

Cyclic Voltammetric Studies on Electrocatalytic Reduction of Bisulfite in Aqueous Buffered Solutions Using a Myoglobin / Surfactant Film Electrode

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Abstract: Background: The surfactant, didodecyl dimethyl ammonium bromide (DDAB), was used to immobilize myoglobin (Mb) onto glassy carbon (GC) electrode surfaces. The Mb/DDAB film on GC electrode showed good charge transport rates. Objectives: Mb in DDAB was utilised as a redox catalyst for reduction reactions requiring multi-electron transfers. Cyclic voltammetry has proved the Mb modified film electrode to be an effective electrocatalyst for the multi-electron reduction of bisulfite in aqueous buffered solutions. Methods: Using cyclic voltammetry technique, bisulfite reduction was studied at various concentrations and scan rates in different aqueous buffer solutions of various pH. Results: The values of k_s of the film and k_{cat} of the catalytic reaction of bisulfite were obtained at different pH values. The variation in the catalytic rate constant values with pH confirmed that the bisulfite is the reactive species. Conclusions: The catalytic bisulfite reduction was an irreversible, diffusion-controlled, and pH dependent process. The reduction of bisulfite by the Mb/DDAB film followed the EC' catalytic mechanism.

Keywords: Mb/DDAB Film, Glassy Carbon (GC) Electrode, Electrochemical Methods, EC' Catalytic Mechanism

1. Introduction

Myoglobin (Mb) is a small heme protein which plays an important role in oxygen transport. Mb contains a single iron heme as the prosthetic group. Purified Mb exchanges electrons very slowly with highly oriented pyrolytic graphite (HOPG) bare electrodes [1]. Also, electron transfers between myoglobin and bare glassy carbon (GC) electrodes were not observed. This behavior was very similar to that reported by Rusling et al. for tin-doped indium oxide (ITO) and pyrolytic graphite (PG) bare electrodes with pure myoglobin in buffered solutions [2-4]. Surfactant films have been shown to be quite effective in enhancing electron transfer rates between electrode surfaces and the myoglobin film. In DDAB films, Mb is used as a redox catalyst because these films behave electrochemically as a thin layer within which Mb is oriented [5]. In aqueous buffered solutions, chromatographically

purified myoglobin gives slow electron transfer rates at bare electrodes surfaces [6-8], though in DDAB surfactant films, Mb could be reduced reversibly in two one-electron steps. The first step involved the divalent myoglobin Fe^{II} reduced species, while the second step involved the formation of monovalent myoglobin Fe^I species [9].

Lin et al. [10] used Mb/DDAB to catalyze the multi-electron reduction of nitrite. Catalytic bisulfite reduction was achieved using a surfactant/protein film, though the details of the reaction mechanism were not studied extensively [11]. The catalytic reduction of bisulfite by a water-soluble iron porphyrin was studied by Kline et al. [12]. Limited studies of model iron porphyrins complexes with bisulfite have been reported [13-16].

Electrochemistry is a good tool to study the redox mediated and film electrocatalyses and the electrochemical methods are straightforward and also utilized to study modified electrode electrocatalyses [17-26].

Using Mb/DDAB film on GC electrode as a redox catalyst, the multi-electron reduction of bisulfite at the potential of the second reduction wave of the film was undertaken using cyclic voltammetry and is reported in this study.

2. Experimental

2.1. Materials and Solutions

Horse skeletal muscle myoglobin from Sigma was dissolved in pH 7.3 Tris buffer solution containing 50 mM KBr. Didodecyl dimethyl ammonium bromide (DDAB, 99 %) was obtained from ACROS. Sodium bisulfite anhydrous was purchased from Sigma (99%), and used as supplied. Potassium bromide was obtained from Aldrich Chemical Company. The supporting electrolytes were 50 mM KBr or 100 mM KCl. The buffers used are Tris (pH 7.3), phthalate (pH 5), phosphate (pH 6), phosphate (pH 8) and borate (pH 9). The pH of the buffers was adjusted with HCl or NaOH solutions. All other chemicals were of analytical grade. The water was purified by Nano pure System to a specific resistance $> 18 \text{ M}\Omega\cdot\text{cm}$.

The working electrode was a DDAB/Mb modified glassy carbon electrode (GC disk, 3 mm in diameter, $A = 0.071 \text{ cm}^2$, Cypress Systems Co.). Before use, the electrode was polished in order to remove any previous adsorbed monolayer and to regenerate the bare surface. Just before modification, the electrode was rinsed with distilled water in a sonicator and treated with 1:3:4 (v/v/v) solution of concentrated $\text{HNO}_3\text{-HCl-H}_2\text{O}$ for 10-15 minutes in order to roughen the electrode so as to increase the amount of the immobilized biocatalyst. A 46.0 mg sample of DDAB was dispersed in 10 mL distilled water and ultrasonicated for at least 6 hours to obtain a clear solution.

Using a $2\mu\text{L}$ DDAB aliquot and $2\mu\text{L}$ myoglobin in pH 7.3 Tris buffer solution containing 50 mM KBr the glassy carbon electrode film was prepared. The modified electrode was dried in air at room temperature for at least 2 hours. The film could be stored in air or in buffer at room temperature.

2.2. Equipment

The cyclic voltammetry experiments were performed using a microprocessor controlled OMNI-101 analog potentiostat (Cypress Systems Co.), connected to an IBM-compatible personal computer by serial port and data were collected with Acquire-101 SER software.

2.3. Electrochemical Methods

The electrochemical cell was used for the electrochemical experiments. The working electrode was a DDAB/Mb modified glassy carbon electrode (GC disk, $d = 3 \text{ mm}$, Cypress Systems Co.). The Ag/AgCl (saturated KCl solution) and a platinum disk (Cypress Systems Co.) were used as reference and auxiliary electrodes, respectively. All solutions were purged with dinitrogen gas, which was also used for stirring the investigated solutions.

3. Results and Discussion

3.1. Characterization of Mb/DDA/GC Modified Electrode

Modifying the GC electrode surface with Mb in the presence of the surfactant didodecyl dimethyl ammonium bromide (DDAB) gives rise to two pair of well-defined, quasi-reversible cyclic voltammetric redox peaks. The peaks potentials are -0.36 V and -1.22 V (Figure 1) versus the Ag/AgCl saturated KCl reference electrode. The redox peaks of the Mb/DDAB film are tentatively assigned to the $\text{MbFe}^{\text{III}}/\text{MbFe}^{\text{II}}$ and $\text{MbFe}^{\text{II}}/\text{MbFe}^{\text{I}}$ redox couples, respectively. The first redox couple occurs in solution while the second redox couple is only observed in Mb/DDAB films. The cyclic voltammetry peaks current for the second reduction wave of the Mb-DDAB modified film at different scan rates is recorded. The relationship between the peak current values of the second reduction wave of the Mb-DDAB modified film and the scan rate is linear (Figure 2), which is diagnostic for an electrode surface with a surface confined species, without any diffusion.

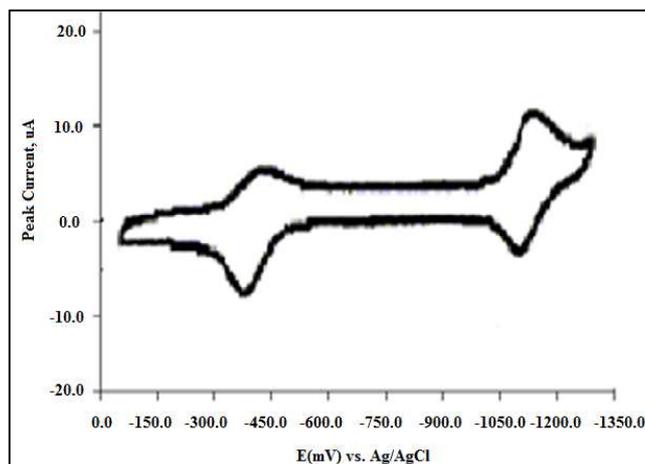


Figure 1. Cyclic voltammogram of Mb/DDAB/GC modified electrode in the absence of bisulfite at scan rate 200 mV/s in pH 7.3 Tris buffer solution containing 100 mM KCl at room temperature.

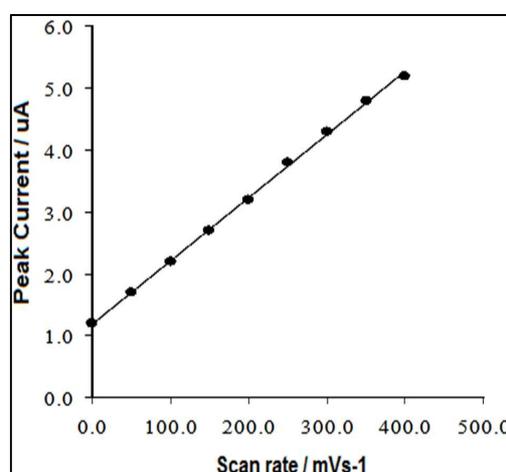
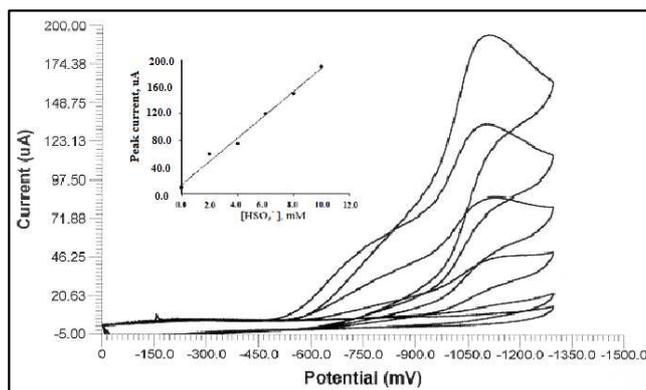


Figure 2. Plot of Peak Current (μA) vs. Scan rate (mV/s) of Mb/DDAB/GC modified electrode in the absence of bisulfite at different scan rates in pH 7.3 Tris buffer solution containing 100 mM KCl at room temperature.

3.2. Mb/DDAB Film Behavior Towards Bisulfite

Cyclic voltammograms of myoglobin-DDAB modified film in the presence of various bisulfite concentrations are shown (Figure 3). In the presence of bisulfite, current at the first wave is unaffected. No catalytic current was observed. At the monovalent Fe wave though, the peak current increases significantly, and a sigmoidal shaped peak wave was observed. These results are typical of a catalytic reduction of bisulfite. The mechanism involves the multi-electron transfer from the Mb in the film to the bisulfite at the limiting current region of the second wave [27]. The peak current for the second wave is to be proportional to the bisulfite concentration up to 10 mM (insert of Figure, 3).



Insert: plot of i_{pc} (μA) vs. $[\text{HSO}_3^-]$, mM.

Figure 3. Cyclic voltammograms of Mb/DDAB/GC modified electrode at various bisulfite concentrations (from bottom to top: 0, 2, 4, 6, 8, and 10 mM, respectively) at scan rate of 50 mV/s in pH 7.3 Tris buffer solution containing 100 mM KCl at room temperature.

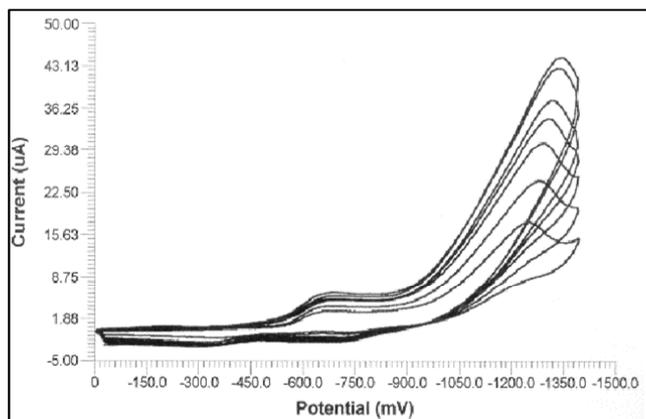


Figure 4. Cyclic voltammograms of Mb/DDAB/GC modified electrode in the presence of 10 mM bisulfite at various scan rates (from bottom to top: 50, 100, 150, 200, 250, 300, and 400 mV/s, respectively) in pH 7.3 Tris buffer solution containing 100 mM KCl at room temperature.

Cyclic voltammograms obtained for bisulfite reduction at Mb/DDAB/GC modified electrode when scan rate is varied are shown (Figure 4). The reduction peak for bisulfite in the Tris buffer solution (pH 7.3) shifted slightly toward more negative potentials as scan rate is increased (Figure 4), a typical characteristics of an irreversible electrochemical reaction of bisulfite. Also, the irreversible catalytic reduction

peak of bisulfite is nearly symmetrical. Figure 5 shows linear plot of peak current vs. square root of scan rate.

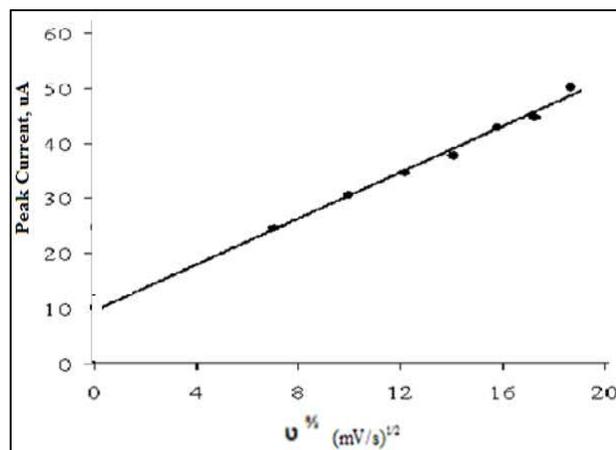


Figure 5. Plot of peak current vs. Square root of scan of Mb/DDAB/GC modified electrode in the presence of 10 mM bisulfite at various scan rates in pH 7.3 Tris buffer solution containing 100 mM KCl at room temperature.

The linearity of the plot (Figure 5) confirmed that the catalytic reduction of bisulfite is diffusion - controlled process. Using equation (1):

$$I_{pc} = (2.99 \times 10^{-5}) n_{\alpha}^{3/2} \alpha^{1/2} A C D^{1/2} \nu^{1/2} \quad (1)$$

for peak current of totally irreversible and diffusion-controlled processes [28], the (n_{α}) involved in the rate determining step in reduction of bisulfite by the Mb/DDAB film on surface of Gassy carbon electrode was obtained ($n_{\alpha} = 1.99 \approx 2$), assuming $D_{\text{HSO}_3^-}$ and α equal to $10^{-5} \text{ cm}^2/\text{s}$ and 0.50 respectively.

As for an irreversible electrode process, and according to Laviron theory, E_{pc} is defined by equation (2) [29]:

$$E_{pc} = E^{0'} - (RT/\alpha n_{\alpha} F) \ln (RT/\alpha n_{\alpha} F k_s) + (RT/\alpha n_{\alpha} F) \ln \nu \quad (2)$$

where α is the charge transfer coefficient, n_{α} is the number of electron transferred in the rate-limiting step, ν the scan rate, k_s is the heterogeneous electron transfer rate constant of Mb, $E^{0'}$ is the formal redox potential of bisulfite. Other symbols have their usual meanings.

The plot of E_{pc} versus $\ln \nu$ (Figure 6) is linear and the value of αn_{α} can be calculate. In this system, the slope value is 0.0284, and taking $T = 298.15 \text{ K}$, $R = 8.314 \text{ J/mol} \cdot \text{K}$, and $F = 96485 \text{ C/mol}$, the αn_{α} is found to be 0.904. Since the number of electrons involved in the rate-limiting reduction step, n_{α} , is 2, the charge transfer coefficient, α , is calculated to be 0.452, and the irreversible catalytic reduction peak of bisulfite was nearly symmetrical. The value of k_s can be determined if the value of $E^{0'}$ is known. The value of $E^{0'}$ in Eqn. 2 can be obtained from the intercept of the E_{pc} vs. $\ln \nu$ plot by extrapolation to the vertical axis at $\ln \nu = 0$ (Figure 6). In this system, the intercept for E_{pc} vs. $\ln \nu$ plot was -1.295 V and $E^{0'}$ is obtained to be -1.100 V. The value of k_s in the present case for the charge transfer for Mb in DDAB in aqueous Tris buffer (pH 7.3) containing 100 mM KCl at room temperature is $29.8 \pm 0.2 \text{ s}^{-1}$ and the catalytic rate constant for bisulfite reduction is

calculated to be $(1.5 \pm 0.1) \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$.

In addition, an alternative expression of I_{pc} in terms of E_{pc} for irreversible electrode processes at varying scan rates is given by equation (3) [30];

$$I_{pc} = 0.227 n F A C_o K_s \exp [-\alpha f (E_{pc} - E^0)] \quad (3)$$

Plot of $\ln I_{pc}$ vs. E_{pc} determined at different scan rates in pH 7.3 Tris buffer solution containing 100 mM KCl at room temperature, (Figure, 7), has a slope of $-\alpha f$ and an intercept proportional to k_s . In this system, the slope was -17.58 with α equal to 0.45. From the intercept, k_s value was obtained to be $30.2 \pm 0.1 \text{ s}^{-1}$. Also, the catalytic rate constant of bisulfite reduction is calculated to be $(1.51 \pm 0.10) \times 10^3 \text{ l. mol}^{-1} \text{ s}^{-1}$.

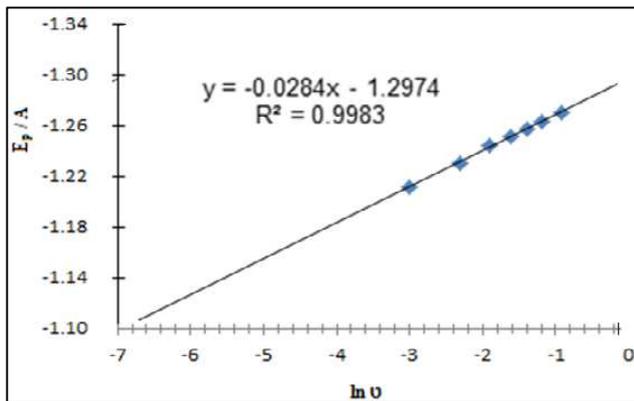


Figure 6. Plot of peak potential vs. \ln scan rate of Mb/DDAB/GC modified electrode in the presence of 10 mM bisulfite at various scan rates in pH 7.3 Tris buffer solution containing 100 mM KCl at room temperature.

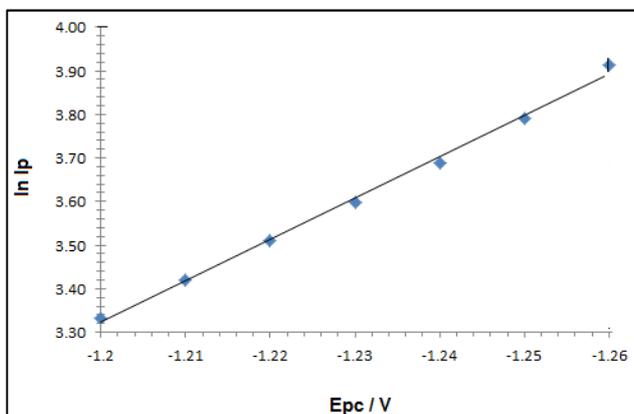


Figure 7. Plot of \ln peak current ($\ln I_p$) vs. peak potential (E_{pc}) of Mb/DDAB/GC modified electrode in the presence of 10 mM bisulfite at various scan rates in pH 7.3 Tris buffer solution containing 100 mM KCl at room temperature.

The catalytic rate constant for bisulfite reduction, k_{cat} , is related to the heterogeneous rate constant of the film, k_s , for Mb/DDAB film by equation (4) [30];

$$K_{cat} = 10^{-3} \times k_s / \Gamma_{Mb} \quad (4)$$

Where: Γ_{Mb} is the surface coverage for the myoglobin in Mb/DDAB film on GC electrode in mol/cm^2 ($\Gamma_{Mb} = Q / n F A$). Based on a value of $2 \times 10^{-11} \text{ mol Mb}/\text{cm}^2$ for surface coverage of Mb in the film calculated from the charge under

the peak, ($Q = 30 \times 10^{-5} \text{ C}$), of the film by cyclic voltammetry at 100 mV s^{-1} in the absence of bisulfite in solutions of buffers. The values of k_s for Mb in DDAB film and k_{cat} for bisulfite in buffered solutions of different pH values (pH 5-9) are calculated (Table 1). The variation in the catalytic rate constant values with pH confirmed that the bisulfite is the reactive species. Figure 8 indicated that the current function ($I_{pc} / v^{1/2} \text{ C}$) values decreased by increasing the potential scan rate and then ($I_{pc} / v^{1/2} \text{ C}$) responses became stable confirming an EC' catalytic mechanism for the bisulfite reduction by the Mb/DDAB film.

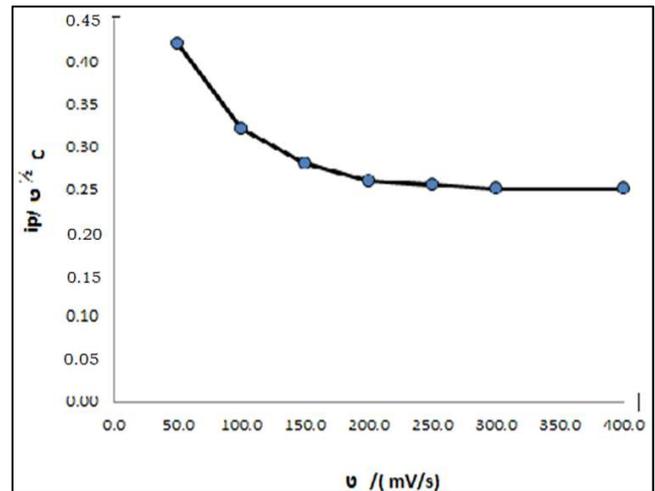


Figure 8. Plot of Current Function ($I_{pc} / v^{1/2} \text{ C}$) vs. Scan rate (v) of Mb/DDAB/GC modified electrode in the presence of 10 mM bisulfite at various scan rates in pH 7.3 Tris buffer solution containing 100 mM KCl at room temperature.

Table 1. The values of k_s for Mb in DDAB and k_{cat} for bisulfite reduction at Mb/DDAB films in different buffer solutions (pH 5-9).

pH	$k_s (\text{s}^{-1})$	$k_{cat} (\text{M}^{-1}\text{s}^{-1})$
5.00	42.0 ± 0.3	$(2.10 \pm 0.02) \times 10^3$
6.00	38.0 ± 0.1	$(1.90 \pm 0.01) \times 10^3$
7.30	29.8 ± 0.3	$(1.50 \pm 0.02) \times 10^3$
8.00	10.0 ± 0.2	$(0.50 \pm 0.02) \times 10^3$
9.00	4.0 ± 0.4	$(0.20 \pm 0.04) \times 10^3$

4. Conclusion

Surfactant film of myoglobin in DDAB formed on the surface of a glassy carbon electrode. Mb in DDAB is reduced reversibly in two one-electron steps. Moreover, the Mb/DDAB/GC electrode revealed a good catalytic behavior towards the multi-electron reduction of bisulfite. The kinetics of bisulfite reduction by surfactant film was examined using cyclic voltammetry and values of k_s of the film and k_{cat} of the catalytic reaction of bisulfite reduction were obtained at different pH values. The variation in the catalytic rate constant values with pH confirmed that the bisulfite is the reactive species. The catalytic bisulfite reduction is an irreversible, diffusion-controlled, and pH dependent process. In addition, catalytic bisulfite reduction by Mb/DDAB film followed EC' catalytic mechanism.

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