

Thin-layer Spectroelectrochemistry of 3, 3', 5, 5' -Tetramethylbenzidine on Pt Minigrid Optically Transparent Electrode

Kui JIAO^{1*}, Tao YANG², Zeng Jian WANG¹

¹College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042

²College of Chemistry and Chemical Engineering, Ocean University of China, Qingdao 266003

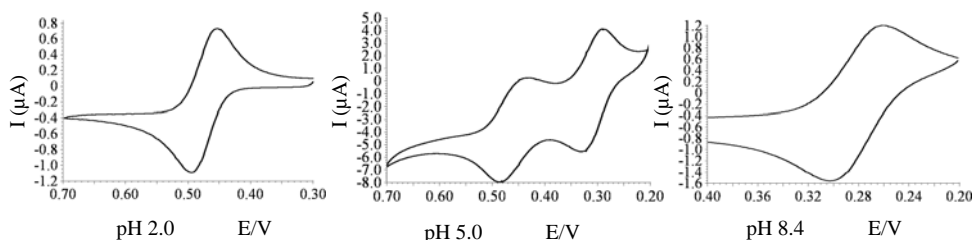
Abstract: The electrooxidation behavior of 3,3',5,5'-tetramethylbenzidine(TMB) was investigated using a platinum minigrid optically transparent thin-layer spectroelectrochemical cell. TMB underwent one two-electron electrooxidation process to yield quinonediimine in the pH range from 2.0 to < 4.0, and two consecutive one-electron electrooxidation processes, gave the mediate product free radical of TMB first, then gave the oxidation product quinonediimine in the pH range from 4.0 to < 7.0. In the pH range from 7.0 to 10.0, the electrooxidation of TMB was also one two-electron electrooxidation process to yield an azo compound. The formal potential $E^{0'}$ and the electron transfer number of the electrooxidation of TMB at pH 2.0 and pH 8.4 were determined by spectroelectrochemical techniques.

Keywords: Thin-layer spectroelectrochemistry, 3,3',5,5'-tetramethylbenzidine, platinum minigrid optically transparent electrode, electrooxidation.

3,3',5,5'-Tetramethylbenzidine(TMB)-H₂O₂-horseradish peroxidase(HRP) voltammetric enzyme linked immunoassay system¹ has very high sensitivity, low detection limit and wide detection range for the determination of some plant viruses. However, the understanding of the mechanism of HRP catalyzed by H₂O₂ oxidizing TMB in this system is of significance. In the previous paper, we have reported the spectroelectrochemical study of TMB on SnO₂:F film optically transparent electrode in the B-R buffer solution in the pH range from 2.0 to 7.0². Due to the limitations of the SnO₂:F film optically transparent electrode, the electrooxidation behavior of TMB in pH > 7.0 buffer solution on optically transparent electrode had not been investigated yet. In this work, we applied platinum minigrid optically transparent electrode to study the electrooxidation behavior of TMB in the B-R buffer solution. The results illustrated that the electrooxidation processes of TMB in pH 2.0 ~ 7.0 B-R buffer solutions on platinum minigrid optically transparent electrode was similar to those on SnO₂:F film optically transparent electrode. Here, we also described the thin-layer spectroelectrochemistry of TMB in pH 7.0 to 10.0 B-R buffer solution on this electrode. This study may contribute to a better understanding of the enzyme-catalyzed mechanism of TMB-H₂O₂-HRP voltammetric enzyme-linked immunoassay system.

* E-mail: kjiao@qust.edu.cn

Figure 1 Cyclic voltammograms of 2.0×10^{-3} mol/L TMB at pH 2.0, pH 5.0, pH 8.4 in the B-R buffer solution



All spectra were recorded with a Cary 50 probe UV-Vis spectrophotometer (Varian, Australia). The potential-control and the voltammetry were performed with a CHI 832 electroanalyzer (CHI, China.), with a platinum minigrid optically transparent electrode as working electrode, an Ag/AgCl electrode as reference electrode and a platinum-wire as auxiliary electrode.

The cyclic voltammetric behavior of TMB in pH 2.0 ~ 10.0 range in the B-R buffer solution on platinum minigrid optically transparent electrode was studied. TMB has a pair of well-defined redox peaks in the pH range from 2.0 to < 4.0, two pairs of redox peaks in the pH range from 4.0 to < 7.0, also a pair of redox peaks in the pH range from 7.0 to 10.0. **Figure 1** shows the cyclic voltammograms of TMB at pH 2.0, pH 5.0, pH 8.4, respectively.

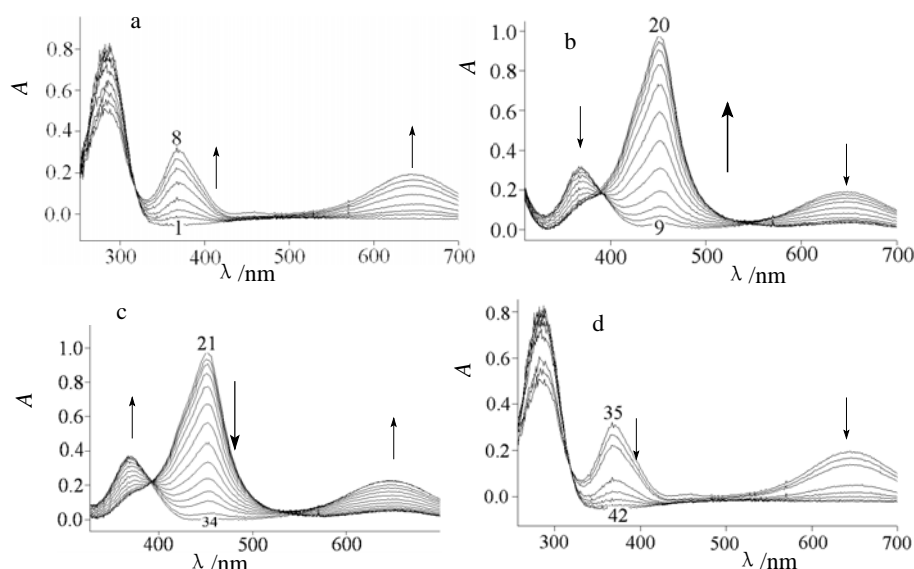
Thin-layer spectroelectrochemistry of TMB at pH 2.0 in the B-R buffer solution

TMB at pH 2.0 has two absorption peaks in the UV-Vis region at 204 nm and 253 nm respectively. From the thin-layer potential-controlled electrolysis absorptometry of TMB at pH 2.0 in the B-R buffer solution, one new absorption peak at 452 nm appeared, which was the absorbance of the oxidative product of TMB. From the absorbance expression of Nernst equation in thin-layer optically transparent spectroelectrochemical cell³, according to the equation $E = E^{0'} + (0.059/n) \lg[(A_2 - A_1)/(A_3 - A_2)]$ (25 °C), the formal potential $E^{0'}$ can be calculated as 0.519 V and the electron transfer number n is 2 from the intercept and the slope of the $E \sim \lg[(A_2 - A_1)/(A_3 - A_2)]$ straight line. TMB has a similar structure to *o*-tolidine. According to the investigation on the oxidation reaction of *o*-tolidine⁴ and the above experimental results, TMB in the acidic solution of pH < 4.0 underwent two-electron oxidation reaction to a quinonediimine product. The results are similar to those on SnO₂:F film optically transparent electrode².

Thin-layer spectroelectrochemistry of TMB at pH 5.0 in the B-R buffer solution

The thin-layer cyclic voltammetric absorption spectra of TMB at pH 5.0 in the B-R buffer solution are shown in **Figure 2**, which shows that the electrooxidation processes of TMB in pH 5.0 B-R buffer solution on platinum minigrid optically transparent electrode are similar to those on SnO₂: F film optically transparent electrode². According to the experimental results at pH 5.0, two consecutive one-electron oxidation

Figure 2 Thin-layer cyclic voltammetric absorption spectra of 2.0×10^{-3} mol/L TMB at pH 5.0 in the B-R buffer solution



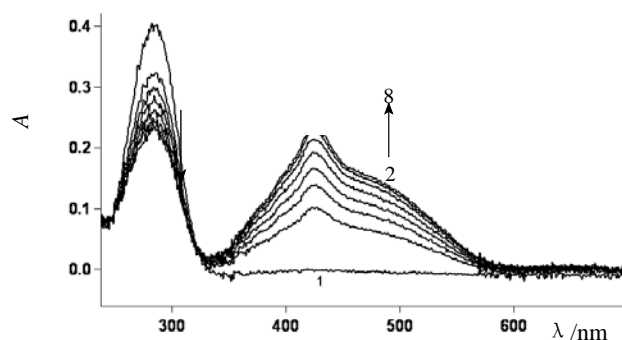
processes^{5,6} occurred, to form a free radical mediate product of TMB (**Figure 2 a**) first, then form the complete oxidation product quinonediimine (**Figure 2 b**). During inverse potential-scan, quinonediimine was electrochemically reduced in two steps, firstly to free radical of TMB (**Figure 2 c**), then to TMB (**Figure 2 d**).

Thin-layer spectroelectrochemistry of TMB at pH 8.4 in the B-R buffer solution

In the thin-layer cyclic voltammetric absorption experiment of TMB at pH 8.4, the potential-scanning range was from 0.20 - 0.70 V. A new absorption peak at 426 nm appeared when the potential was scanned positively to 0.23 V, indicating that TMB began to be oxidized. The new absorption peak became gradually bigger with the continuous positive scanning of the potential. The peak reached the maximum when the potential was scanned to 0.41 V. When the potential was scanned negatively to 0.45 V from 0.70 V the absorption peak at 426 nm began to decrease. The peak at 426 nm decreased continuously with the continuous negative scanning of the potential, and decreased to the nearly initial value when the potential was scanned to 0.20 V.

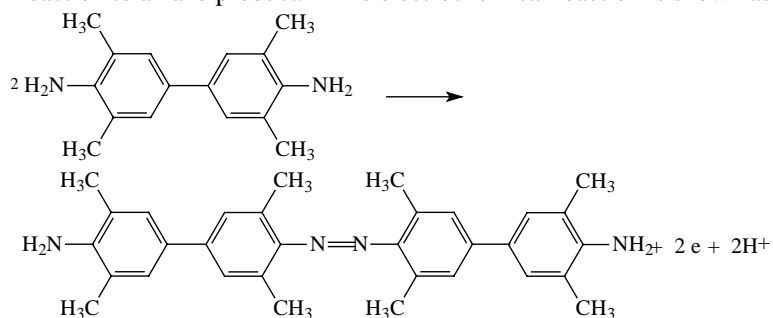
The thin-layer single-potential-step chronoabsorption spectra of TMB at pH 8.4 in B-R buffer solution were shown in **Figure 3**. After the potential was stepped from 0 V - 0.45 V one minute later the absorption spectrum in 250 nm ~ 700 nm was run every other 15 s. Curve 1 was the absorption spectrum at 0 V. Curves from 2 to 8 were the absorption spectra from 75 s to 165 s, respectively. An obvious absorption peak at 426 nm could be found in the absorption spectrum recorded at 75 s and the absorption peak at 283 nm decreased. The absorption peak at 426 nm reached the maximum at 165 s. It indicated TMB had been oxidized entirely.

Figure 3 Thin-layer single-potential-step chronoabsorption spectra of 2.0×10^{-3} mol/L TMB at pH 8.4 in B-R buffer solution



From the thin-layer potential-controlled electrolysis absorptometry of TMB at pH 8.4 in the B-R buffer solution, one new absorption peak at 426 nm appeared. According to the same method at pH 2.0, $E^{0'}$ was obtained to be 0.321 V and $n = 2$.

In view of the previous investigation¹ on the oxidation reaction of TMB and the above experimental results, TMB in the B-R solution of pH 8.4 underwent two-electron oxidation reaction to an azo product. The electrochemical reaction is shown as follows:



Acknowledgment

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