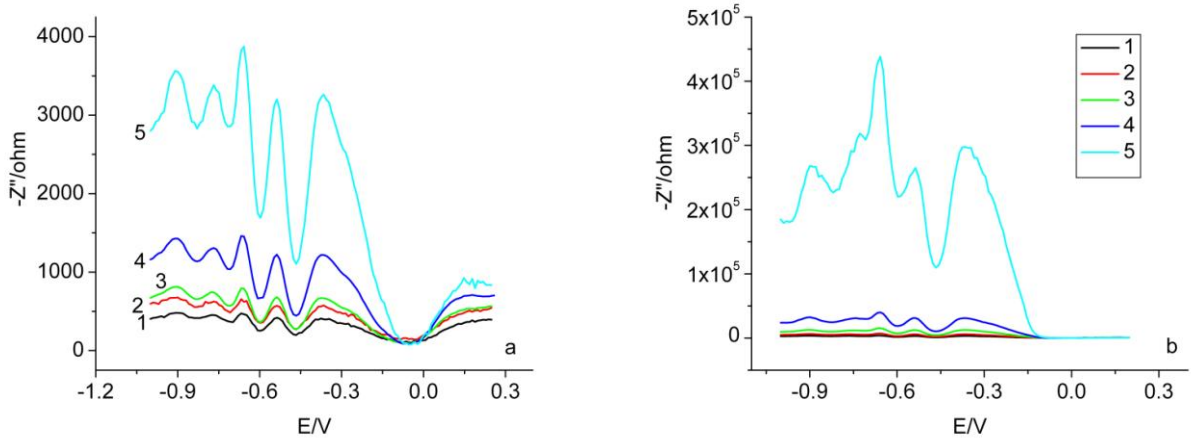

Figure 4. Impedance of 0.19 M L-histidine, pH 7.4 at a) 1) 1000 Hz, 2) 750 Hz, 3) 500 Hz, 4) 250 Hz, 5) 100 Hz; b) 1) 100 Hz, 2) 50 Hz, 3) 25 Hz, 4) 10 Hz, 5) 1 Hz

Studies of interactions of histidine-histidine dipeptide in gas phase using density functional theory method with Gaussian 98 program have revealed the presence of six rings, four resulting from intramolecular hydrogen bonds and two from the imidazole ring [15]. Even though we did not use a dipeptide, it is possible to have similar arrangements with two histidine molecules near the double layer with hydrogen bonding with water. At negative potentials it is possible to have both the protonated and nonprotonated forms of the histidine to be near the double layer competing with water (dipole). These hydrogen bonded structures may be disturbed with changing frequencies and may explain the observed increase in admittance with decreasing frequencies. Of course it is not clear at this time the nature of the next neighboring molecules at the double layer.

The impedance spectra of 0.19 M histidine are shown in Figure 4. It is clear that the impedance increased monotonically with decreasing frequencies. Also there were no apparent shifts in potentials corresponding to the impedance maximum.

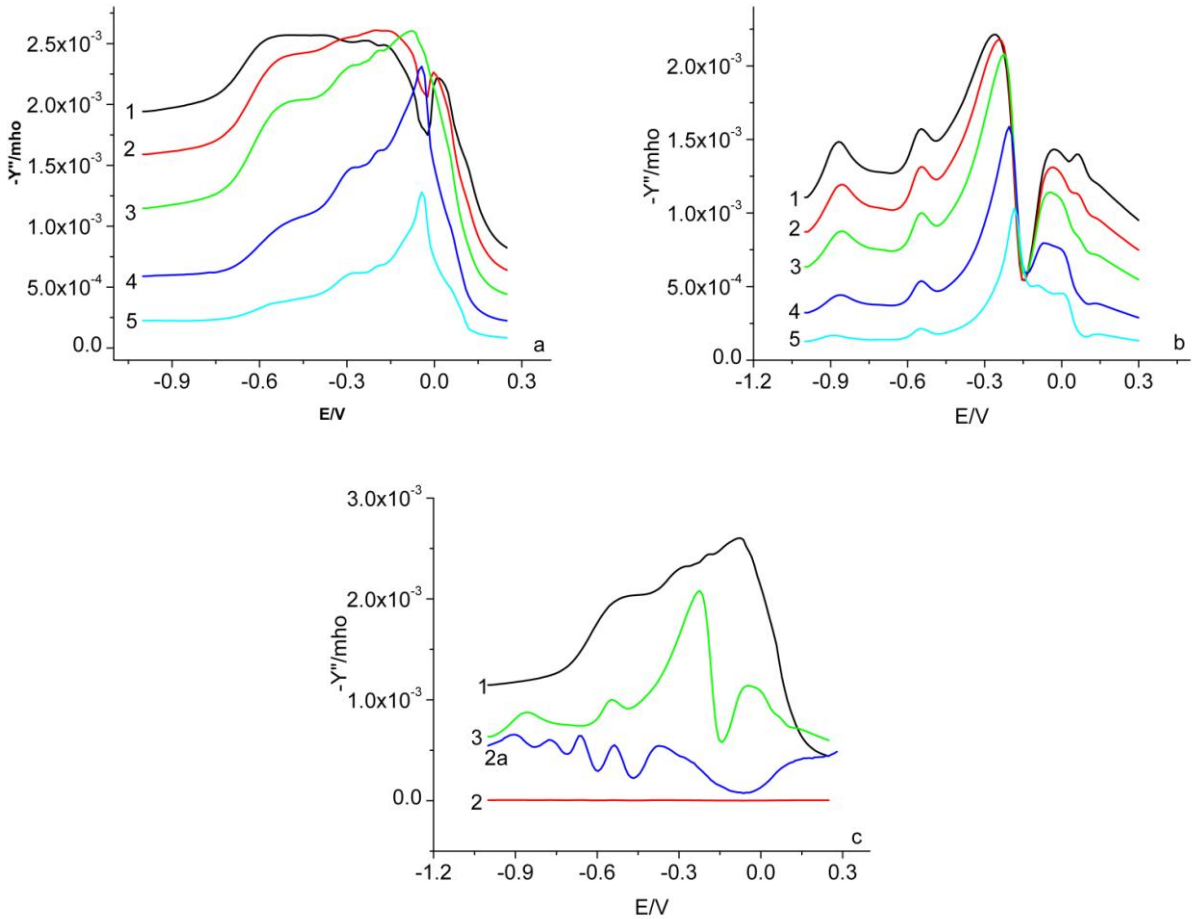

Figure 5. Admittance of a) 0.095 M sodium molybdate; b) 0.19 M histidine - 0.095 M sodium molybdate, at 1) 1000 Hz, 2) 750 Hz, 3) 500 Hz, 4) 250 Hz, 5) 100 Hz; c) 1, 0.095 M molybdate; 2, 0.19M L-histidine; 2a, data of 2 multiplied 100 times for easy comparison with other curves; 3, 0.19 M histidine – 0.095 M sodium molybdate; all at 500 Hz

It is known that histidine kinase interacts with DNA at the phosphate group [16] and 1-phoshpo and 3-phospho histidines are known. Our earlier investigations of molecules such as cysteine and

lysine had revealed strong interactions with molybdate [1, 12]. The presence of molybdate had completely changed the electronic character of these systems by providing negative differential resistance in the impedance and thus being involved in long range signaling. The admittance results for histidine with molybdate are shown in Figure 5.

For comparison, the admittance spectra of 0.095 M sodium molybdate are shown in Figure 5a. The admittance spectra of 0.19M histidine and 0.095 M sodium molybdate are shown in Figure 5b. A comparison of the admittance spectra of histidine, molybdate and their combination at 500 Hz are shown in Figure 5c. Since the admittance of histidine was much less than that of the other two, its data were multiplied 100 times for the purpose of comparison (curve 2a in Figure 5c).

Unlike the data in Figure 2 for histidine, the data for molybdate and histidine-sodium molybdate indicated a decrease in admittance with decreasing frequencies and thus masked the opposite effect observed for histidine without any background electrolyte. This is probably due to the effect of the positively charged sodium ions near the double layer at cathodic potentials. Also the number of admittance peaks decreased from five for histidine to three for the histidine-sodium molybdate system. We had suggested earlier the presence of both protonated and nonprotonated histidines at the double layer for pure histidine responsible for the five peaks and a shoulder. The decrease in the number of peaks suggested strong histidine-molybdate interactions with water dipoles at the double layer instead of histidine-histidine whether they are protonated or not. This is more clearly indicated in curves 2a and 3 in Figure 5c. The histidine-molybdate interactions were also evident from the fact that admittance was much less for histidine-molybdate system when compared with the same concentration of sodium molybdate. With decreasing frequencies, the first peak and third admittance peaks were shifted slightly anodic and the middle second admittance peak was shifted slightly cathodic.

4. CONCLUSIONS

We have utilized admittance measurements for the systems, L-histidine-water and L-histidine-molybdate-water to probe the interactions of these molecules with water at the double layer. At biological pH L-histidine has significant amounts of its protonoted form, which can resonate in the imidazole ring. This resonance and the presence of the nonprotonated form complicated the observed admittance spectra around biological pH. The admittance of histidine increased with decreasing frequencies from 1000 to 25 Hz and then decreased. Five admittance peaks and a shoulder were observed in the spectra. With decreasing frequencies, the observed shift in potentials corresponding to the admittance maximum was attributed to cathodic and anodic shifts arising from the interaction of water with the δ_+ and δ_- of the dipoles of the imidazole ring in histidine. The interaction of molybdate which can bind to histidine reduced the number of admittance peaks from five to three. This suggested the possibility of less histidine-histidine and more histidine-molybdate interactions at the double layer.

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