

loaded polyethylene was evacuated at room temperature and 10^{-2} Torr and the products were passed through a series of three traps kept at 0 °C, -20 °C and -78 °C. The trap kept at 0 °C contained 27 mg (4%) of **6**; the trap kept at -20 °C contained additional **6** (7%; $\delta=2.28$) as well as **7** (11%; $\delta=4.66$), acetone (21%; $\delta=2.18$) and acetic acid (58%; $\delta=2.10$) in the relative proportions reported in parenthesis, as determined by ^1H NMR spectroscopy. Separation of this mixture by flash chromatography (silica gel; n-pentane/diethyl ether, in a volumetric ratio of 10:1) gave 12 mg (3%) of **7**. The assignments of the above mentioned products were in each case **11**, **12** confirmed by the comparison of their ^1H NMR data with those of reported^{11,12}; the identity of the non-isolated products acetone and acetic acid was additionally confirmed by their GC- t_R data.

Acetonitrile (**6**). Colorless solid, mp 45-46 °C (lit.¹¹ mp 44-45 °C); ^1H NMR (CDCl_3 , TMS): $\delta=2.28$ (s, 3H), 4.92 (s, 2H); EI-MS (%) $m/e=207$ (100) ($M+1$)⁺.

Acetoxyacetone (**7**). ^1H NMR (CDCl_3 , TMS): $\delta=2.17$ (s, 3H), 2.18 (s, 2H), 4.66 (s, 2H); EI-MS (%) $m/e=116$ (13) M^+ .

Acetone. ^1H NMR (CDCl_3 , TMS): $\delta=2.18$ (s); EI-MS (%) $m/e=58$ (34) M^+ ; GC- $t_R=2.59$ min (condition a).

Acetic acid. ^1H NMR (CDCl_3 , TMS): $\delta=2.10$ (s); EI-MS (%) $m/e=60$ (31) M^+ ; GC- $t_R=8.73$ min (condition a).

Ozonolysis of 1-methyl-2-cyclopentenyltriflate (14). Ozonolysis of 780 mg (3.39 mmol) of **14** on 65 g of polyethylene at -78 °C was carried out for 6 h, and the products were collected as described above. The trap kept at -78 °C contained 318 mg of a colorless liquid, which was composed of acetic acid (60%; $\delta=2.09$; EI-MS: $m/e=60$, M^+) and formic acid (40%; $\delta=8.03$; EI-MS: $m/e=46$, M^+).

The residual products on polyethylene were isolated by extraction with diethyl ether and subsequent removal of the solvent at room temperature and reduced pressure to leave 412 mg of a colorless liquid residue. ^1H NMR analysis showed the presence of **15** (25%; $\delta=2.31$, s) and **16** (75%; $\delta=2.16$, s) in the relative proportions reported in parenthesis. The assignments of **15**¹³ and **16**¹⁴ were confirmed by comparison of their ^1H NMR, data with those of reported.

5-Acetylbutyrolactone (15). ^1H NMR (CDCl_3 , TMS): $\delta=2.50-2.61$ (m, 4H), 2.31 (s, 3H), 4.79-4.85 (m, 1H); EI-MS (%) $m/e=128$ (15) M^+ ; GC- $t_R=14.97$ min (condition b).

5-Oxohexanoic acid (16). ^1H NMR (CDCl_3 , TMS): $\delta=1.90$ (quint, $J=7.1$ Hz, 2H), 2.16 (s, 3H), 2.43 (t, $J=7.1$ Hz, 2H), 2.56 (t, $J=7.1$ Hz, 2H); EI-MS (%) $m/e=130$ (2) M^+ ; GC- $t_R=16.28$ min (condition b).

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pH Dependence of Electrochemical Behaviors of Methylene Blue on Self-Assembled Monolayers

Myoung-ho Pyo* and Sook-Hee Jeong

Department of Chemistry, Sunchon National University, Junam 540-742, Korea

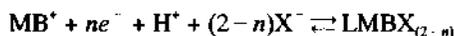
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Spectroscopic¹⁻³ and electrochemical⁴⁻⁶ properties of methylene blue (MB) have been widely studied mainly due to its specific interaction with deoxyribonucleic acid (DNA). The driving force of these works is based on the fact that small and planar organic molecules can be used for analytical, diagnostic, and therapeutic purposes⁷ since the probe molecule is electroactive and specifically bound to double-strand-

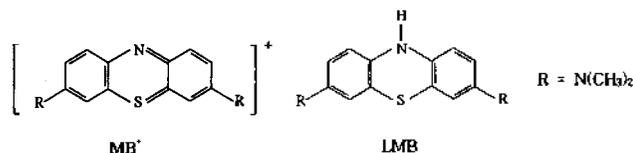
ed DNA molecules.

Important studies on the electrochemical behaviors of MB have been performed by Chevalet *et al.*⁴⁻⁶ a decade ago. They addressed, from the voltammetric results, that the insoluble reduction product, a mixed valence salt of the leucomethylene blue (LMB) and its cation radical (LMB^{•+}), forms two polymorphic conductive structures on Au and Pt

electrodes and the redox process includes anion transport as well as polymorphic transformation. When the reduction potential is higher than -0.498 V vs. SCE, the proposed mechanism can be simplified as



where the n -value of *ca.* 1.5 was obtained from the slope of the rising part of the cyclic voltammogram.



In this report, we address electrochemical behaviors of methylene blue on polycrystalline Au modified with self-assembled monolayers (SAMs) in phosphate buffer solutions of various pHs. Additional supporting electrolytes were not added on purpose to avoid complex electrochemical behaviors as reported previously.⁴⁻⁶ The voltammetric results of MB on various SAM modified Au were compared and the effects of solution pH were investigated.

Experimental

Methylene blue (3,7-bis(dimethylamino)phenothiazin-5-ium-chloride, MB) and octadecanethiol ($\text{C}_{18}\text{H}_{37}\text{SH}$), purchased from Aldrich Chemical Co., were used as received. 11-mercaptoundecanoic acid was synthesized according to a literature procedure.⁸ Other chemicals were of reagent grade or better. Phosphate buffer solutions (50 mM, various pH) were used for all measurements. Water was purified through an Milli-Q Plus system (Millipore Co.) until its resistivity was over $18 \text{ M}\Omega\text{cm}$.

For electrochemical measurements, polycrystalline gold (Au) disk electrode (Valpey Fisher Co., geometrical area = 0.71 cm^2) was used as a working electrode. The reference electrode was an Ag/AgCl electrode (saturated KCl) and the counter electrode was a platinum flag. All electrochemical experiments were performed with a Bioanalytical Systems (BAS) Model CV-50 W electrochemical analyzer.

The gold thin film electrode was cleaned by immersing into piranha solution (3:1 mixture of conc. H_2SO_4 and 30% H_2O_2) prior to use. After rinsing thoroughly with deionized water, the Au electrode was dipped into an ethanol solution of 10 mM 11-mercaptoundecanoic acid or a hexane solution of 10 mM octadecanethiol for 4 hours at room temperature, respectively. Each modified electrode was washed thoroughly with solvent and subsequently with the buffer solution and immediately placed in the electrochemical cell filled with 50 mM phosphate buffer solutions of 0.1 mM MB. All the measurements were conducted under Ar blanket at room temperature.

Results and Discussion

The bare Au electrode and SAM modified electrodes were cycled at 200 mV/s in phosphate buffer solutions of various pHs containing 0.1 mM MB. In order to avoid the structure transformation of the LMB layers deposited during MB reduction, the potential window was limited between -400 mV and $+600$ mV and no extra supporting elec-

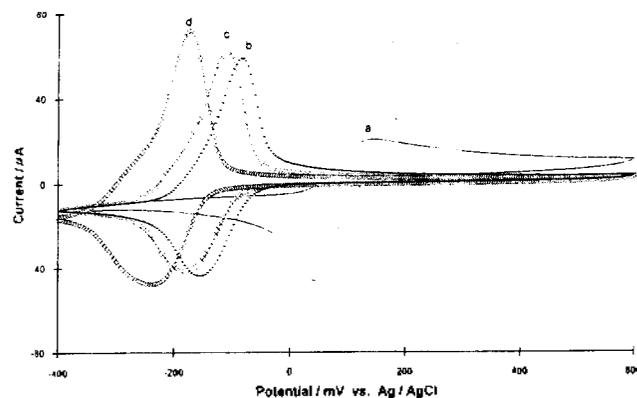


Figure 1. Cyclic voltammograms of 0.1 mM MB in phosphate buffer solutions of pH (a) 2.5, (b) 5.6, (c) 7.7, and (d) 9.0 on $\text{C}_{18}\text{H}_{37}\text{SH}$ modified electrodes. $v=200$ mV/s.

trolyte was added. Chevalet *et al.*⁴⁻⁶ claimed that the reduction postwave, which leads to polymorphic transformation, is shown at more negative potentials than -400 mV and much less pronounced without added supporting electrolytes. Using an electroreflectance technique, it has been also disclosed by Sagara *et al.*⁹ that MB molecules are deposited into the dodecanethiol monolayer interior, but not in direct contact with the underlying Au. Figure 1 shows the cyclic voltammograms with the octadecanethiol ($\text{C}_{18}\text{H}_{37}\text{SH}$) modified electrode in phosphate buffer solutions of pH=(a) 2.5, (b) 5.6, (c) 7.7, and (d) 9.0. Obviously, redox potentials move to the negative direction as the increase of pH. The effects of solution pH on $E_{1/2}$ shifts will be discussed below. The solution pH also affects the peak currents. While the cathodic peak currents (i_{pc}) and total charges passed during MB reduction are independent of the solution pH, the anodic peak currents (i_{pa}) suddenly decrease in pH=2.5, as shown in Figure 1a. It should be noted that, although i_{pa} is substantially reduced in pH=2.5, the total charge during the anodic process is similar to the results in higher pH solutions. The charge was determined by graphical integration of the voltammetric wave.

The scan rate (v) dependence of i_{pa} also demonstrates distinguishable behaviors with pH. The potential was fixed at -0.4 V for 30 sec and scanned to the positive direction at different rates. The i_{pa} was proportional to v in pH=5.6 or higher, indicative of thin layer behavior, but semi-infinite diffusion behavior predominates in pH=2.5 within the range of scan rates examined (25 mV/s to 1 V/s). The cyclic voltammograms with bare Au do not show the reduction of the anodic peak currents and semi-infinite diffusion behavior in the pH range examined. This seems to suggest that the stripping of LMB deposited at the SAM surface is the dominant anodic process in relatively high pH solutions, but the deposition of MB during cathodic process does not occur in low pH solutions. It should be mentioned that the amount of spontaneously adsorbed MB at the solution-SAM interface is negligible since the $\text{C}_{18}\text{H}_{37}\text{SH}$ modified electrode, which was dipped in 0.1 mM MB solutions for 30 min and subsequently placed in MB-free solutions, does not show the comparable MB redox peaks.

The effect of electrode surface modification on MB redox was further examined with 11-mercaptoundecanoic acid

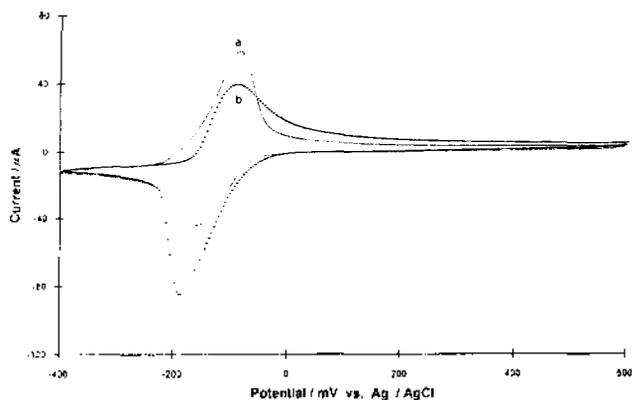


Figure 2. Cyclic voltammograms of 0.1 mM MB in phosphate buffer solutions of pH=5.6 on (a) $C_{18}H_{37}SH$ and (b) $HOCC_{10}SH$ modified electrodes. $v=200$ mV/s.

($HOCC_{10}SH$) modified electrodes. While the cyclic voltammogram in buffer solutions of pH=2.5 was similar to Figure 1a, the peak height and shape were greatly different in pH=5.6 or higher. Figure 2 exhibits the representative example in pH=5.6. The i_{pc} and total charge on the $HOCC_{10}SH$ modified electrode are greater than those on the $C_{18}H_{37}SH$ modified electrode. Meanwhile, compared with the results on the $C_{18}H_{37}SH$ modified electrode, the i_{pa} on the $HOCC_{10}SH$ modified electrode is slightly lower, but the total charge during the anodic process is identical. These differences result from the surface charge which does not exist in low pH. The concentration of MB at the SAM-solution interface could be high since it can serve as counter ions of carboxylate anions in higher pH. These surface-enriched MB contribute to the increase of the i_{pc} and charge during the cathodic scan. On the other hand, the negatively charged SAM surface seems to hamper the deposition of LMB on the SAM surface, leading to the reduced i_{pa} and semi-infinite diffusion behavior.

The effects of pH on the MB redox peak shift were also investigated. Figure 3 shows the $E_{1/2}$ shift vs. solution pH when (a) $C_{18}H_{37}SH$ and (b) $HOCC_{10}SH$ modified electrodes are cycled at 200 mV/s. Although i_{pa} in lower pH is not obvious as in higher pH, the determination of E_{pa} was still possible from $1/2(E_{pa}+E_{pc})$. The slopes ($\Delta mV/\Delta pH$) are bent at ca. pH=5.2-5.4, regardless of surface charges, suggesting that the reason of the slope change with pH is an intrinsic property from hydrophobic interaction of MB and SAM rather than deprotonation of $HOCC_{10}SH$ in high pH. When the solution pH is higher than 5.2, the slopes are -29 and -19 mV/pH for $C_{18}H_{37}SH$ and $HOCC_{10}SH$ modified electrodes, respectively. The value of -29 mV/pH is close to the 2-electron and 1-proton process ($MB^+ + 2e^- + H^+ \rightleftharpoons LMB$) without the anion involvement. The reason of slight deviation from this process on the $HOCC_{10}SH$ modified electrode is not clear, but most likely due to the surface charges. On the other hand, in the solutions of pH lower than 5.2, the slopes (ca. -65 mV/pH) are similar in Figure 3a and 3b. Since the total charges were invariant with solution pH and surface modification in low pH, as shown in Figure 1, the 2-electron involvement during MB redox processes can be assumed. Therefore, the MB redox

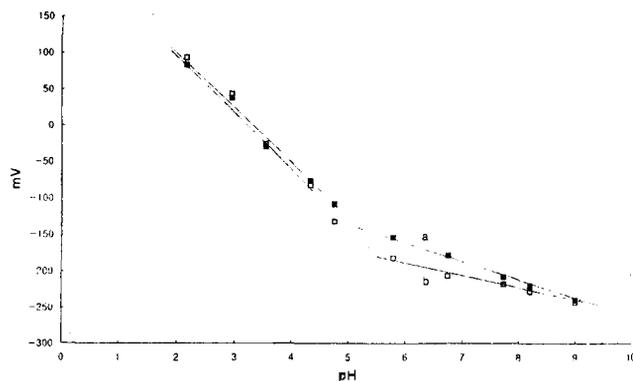


Figure 3. $E_{1/2}$ shifts of MB redox vs. solution pH (a) $C_{18}H_{37}SH$ and (b) $HOCC_{10}SH$ modified electrodes.

mechanism in low pH solutions can be considered as 2-electron, 2-proton, and 1-anion process ($MB^+ + 2e^- + 2H^+ + X^- \rightleftharpoons LMBX$). This mechanism corresponds to the result of Figure 1a, in which i_{pa} and i_{pc} were greatly different, since the diffusion coefficient of MB will differ from that of LMBX. These results seem to indicate a variation of MB reduction products with the solution pH. While a mixed valence salt of LMB and LMB^+ is a major reduction product of MB in low pH solutions, only LMB is involved during MB reduction in high pH solutions.

We investigated the electrochemical behaviors of MB in phosphate buffer solutions of various pH. Different voltammetric behaviors were revealed with solution pH and surface charges of SAM. In low pH solutions, the MB redox process was found to be $MB^+ + 2e^- + 2H^+ + X^- \rightleftharpoons LMBX$. For the $C_{18}H_{37}SH$ modified electrode in high pH solutions, no anion is involved in the MB redox process, leading to $MB^+ + 2e^- + H^+ \rightleftharpoons LMB$. The $HOCC_{10}SH$ modified electrode, on the other hand, does not strictly follow the 2-electron and 1-proton mechanism in high pH due to surface charges.

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