

Spatiotemporal Oscillations in Biological Molecules: Molybdate-L-Cysteine

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The interactions between sodium molybdate and L-cysteine at different pH values have been investigated by using cyclic voltammetry and frequency response analysis. With increasing pH values, the decreasing redox interaction was demonstrated by decreasing color, and admittance behavior, as tending towards that of L-cysteine. At pH values close to neutral and above, the two broad admittance maxima moved in opposite directions with decreasing frequencies and merged into one at around 250 Hz. This compares to the similar behavior for L-cysteine alone at pH = 5.18 and at around 10 Hz. At pH = 9.34, two pairs of maxima moving in opposite directions with decreasing frequencies and merging into two peaks were observed. The first pair was assigned to the interactions between $\alpha\text{-NH}_3^+$ and $\alpha\text{-COO}^-$ and the second pair to Na^+ and sulfhydryl S^- . The impedance exhibited negative differential resistance: impedance loci occurring in two or more quadrants at different pH values. When the potential was fine-tuned, the impedance loci extended after the first 4 quadrants again into the first and second quadrants for a total of 6 quadrants, a phenomenon observed for the first time in impedance studies. Increasing the surface area decreased the impedance but followed the same multi-quadrant impedance. When the same used Hg drop was used again for repeat measurements, the same phenomenon was observed, indicating that there was no need to have a fresh Hg drop for each experiment. A comparison of the data in the presence of sulfate instead of chloride, and the negative differential resistance observations at different pH values, suggested that heptamolybdate complexes of L-cysteine were more active. Also the presence of chloride gave smoother impedance data in the 4th and 5th quadrants at low frequencies. These data suggested that we should consider the electronic properties of these molecules as complement to enzymatic process in biological systems.

Keywords: Spatiotemporal oscillations, Molybdate-L-cysteine, Impedance in 6 quadrants, Admittance

1. INTRODUCTION

In order to understand cell signaling it is important to probe into the electronic nature of simple biological molecules from which complex enzymes and other proteins are synthesized. Since all these

complex molecules are in a milieu of some simple aqueous electrolytes, mostly NaCl, solute-solute and solute-solvent interactions play a crucial role in the signaling process. Our recent electrochemical studies using DNA, aqueous electrolytes, basic amino acids, collagen and prothrombin have demonstrated that impedance measurements can offer a powerful tool to elucidate solute-solvent interactions as well as semi-conduction in biological systems [1-5].

We have chosen mercury as the working electrode because a vast amount of data on the double layer behavior is available for this system. Also, it is relatively easy to get a fresh drop of mercury each time and thus to minimize surface inhomogeneities. The mercury drop approach also offers opportunities to study the influence of surface area more easily because the size of the drop can be easily changed.

Earlier results of proteins, collagen and prothrombin, showed a profound influence of NaCl on their electronic behavior [1, 2]. While NaCl promoted the electronic behavior of collagen, it was detrimental to prothrombin. This is evidenced by the occurrence of impedance loci in the first two quadrants, a characteristic of negative differential resistance, and probable resonant tunnel diode behavior. We have also reported the admittance and impedance behavior of aqueous 0.01M KCl, KBr, and KI using a static mercury drop electrode [3]. As expected, the interaction of mercury was observed to increase from chloride to bromide to iodide near the passivation region. Both p-type and n-type semi conduction in these systems could be observed from Mott-Schottky plots.

In order to explain the aqueous solution behavior of simple electrolytes at or near the double layer we recently introduced a new concept of "potential-induced ion pairing" [4]. This concept when coupled with the traditional Coulombic ion pairing, localized hydrolysis (or ion pairing through an intervening water molecule), and water-structure-enforced ion pairing, could explain the admittance data at high and low frequencies for alkali halides and tetra alkyl ammonium halides.

Further, we have also investigated the phenomenon of impedance with negative differential resistance or impedance loci occurring in 4 quadrants in many molecules of biological origin. This includes DNA-H₂O₂, DNA-Lysine, arginine, and histidine, peroxocomplexes of molybdenum as well as molybdate with lysine, EDTA and basic amino acids [5].

Dynamical spatiotemporal oscillations or periodicities observed in all living systems are often characterized by negative differential resistance and impedance loci in four quadrants [6-9]. Many other electrochemical systems such as anodic metal dissolution, cathodic metal deposition, oxidation of small organic molecules and reduction of hydrogen peroxide [8], also exhibit this phenomenon and are used as models for biological oscillations.

The $pK_1(\alpha\text{-COOH})$, $pK_2(\text{thiol or sulfhydryl})$ and $pK_3(\alpha\text{-NH}_3^+)$ values of L-cysteine are 1.92, 8.37 and 10.70 respectively [10]. At the zwitterionic pH of 5.02 there is no net charge on the molecule. At pH below and above 5.02, the molecule possesses predominantly cationic and anionic properties, respectively. At higher pH values the sulfhydryl group also ionizes and possesses a negative charge. Thus the double layer behavior of this molecule should depend on concentration, pH, counter ions and the applied potential.

We also reported that impedance measurements could be used as a tool to explore the self-assembly process of large Keplerate type molybdenum clusters of $(\text{NH}_4)_{42}[\text{Mo}^{\text{VI}}_{72}\text{MoV}_{60}\text{O}_{372}(\text{CH}_3\text{COO})_{30}(\text{H}_2\text{O})_{72}] \cdot \text{ca.}300\text{H}_2\text{O} \cdot \text{ca.}10\text{CH}_3\text{COONH}_4$ [11]. It has been

reported that sodium molybdate in the presence of cysteine hydrochloride acting as an educt and reducing agent reacts at low pH values of ~ 1.5 to produce a nanosized ring-shaped cluster of $\text{Na}_3[\text{Mo}_{154}\text{O}_{462}\text{H}_{14}(\text{H}_2\text{O})_{48}(\text{HO}_2\text{C}-(\text{NH}_3^+)\text{HC}-\text{CH}_2-\text{S}-\text{S}-\text{CH}_2-\text{CH}(\text{NH}_3^+)-\text{COO}^-)_{11}].x \text{H}_2\text{O}$ ($x \approx 250$) [12]. This interesting structure has multiphilic ligands such as $-\text{NH}_3^+$, CO_2^- , CO_2H and $-\text{S}-\text{S}-$ attached to the inner wall of the cluster through a carboxylate group. As a prelude to the electrochemical investigation of the self-assembly process of this cluster, we wanted to investigate the L-cysteine and molybdate-L-cysteine at different pH values. Even though there were numerous electrochemical studies on L-cysteine [13-19], they were all carried out in buffers or some other electrolytes at fairly high concentrations and the charge interactions between the L-cysteine and the counter ions were neglected in interpreting the data. We had carried out electrochemical measurements of simple molybdate at different pH values earlier [20]. Recently we reported cyclic voltammetry and impedance data for L-cysteine in the absence and presence of counter-ions such as chloride at different pH, bromide at pH 5.15, and phosphate from buffer at pH 7.03. This was in order to elucidate solute-solvent interactions and interactions between L-cysteine and counter ions [21]. We report here the cyclic voltammetric and impedance data on the interaction between sodium molybdate and L-cysteine, a nonessential α -amino acid, before embarking on the Keplerate type molybdenum structure containing L-cysteine.

Cysteine has also been evaluated as a corrosion inhibitor for copper in neutral and acidic chloride solutions [22, 23]. Several studies explored the possibility of corrosion prevention or minimization by using the environmentally friendly molybdates instead of the toxic chromates [24-33]. The combination of L-cysteine and molybdate offers another possibility. Our fundamental electrochemical studies on these substrates and their combinations should provide valuable background information for such an undertaking.

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 were used for cyclic voltammetric and electrochemical admittance measurements at 298 K. Sigma $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, L-cysteine and distilled water were used for preparation of all solutions. NaOH and HCl were used to adjust pH. No other background electrolyte was used for the electrochemical measurements. The solutions were purged with N_2 for about 10 minutes before the experiment. Admittance measurements were carried out using about 7 mL solutions in the frequency range 1000 Hz to 10 mHz. The amplitude of the sinusoidal perturbation signal was 10 mV.

3. RESULTS AND DISCUSSION

3.1 Cyclic Voltammetry

These measurements were made for 0.1 M aqueous solutions of sodium molybdate-L-cysteine mixtures of different pH at a scan rate of 100 mV/s in the potential range 0.0 to -1.0 V and 0.3

to -1.0V. The pH was adjusted using HCl or NaOH. The results are shown in Figures 1a, 1b, 1c and 1d.

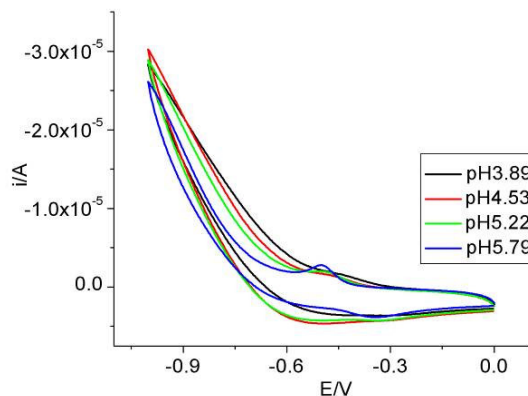


Figure 1a. Cyclic voltammetry of 0.1 M Molybdate-L-cysteine, scan rate 100 mV/s; -1.0 to 0.0 V, scan 3, after 24 hours of preparation of solution

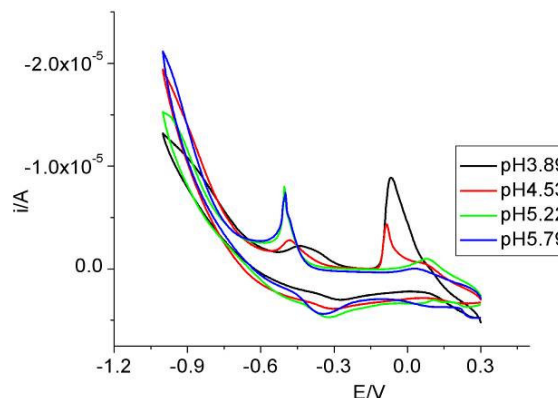


Figure 1b. Cyclic voltammetry of 0.1 M Molybdate-L-cysteine, scan rate 100 mV/s; -1.0 to 0.3 V, scan 3, after 24 hours of preparation of solution

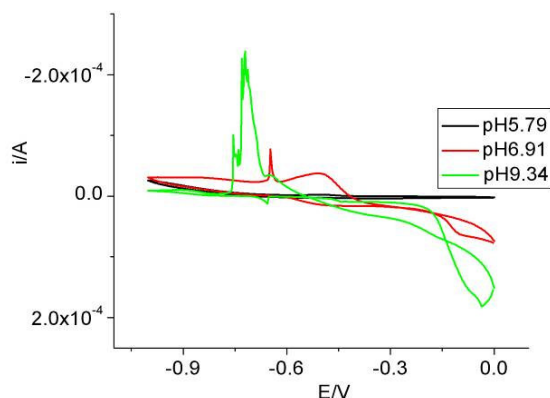


Figure 1c. Cyclic voltammetry of 0.1 M Molybdate-L-cysteine, scan rate 100 mV/s; -1.0 to 0.0 V, scan 3, after 24 hours of preparation of solution

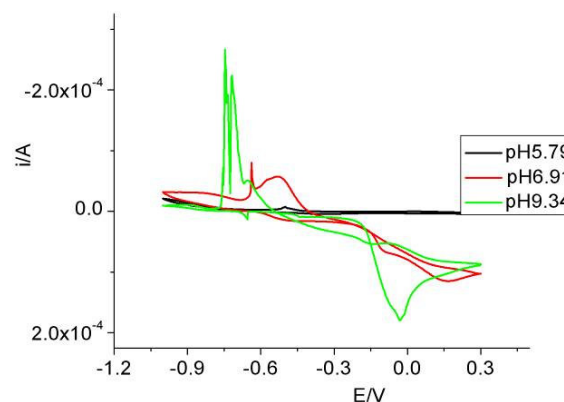


Figure 1d. Cyclic voltammetry of 0.1 M Molybdate-L-cysteine, scan rate 100 mV/s; -1.0 to 0.3 V, scan 3, after 24 hours of preparation of solution

For the sake of continuity, the data for pH = 5.79 were repeated, as shown in Figures 1b and 1d. The solutions contained 0.15 M, 0.125 M, 0.1 M, 0.05 M, and 0.0 M HCl for obtaining pH values of 3.89, 4.53, 5.22, 5.79 and 6.91 respectively and 0.09 M NaOH for pH = 9.34. With increasing pH values, the color of the solutions ranged from greenish blue, deep orange yellow, yellow, pale yellow and very pale yellow and these measurements were carried out after waiting for about 24 hours.

When the scan was carried out from 0.0 to -1.0 V and back, very little change in cathodic and anodic activities were observed with change in pH, as shown in Figure 1a. The influence of the chloride at the lower pH values was observed by additional cathodic peaks seen in Figure 1b. Also a

pH dependent cathodic shift with increasing pH was observed here in Figure 1c. Above pH 6, a cathodic current spike was observed and there was a small cathodic current oscillation at pH 9.34.

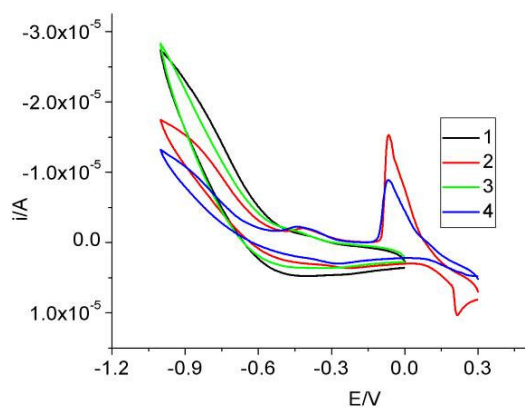


Figure 2a. Cyclic voltammetry of 0.1 M Molybdate-L-cysteine, scan rate 100 mV/s; scan 3; Immediately after preparation of solution, pH 3.29; 1, 2; after 24 hours of preparation of solution, pH 3.89; 3, 4

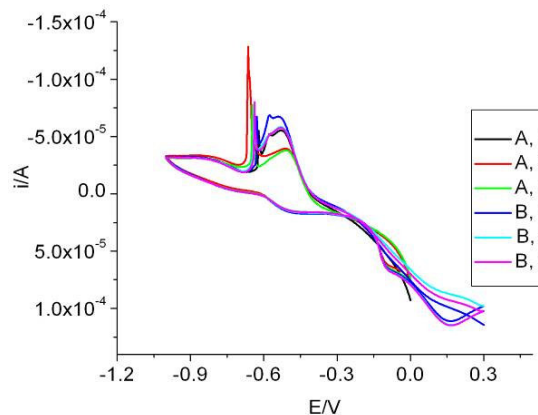


Figure 2b. Cyclic voltammetry of 0.1 M Molybdate-L-cysteine, pH 6.91, scan rate 100 mV/s; scan 1, 2, 3 for the range A) -1.0 to 0.0 V and B) -1.0 to +0.3 V

In order to find the influence of the waiting period on color development and consequently on the cyclic voltammograms, measurements were also carried out immediately after preparation of the solution. A typical example is shown in Figure 2a. The results were similar in both cases. Figure 2b shows the influence of different scans. Scan 1 gave a higher cathodic current, irrespective of the starting potential of the scan, and scans 2 and 3 gave similar currents.

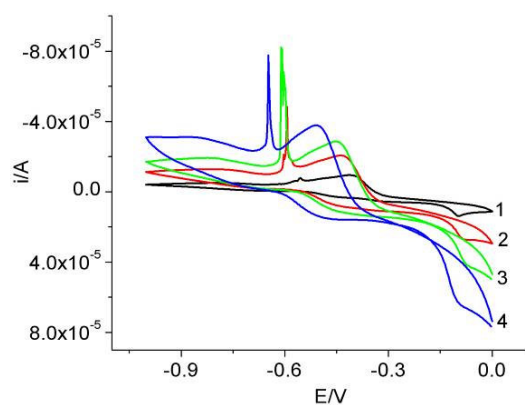


Figure 3a. Cyclic voltammetry of 0.1 M Molybdate, L-cysteine (1)0.01 M (2)0.03 M (3)0.05 M (4)0.1 M; scan 3, scan from 0.0 -1.0 V and back, immediately after preparation, pH ~ 6.8

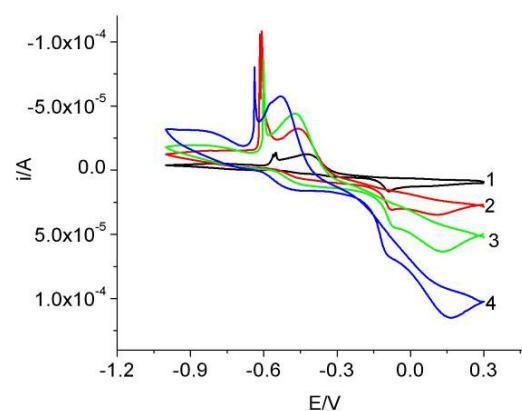


Figure 3b. Cyclic voltammetry of 0.1 M Molybdate, L-cysteine (1)0.01 M (2)0.03 M (3)0.05 M (4)0.1 M; scan 3, scan from 0.3 to -1.0 V and back, immediately after preparation, pH ~ 6.8

Figures 3a and 3b show the cyclic voltammograms for L-cysteine concentration dependence in 0.1 M sodium molybdate. With increasing concentration, both the cathodic and anodic currents

increased as expected. The cathodic current spikes also increased initially. Even though the pH was nearly the same, there was a slight cathodic shift in the peaks and spikes with increasing concentration. With still higher concentrations of 0.2 and 0.4 M L-cysteine in 0.1 M sodium molybdate, shown in Figures 3c and 3d, current spikes became broader. In the concentration range of 0.1 and 0.4 M L-cysteine, the peak currents were nearly the same with spike currents, being significantly different if the starting potential of the scan was 0.3 V.

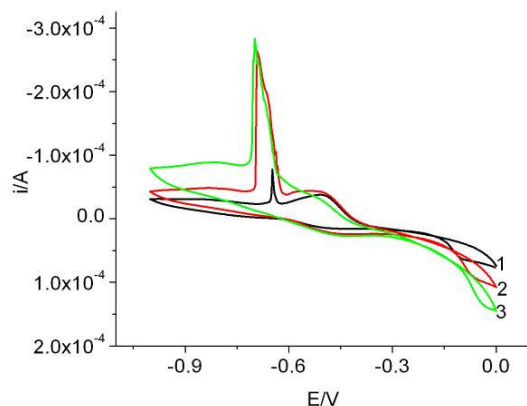


Figure 3c. Cyclic voltammetry of 0.1 M Molybdate, L-cysteine (1)0.1 M (2)0.2 M (3)0.4 M; scan 3, scan from 0.0 to -1.0 V and back, immediately after preparation, pH ~ 6.8

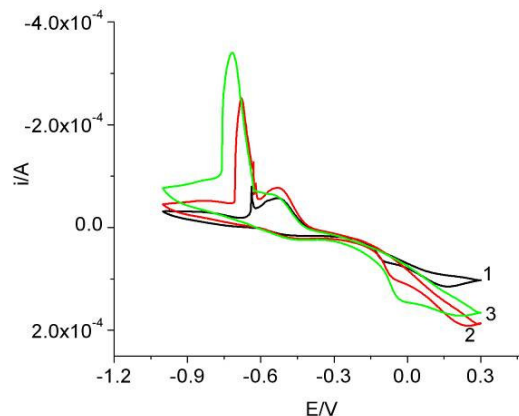


Figure 3d. Cyclic voltammetry of 0.1 M Molybdate, L-cysteine (1) 0.1 M (2) 0.2 M (3) 0.4 M; scan 3, scan from 0.3 to -1.0 V and back, immediately after preparation, pH ~ 6.8

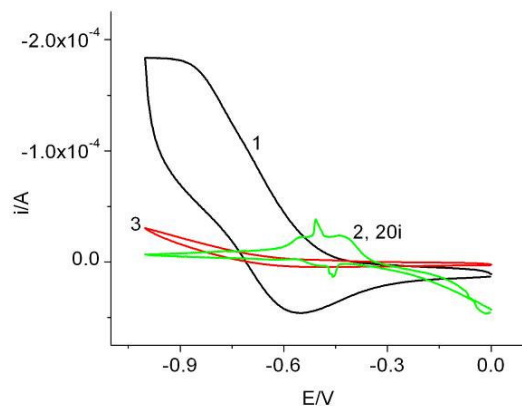


Figure 4a. Cyclic voltammogram for (1) 0.095 M sodium molybdate in 0.12 M HCl, pH 4.51; (2) 0.1 M L-cysteine in 0.1 M NaCl, pH 5.18; current multiplied 20 times (3) 0.1 M sodium molybdate, 0.1 M L-cysteine, 0.125 M HCl pH 4.53, scan 3, scan from 0 to -1.0 V and back, scan rate 100 mV/s

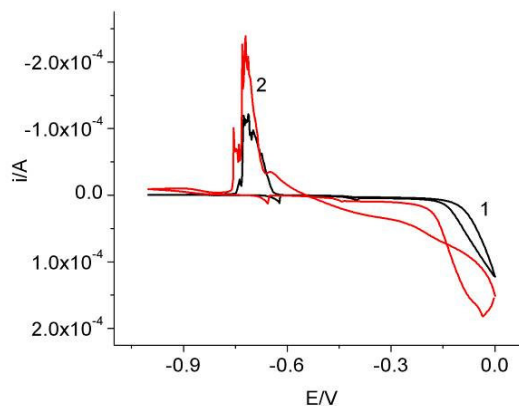


Figure 4b. Cyclic voltammogram for (1) 0.1 M L-cysteine in 0.1 M NaOH, pH 9.34; (2) 0.1 M sodium molybdate, 0.1 M L-cysteine, (3) 0.09 M NaOH, pH 9.31, scan 3, scan from 0.0 to -1.0 V and back, scan rate 100 mV/s

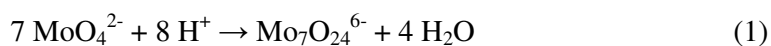
A comparison of the cyclic voltammograms for 0.095 M sodium molybdate in 0.12 M HCl at pH = 4.51, 0.1 M L-cysteine in 0.1 M NaCl at pH = 5.18 and 0.1M sodium molybdate-L-cysteine in 0.125 M HCl at pH = 4.53 are shown in Figure 4a. Since the current for L-cysteine in 0.1 M NaCl was small, it was multiplied by 20 times in Figure 4. The redox activity of condensed molybdate was considerably reduced in the mixture. At pH 9.3, the data shown in Figure 4b indicated a lack of interaction between molybdate and L-cysteine. This was also obvious from the lack of color in the solution.

3.2 Frequency Response Analysis

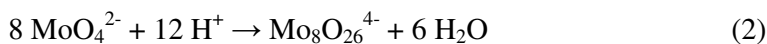
3.2.1 Admittance

Admittance (Y) and impedance are interrelated, $Y \equiv Z^{-1} \equiv Y' + jY''$. Since its introduction in 1969 by Bauerle for the determination of accurate conductivity of solid electrolytes [34], we are not aware of any major effort to utilize its usefulness. We have utilized this plot extensively in order to try to understand solute-solvent interactions near the electrode double layer [1-4, 9, 11, 20, 21]. On scanning from -1.0 to 0.0 V, the surface charge of the mercury electrode slowly changed from negative to positive. Correspondingly the orientation of the water molecule towards the electrode slowly changed from the positively charged hydrogen to the negatively charged oxygen. It could also be possible to have either one or both of the hydrogens oriented towards the mercury electrode depending on the nature of other species in solution near the double layer. This shift from hydrogen to oxygen pointing towards the electrode should be gradual, and could be decided by the changes in the scanning potential. The nature of the counter-ions and their hydration near the double layer could also influence the measured admittance. The complexity of the present system was obvious because of the presence of Na^+ , MoO_4^{2-} , and cysteine with charges such as $\alpha\text{-NH}_3^+$, $\alpha\text{-COO}^-$, and S^- . Other ions such as, Cl^- , OH^- , condensed molybdates with and/or without protonation, condensed molybdates coordinated with L-cysteine, and cystine with or without coordination with molybdates may be present depending on the solution pH. One should not overlook interactions between the positively charged amine group in L-cysteine and the negative counter-ions, such as chloride and molybdate. There can also be interactions between the positively charged sodium ion and the negatively charged carboxyl group and sulfhydryl group. It is also possible to have the interaction between the carboxyl group and sodium mediated by a molecule of water. We have made a modest attempt to sort out some of these effects.

The admittance data for 0.1M sodium molybdate-L-cysteine solutions at different pH values are shown in Figures 5-7. The comparison data at 1000 Hz and 100 Hz with sodium molybdate and L-cysteine are shown in Figures 8 and 9. We know that below pH = 7.0, MoO_4^{2-} begins to condense by the following reaction:



$\text{Mo}_7\text{O}_{24}^{6-}$ begins to protonate on further lowering the pH and produces species such as $\text{HMo}_7\text{O}_{24}^{5-}$ and $\text{H}_2\text{Mo}_7\text{O}_{24}^{4-}$. At pH values around 4.5 and below, octamolybdates are being formed by the reaction:



$\text{Mo}_8\text{O}_{26}^{4-}$ begins to protonate on further lowering the pH and produces species such as $\text{HMo}_8\text{O}_{26}^{3-}$ and $\text{H}_2\text{Mo}_8\text{O}_{26}^{2-}$. Also there are at least 3 forms of octa-molybdates, α -, β -, and γ -type, where the γ -form is an intermediate in the $\alpha \leftrightarrow \beta$ inter-conversion. All of these hepta and octamolybdates and their protonated forms can interact with cysteine and or cystine. These condensation and protonation processes increase with decreasing pH [20]. We have studied in detail the admittance behavior of condensed molybdates [20]. Except for the absolute values, the data at pH 3.89, 5.22 and 5.90 for the complexed molybdate with L-cysteine were somewhat similar to that of molybdate. We have seen evidence for the complex formation by cyclic voltammetry (Figure 4) and also from the increase in color intensity with decreasing pH values.

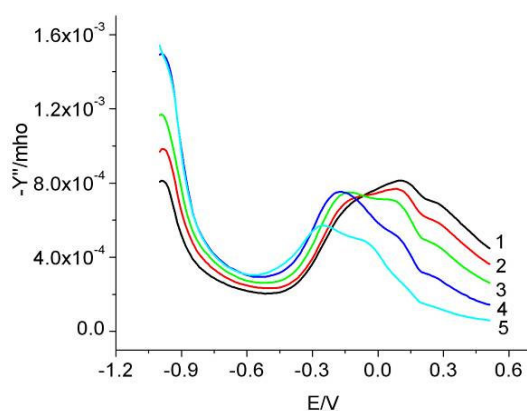


Figure 5a. Admittance of 0.1 M molybdate-L-cysteine, 0.15 M HCl, pH 3.89, after 24 hours of preparation, 1) 1000 2) 750 3) 500 4) 250 5) 100 Hz

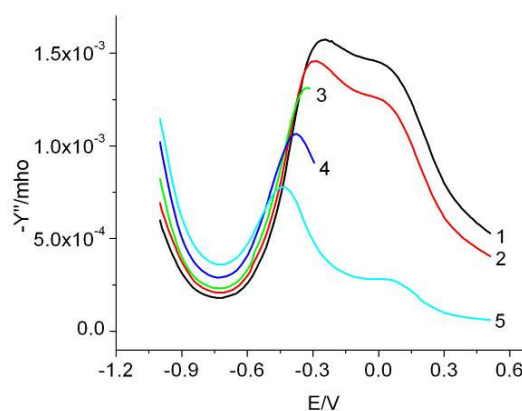


Figure 5b Admittance of 0.1 M molybdate-L-cysteine, 0.1 M HCl, pH 5.22, after 24 hours of preparation, 1) 1000 2) 750 3) 500 4) 250 5) 100 Hz

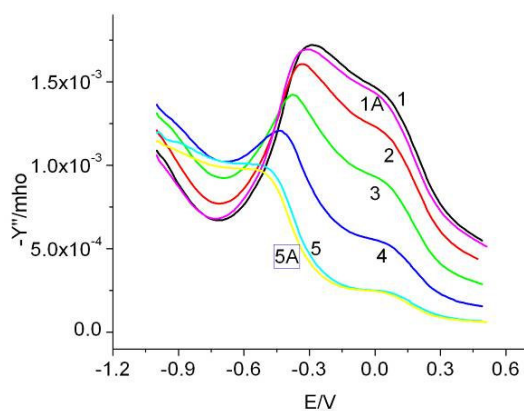


Figure 6a Admittance of 0.1 M molybdate-L-cysteine, 0.05 M HCl, pH 5.90, immediately after preparation, 1) 1000 2) 750 3) 500 4) 250 5) 100 Hz; After 24 hours of preparation, 1A, 1000 Hz, 5A, 100 Hz

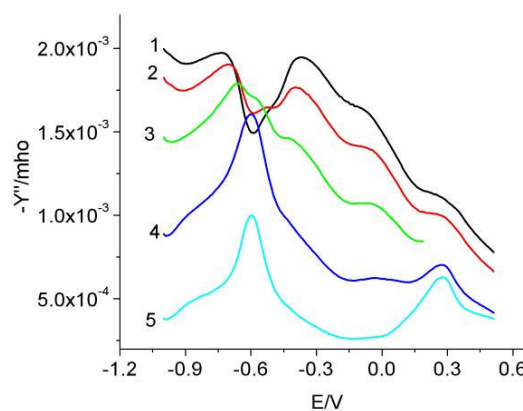


Figure 6b. Admittance of 0.1 M molybdate-L-cysteine, 0.0 M HCl, pH 6.91, after 24 hours of preparation, 1) 1000 2) 750 3) 500 4) 250 5) 100 Hz

The admittance data at pH = 6.91 as shown in Figure 6b, were clearly different. The comparison data with molybdate, as shown in Figures 8a and 8b, curves 1 and 4, also confirmed this finding. The data were now more similar to that of L-cysteine, as shown in Figure 9a, curves 2 and 3. Since there was still color in the solutions we had to assume significant interactions. However instead of interactions with condensed molybdate, it should be with the monomer, MoO_4^{2-} . Also the two broad maxima moved in opposite directions with decreasing frequency and merged into one below 500 Hz compared to L-cysteine only where the merging took place at frequency around 1 Hz [21]. It could be quite possible that the charged interaction might also involve MoO_4^{2-} and $\alpha\text{-NH}_3^+$ along with redox reaction as evidenced by the presence of color.

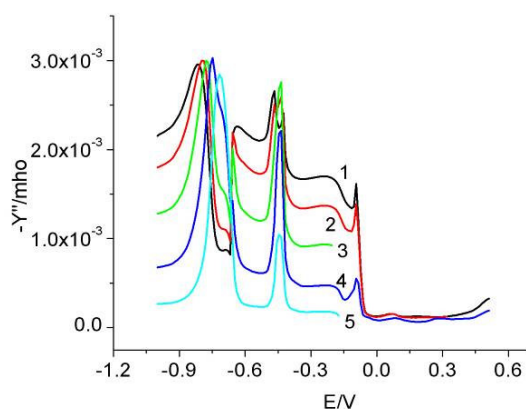


Figure 7. Admittance of 0.1 M molybdate-L-cysteine, 0.09 M NaOH, pH 9.34, after 24 hours of preparation, 1) 1000 2) 750 3) 500 4) 250 5) 100 Hz

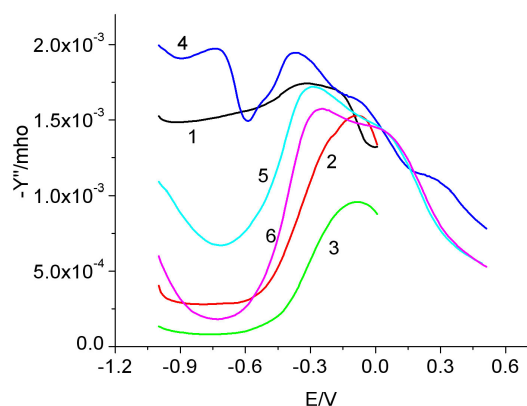


Figure 8a. Admittance, 1000 Hz, 0.095 M molybdate, pH 6.95(1), 6.04(2), 5.04(3) and 0.1 M molybdate-L-cysteine, pH 6.91(4), 5.90(5), 5.22(6)

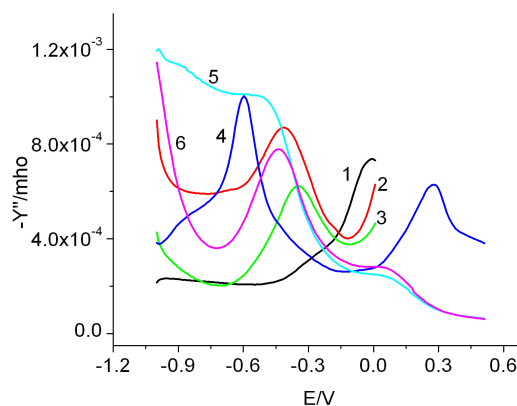


Figure 8b. Admittance, 100 Hz, 0.095 M molybdate, pH 6.95(1), 6.04(2), 5.04(3) and 0.1 M molybdate-L-cysteine, pH 6.91(4), 5.90(5), 5.22(6)

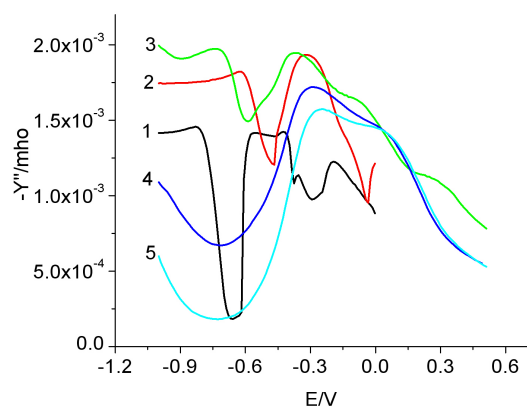


Figure 9a. Admittance, 1000 Hz, 0.1 M L-cysteine, pH 8.23(0.05 M NaOH and 0.05 M NaCl)(1), 5.18 (0.1M NaCl)(2), and 0.1 M molybdate-L-cysteine, pH 6.91 (3), 5.90(0.05 M HCl)(4), 5.22(0.1M HCl)(5)

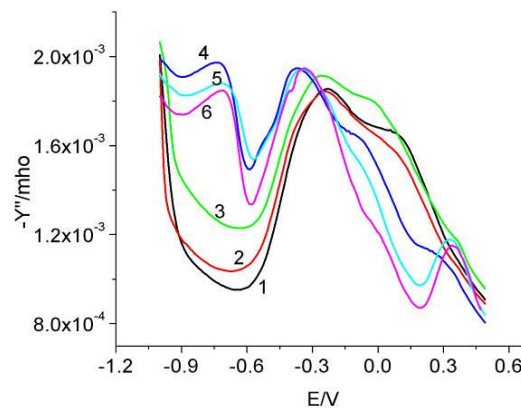


Figure 9b Admittance, 1000 Hz, 0.1 M molybdate with L-cysteine 1) 0.01 M 2) 0.03 M 3) 0.05 M 4) 0.1 M 5) 0.2 M and 6) 0.4 M; pH ~ 6.8

Figure 7 gives the admittance data at pH = 9.34. The influence of the ionized sulfhydryl group was clearly evident here. At this pH value there was only an extremely pale yellow color. One very interesting aspect was the presence of 2 pairs of two broad maxima. In both pairs there were both cathodic and anodic shifts with decreasing frequencies. The first, more cathodic pair merged into one peak around 250 Hz whereas the second, more anodic pair merged into one peak around 500 Hz. We assigned the second pair of peaks at comparatively more anodic potentials, to the presence of the ionized sulfhydryl groups. We have not seen such spectacular indications of the presence of charged groups in electrochemical measurements, except in admittance data reported here. We are exploring more on this observation with pure L-cysteine itself at higher pH values and plan to report the findings separately.

We also looked at the influence of the concentration of L-cysteine on its interactions with molybdate, as shown in Figure 9b. The ratio of molybdenum: L-cysteine ranged from 10:1, 10:3, 10:5, 1:1, 1:2 and 1:4. The data were somewhat similar even though they could be split into a pair, the first 3 and the last 3. A comparison of the pair suggested that the broad first maxima were influenced by the presence of $\alpha\text{-NH}_3^+$ group. Then, the second broad maximum could be that of the ionized carboxylate group. The third maximum might be due to the presence of the sodium ions near the charged sulfhydryl group, and the fourth one due the charge of the sulfhydryl group. This assignment will explain the opposite anodic (for $\alpha\text{-NH}_3^+$ and Na^+) and cathodic (for COO^- and sulfhydryl S^-) shifts with decreasing frequencies, as seen in Figure 7. This is because the orientation of the water molecules around these ions must have opposite orientations due to their differences in charges. The data in 9b suggested that Na^+ was competing with $\alpha\text{-NH}_3^+$ group when L-cysteine was much less than sodium molybdate.

3.2.2. Nyquist plots

3.2.2.1. Influence of pH

Cyclic voltammetry data as well as admittance data at different pH values resembled molybdate behavior in acidic conditions and L-cysteine behavior in basic solutions. An example of impedance behavior is shown in the Nyquist plot in Figure 10. At the same anodic potential, the impedance loci occurring in 4 quadrants were best at pH 5.22 and 5.79. It became more chaotic in the 4th quadrant at pH 6.91 and the chaotic nature dominated at the beginning of the 2nd quadrant at pH 9.34. We were not able to get a strict comparison of the pH behavior because there was a slight shift in the best anodic potential at which the best Nyquist plot appearing in several quadrants was obtained. Since the impedance was less at pH = 5.79 than that at pH = 5.22, the best Nyquist plot was at the former pH. We should also point out that we had chloride in some of these solutions (0.1, 0.05, 0.0 M HCl) at pH 5.22, 5.79 and 6.91 and additional sodium ions at pH 9.34 (0.09 M NaOH). There was no chloride at pH 6.91 and the data were free from any effects due to chloride.

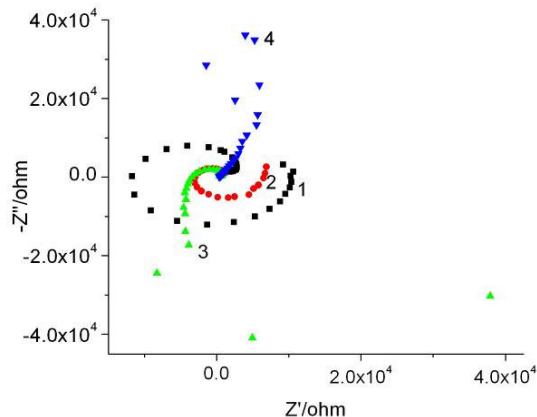


Figure 10. Nyquist plot for 0.1M molybdate-L-cysteine at pH 1) 5.22 2) 5.79 3) 6.91 4) 9.34; 0.3 V, 1000 Hz – 136 mHz

The data also suggested that impedance loci occurring in 4 or more quadrants preferred a pH value of about 6. On either side we got impedance loci in less than 4 quadrants. Of course we had to deal with the influence of the chloride introduced during lowering of the pH value, and with ionization of the sulfhydryl group at higher pH values, and at still higher pH values with the deprotonation of the amine group. Another problem was the condensation of molybdates into hepta and octamolybdates with varying degrees of protonation, and the corresponding interactions with L-cysteine. The data seemed to favor heptamolybdates with much less protonation because we got the best results around pH ~ 6.0 in 0.05 M HCl.

3.2.2.2 Surface of mercury

We were curious as to whether the observed phenomenon was due to the passivated surface of mercury with or without any adsorbed molybdate-cysteine (or cystine) complex. Mercury is known to

have strong attraction for sulfur. It is also possible to have some form of self-assembled layer of molybdate-L-cysteine (or cystine) near the double layer. We do not have evidence for this type of cluster formation for molybdate-L-cysteine except that of 0.1 M molybdate-L-cysteine dried on a glass slide and viewed by a phase microscope as shown in Figure 11.

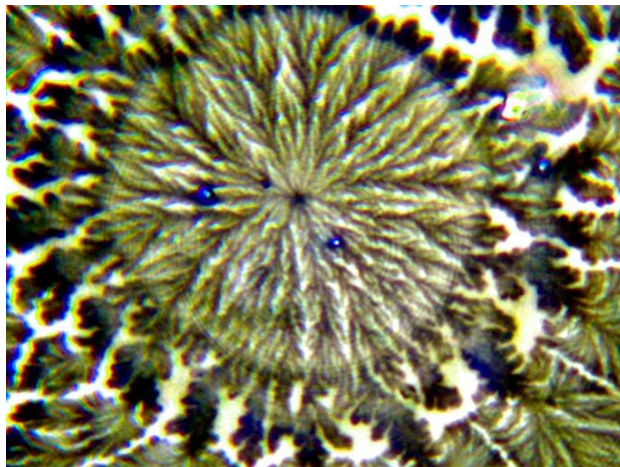


Figure 11. Phase microscopy (300X) of 0.1 M molybdate-L-cysteine, pH 5.79

The data shown in Figure 12 suggested that the adsorbed layer of the molybdate-cysteine (or cystine) complex or the self-assembled material remained intact and its properties were reversible. Whatever changes took place at lower frequencies do not affect its nature because the impedance data were quite reproducible when repeated from 1000 Hz.

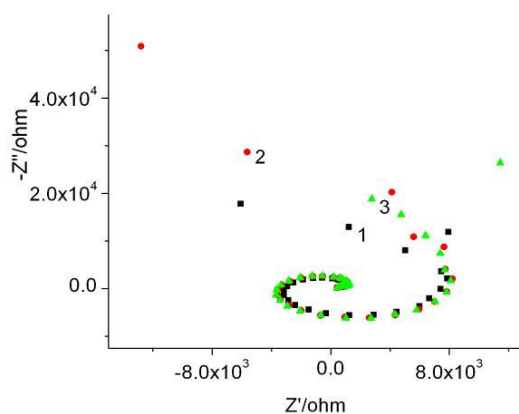


Figure 12. Nyquist plot for 0.1 M molybdate-L-cysteine, pH 5.79 (1) fresh Hg, (2) used Hg drop from (1), (3) used Hg drop again from (2); 0.25 V

3.2.2.3. Potential Dependence of Impedance Loci in more than 2 Quadrants

The sensitive nature of the impedance loci occurring in several quadrants was investigated by carefully changing the potential at which the impedance was measured. The results are shown in Figures 13 and 14. We have not seen similar extensive studies before. Our results indicated 3

capacitive loops at 0.0 and 0.1 V which can be reasonably fitted, with the equivalent circuit R1(R2C1)(R3C2)(R4C3)(R5C4)(R6C5). At 0.2 and 0.35 V, shown in Figure 13b, we observed the 3 capacitive loops followed by an inductive loop. In between these two potentials, we observed chaotic impedance loci occurring in 4 quadrants, as shown in Figure 14a. The impedance loci occurring in 4 quadrants was an optimum, in our case, at about 0.28 V. The impedance increased on both sides of this range. If we went much further on both sides, the results were entirely different, as shown in Figures 13b.

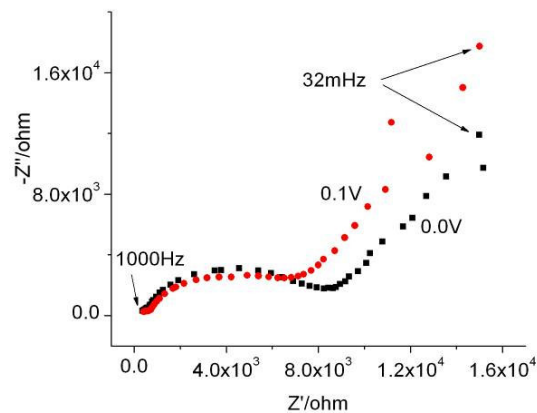


Figure 13a. Nyquist plot, 0.1 M molybdate-L-cysteine, 0.125 M HCl, pH 4.53, 3 capacitive loops

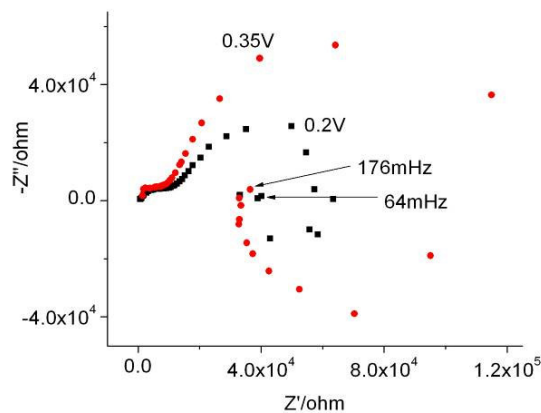


Figure 13b. Nyquist plot, 0.1 M molybdate-L-cysteine, 0.125 M HCl, pH 4.53; 3 capacitive loops and one inductive loop

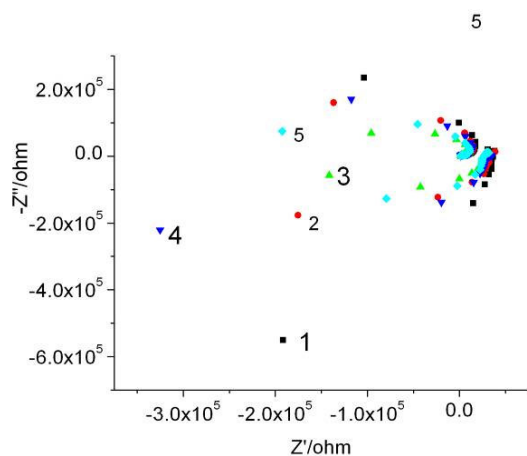


Figure 14a. Nyquist plot, 0.1 M molybdate-L-cysteine, 0.125 M HCl, pH 4.53, 1) 0.25 V 2) 0.27 V 3) 0.28 V 4) 0.29 V 5) 0.3 V, 1000 Hz - 136 mHz

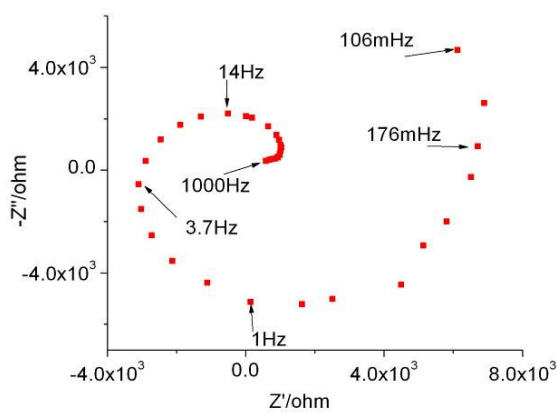


Figure 14b. Nyquist plot, 0.1 M molybdate-L-cysteine, 0.05 M HCl, pH 5.79, 0.3 V, 24 hours after preparation of solution

Figure 14b illustrates the need to optimize the pH to get very smooth impedance data that span 5 quadrants. For the sake of clarity, we have omitted the data in the 6th quadrant.

3.2.2.4. Concentration dependence of L-cysteine

The cyclic voltammetric data, as shown in Figure 3, and the admittance data, as shown in Figure 9b, separated themselves into two groups, one with L-cysteine less than 1:1 with respect to molybdenum, and the other one with more than 1:1. The corresponding impedance data are shown in Figure 15.

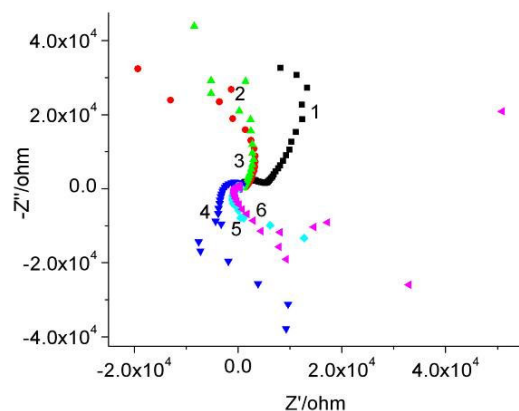


Figure 15. Nyquist plot, pH ~ 6.8, 0.1M molybdate, Concentration of L-cysteine, M, 1) 0.01, 2) 0.03, 3) 0.05, 4) 0.1, 5) 0.2, 6) 0.3; corresponding potentials of measurement, V, 1) 0.33, 2) 0.28, 3) 0.25, 4) 0.25, 5) 0.3, 6) 0.3

Instead of selecting data at one potential, we selected the best potential for the chosen ratio of Mo:L-cysteine at which impedance loci were observed in more than one quadrant. Here again the data could be clearly separated into two groups. The data suggested that in order to observe negative differential resistance or to exhibit impedance in more than one quadrant, the Mo:L-cysteine ratio should at least be 1:1.

SURFACE AREA

Solid electrodes pose an extremely difficult problem to investigate the influence of surface area on the phenomenon of spatiotemporal periodicities. On the other hand, our approach of using a mercury drop as the working electrode offers a great advantage. In the PAR 303A SMDE trielectrode system, there are small, medium and large size drop controls. Also for impedance measurements, we can adjust the size of the drop by pushing the dispense button for the appropriate number of times before the mercury falls. Thus, this system allows us to use a fresh surface as well as a controlled surface area each time. Our results are shown in Figure 16a. It is clear that the larger the surface area the better the observed periodicity and the lower the observed impedance.

Figure 16a shows our data on the influence of surface area on the observed impedance occurring in more than 5 quadrants. It is indeed surprising that such a complicated phenomenon yields amazingly smooth curves. As expected, the impedance became less and less with increasing surface area.

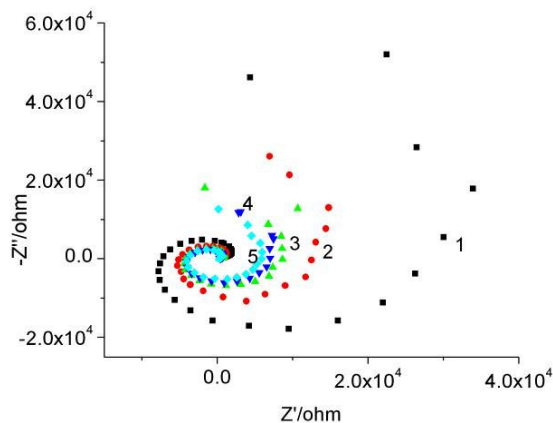


Figure 16a. Nyquist plot, 0.1 M Na_2MoO_4 -L-cysteine, 0.05 M HCl, pH 5.90, immediately after prep., 0.25 V, surface area in cm^2
1)0.011 2)0.017 3)0.022 4)0.027 5)0.031

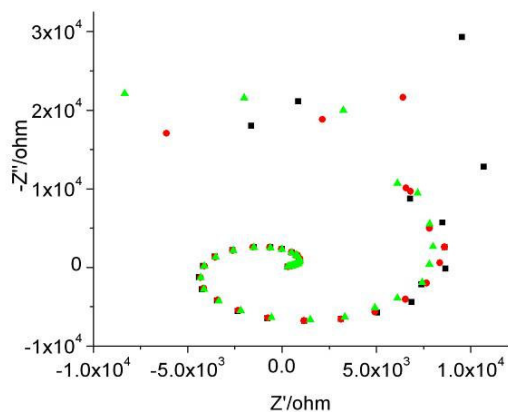


Figure 16b. Nyquist plot, 0.1 M Na_2MoO_4 -L-cysteine, 0.05 M HCl, pH 5.90, immediately after prep., 0.25 V, Stability of 3 data sets

STABILITY

The results for a set of 3 experiments in the frequency range 1000 Hz to ~ 5 mHz are shown in Figure 16b. It is remarkable that the impedance loci occurring in 5 quadrants was quite reproducible with little scatter. There was some scatter for the data in the 6th quadrant. We had pointed out earlier [3] that depending on the nature of the curve chosen for reproducibility, one could manipulate the visual observation. If we were to use the Bode plots to show reproducibility, the scatter was much less than the one shown in Figure 16b. The purpose of our experiment was to show the impedance loci in more than 4 quadrants rather than using that data for extensive calculations.

pH adjustment with H_2SO_4 instead of HCl

We observed the impedance loci occurring in 6 quadrants at pH = 5.8 in 0.1 M sodium molybdate-L-cysteine containing 0.05 M HCl. In order to assess whether this unique observation was due to the presence of chloride or the condensation of molybdate, we carried out a similar experiment in 0.05 N H_2SO_4 and the results are shown in Figure 17.

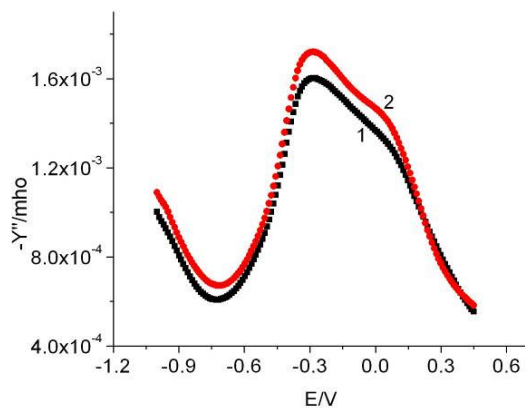


Figure 17a. Admittance, 1000 Hz, pH 5.9, 0.1 M molybdate-L-cysteine in (1) 0.05 N H₂SO₄, (2) 0.05 M HCl, immediately after preparation

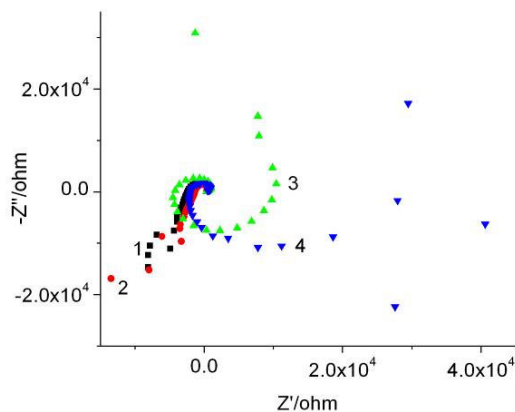


Figure 17b Nyquist plot, pH 5.9, 0.1M molybdate -L-cysteine in (1) 0.05 N H₂SO₄, 0.25 V, (2) 0.3 V ; in (3) 0.5 M HCl, 0.25 V, (4) 0.3 V; immediately after preparation

The admittance data in the presence of sulfate were remarkably similar to that of chloride. However, we were not able to obtain impedance loci in more than 4 quadrants. The data in the 4th quadrant were also somewhat chaotic. Since we were observing negative differential resistance behavior or impedance loci in more than 2 quadrants we could conclude that the effects were real and were probably due to complexation of L-cysteine with condensed heptamolybdate, instead of octamolybdate. Chloride did seem to provide very smooth impedance data even in the 4th and 5th quadrants.

4. CONCLUSIONS

We have investigated the interactions between sodium molybdate and L-cysteine at different pH values using cyclic voltammetry and frequency response analysis. With increasing pH values, the decreasing redox interaction was demonstrated by decreasing color, and admittance behaviour tending towards that of L-cysteine. At pH values close to neutral and above, the two broad admittance maxima moved in opposite directions with decreasing frequencies and merged into one around 250 Hz. This compares to the similar behaviour for L-cysteine only at pH = 5.18 and at around 10 Hz. At pH = 9.34, two pairs of maxima moving in opposite directions with decreasing frequencies and merging into two peaks were observed. The first pair was assigned to the interactions between α -NH₃⁺ and α -COO⁻ and the second pair to Na⁺ and sulfhydryl S⁻. The impedance exhibited negative differential resistance or impedance loci occurring in two or more quadrants at different pH values. When the potential was fine tuned, the impedance loci extended after the first 4 quadrants again into the first and second quadrants for a total of 6 quadrants, a phenomenon observed for the first time in impedance studies. Increasing the surface area decreased the impedance but followed the same trend. When the same used Hg drop was used again for repeat measurements, the same phenomenon was observed, indicating that there

was no need to have a fresh Hg drop for each experiment. A comparison of the data in the presence of sulfate instead of chloride, and the negative differential resistance observations at different pH values, suggests that heptamolybdate complexes of L-cysteine are more active. The presence of chloride gave smoother impedance data in the 4th and 5th quadrants at low frequencies. These data suggested that we should consider the electronic properties of those molecules to complement the enzymatic process in biological systems.

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