A Novel Electrochemical Method for Lentinan Determination Based on its Interaction with Medaylene Blue

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Abstract: *This contribution proposed a novel electrochemical approach for lentinan (LT) determination using medaylene blue (MB) as probe. The interaction conditions of LT with MB were carefully studied by cyclic voltammetry. Under the optimum conditions, the cyclic voltammetric curve of MB showed a pair of convertible oxidation and deoxidization peaks with the peak potentials of -0.157 V and -0.191 V, respectively. After LT was mixed to the MB solution, the peak potential of MB was negatively moved weakly meanwhile the oxidation peak current decreased corresponding to the addition of LT, and consequently a novel method for LT determination was established. The liner equation between the decrease of current (Δip) and LT concentration was obtained as $\Delta ip(\mu A)=1.00C(\mu g/mL)+5.50$ (n=10 , r=0.996). The linearity range and detectability (3σ) were 0.8~8.0 μg/mL and 0.45 μg/mL. The effects of coexisting materials on the new method were carefully studied and the detection results for LT injection sample were satisfactory. The interaction mechanism was also discussed.*

Keywords: *Lentinan, Medaylene Blue, Voltammetry, Interaction, Determination*

1. INTRODUCTION

Lentinan is an effective component for medical purpose with the structure of β (1, 3)-D-glucan[1]. It showed some active functions such as improving body immunity, enhancing the phagocytic activity of macrophages, promoting the formation of antibodies, anti-tumor metastasis and so on[2,3]. The combined use of lentinan and chemotherapy as an adjuvant treatment showed very good therapeutic effects to acute and chronic leukemia, gastric cancer, lung cancer and other tumors[4]. At present, the methods for quantitative and structure analysis of polysaccharide include high performance liquid chromatography (HPLC), capillary electrophoresis (CE), nuclear magnetic resonance (NMR) and mass spectrometry (MS), ion selective electrode (ISE), spectrophotometry and biological method[5-7].

Methylene blue (MB) is a kind of basic biological stain, which is often used as the antidote medicine for ammonia, nitrite, sulfonyl cyanide and carbon monoxide poisoning in clinical diagnosis. In the life sciences, MB is often used as an electrochemical probe for the research of biological molecular[8]. Due to the advantages of high sensitivity, wide linear range and simple equipment, electrochemical method has been widely used to study the large biological molecules[9-12]. In recent years, with the continuous development of electrochemical analysis technology and the extension of research scope, the studies on drug analysis and quality control have aroused widespread concern[13,14].

In this paper, cyclic voltammetry was applied to investigate the electrochemical behavior of MB and its interaction mechanism with LT. And a novel electrochemical method for LT detection was established using MB as probe.

2. METHODS

2.1. Apparatus

Electrochemical measurements were all carried out by an electrochemical workstation (EC 550, Wuhan Gaossunion Technology Co., Ltd., China). A traditional three-electrode system was used in the measurements, which consists of a glassy carbon electrode (GCE) as the working electrode, Pt as the auxiliary electrode, and a saturated calomel electrode (SCE) as the reference electrode. pH values were all measured by pHS-25 meter (Shanghai LeiCi Instrumentation Co., Ltd., China).

2.2. Reagents

Lentinan reagent (1.00 mg/mL, Zhejiang Fangge Pharmaceutical Co. Let., China), medaylene blue (Tianjin Kemiou Chemical Reagent Co., Ltd., China) were used directly. All other reagents were of analytical reagent grade. The working solutions were prepared by diluting appropriate mass chemicals with deionized water. 0.2 mol/L phosphate buffer saline (PBS) with different pH values was used to control acidity. Deionized water were used throughout this study.

2.3. Electrochemical Measurements

0.60 mL 1.0×10^{-4} mol/L MB, 3.0 mL pH 6.0 PBS buffer and a moderate amount of LT (or sample) were added into a 10 mL cuvette in order. Then dilute the solution to the scale with water and mingled completely. 20 minutes later, the three-electrode system was immersed into the solution. The voltametric signals were scanned from -0.6V to 0.1V at 50 mV/s. The oxidation peak currents of MB and MB–LT complex appeared at -0.157 V (vs. SCE) were recorded as ip_0 and ip. The difference between ip_0 and ip was denoted as Δip, which was further used for LT determination. All of the measurements were conducted at room temperature.

3. RESULTS AND DISCUSSION

3.1. Electrochemical Behaviors of MB at GCE

The cyclic voltammograms of MB at bare GCE with 0.20 mol/L pH 6.0 PBS buffer as supporting electrolyte were shown in Fig.1. It can be seen that a pair of asymmetric redox peaks were got at -0.157 and -0.191V, respectively, and ΔE_p was calculated as 34 mV. The research was consistent with Zutic V[15], which showed that the electrode reaction of MB was a two electron transfer quasi-reversible process.
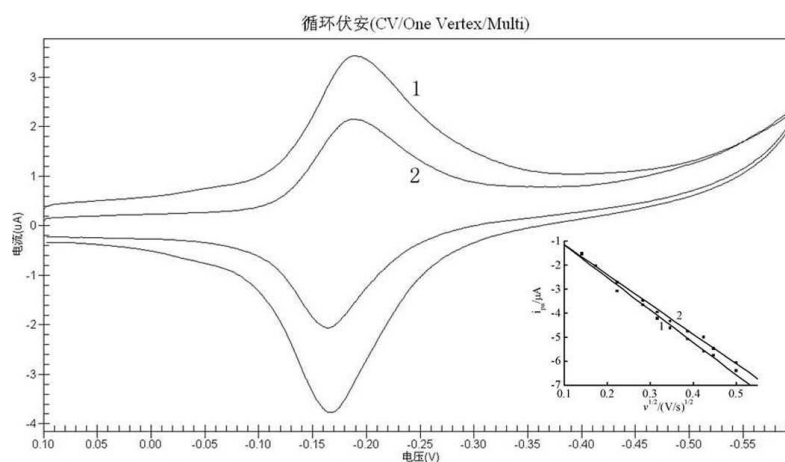

Fig1. *Cyclic voltammograms of MB (curce 1, 6.0×10^{-5} mol/L MB+3.0 mL pH 6.0 PBS) and MB-LT(curve 2, 6.0×10^{-5} mol/L MB+3.0 mL pH 6.0 PBS+30 mg/L LT) ; Insert is the relationship of ip with the square root of scan rate ($v^{1/2}$).*

The acidity effect on the electrochemical response of MB was also studied. The results showed that the oxidation peak potentials (E_{pa}) moved negatively in the pH range from 3.0 to 7.0, and a liner regression equation of $E_{pa}=0.1672-0.051$pH was got. According to the equation of $E_{pa}=E_0-(0.059m/n)$pH[16], suggesting that two protons and two electron were related to the electrochemical reaction.

The scan rate (v) experiments displayed that the electrochemical response of MB changed regularly while the scan rate of both MB and MB-LT systems increased. The oxidation peak current (ip_a) values were in good linear relationship with $v^{1/2}$ (inset of Fig 1). The regression equations were obtained as $ip_a(\mu A)=-13.52v^{1/2}(v^{1/2}/s^{1/2})+0.197$ (r=0.997,curve 1) and $ip_a(\mu A)=-12.41v^{1/2}(v^{1/2}/s^{1/2})+0.097$ (r=0.998, curve 2) respectively for free MB and MB-LT complex, which suggested that the electrochemical processes of MB and MB-LT complex on the electrode surface were both controlled by diffusion[17].

According to the proposed method[18], which supposed that only a simple complex was formed between MB and LT with a model of LT-mMB. The binding coefficient (m) and the equilibrium value (β_s) of the interaction can be inferred as follows:

$$\text{LT} + m\text{MB} \rightarrow \text{LT} - m\text{MB} \tag{1}$$

β_s is deduced by equilibrium equation:

$$\beta_s = \frac{[\text{LT} - m\text{MB}]}{[\text{LT}][\text{MB}]^m} \tag{2}$$

as:

$$\Delta i_{max} = kC_{LT} \tag{3}$$

$$\Delta i = k[\text{LT} - m\text{MB}] \tag{4}$$

$$[\text{LT}] + [\text{LT} - m\text{MB}] = C_{LT} \tag{5}$$

Therefore:

$$\Delta i_{max} - \Delta i = k(C_{LT} - [\text{LT} - m\text{MB}]) = k[\text{LT}] \tag{6}$$

By Eqs. (2), (4) and (6), Eq(7) is got as:

$$\log(\Delta i / (\Delta i_{max} - \Delta i)) = \log\beta_s + m\log[\text{MB}] \tag{7}$$

Where Δi corresponds the difference of peak current between the LT-mMB complex and MB, and Δi_{max} is the maximum value of Δi. C_{LT}, [LT-mMB] and [LT] are the total, equilibrium and free concentrations of LT, respectively. Figure 2 showed the relationship between ip, Δip and C_{MB}. From equation (7), a linear regression equation between s and $\log[\text{MB}]$ was got as $\log(\Delta i / (\Delta i_{max} - \Delta i)) = 0.996\log[\text{MB}] + 4.389\,(n = 5, \gamma = 0.991)$. The values $m=1$ and $\beta_S = 2.5 \times 10^4$ were deduced from the intercept and the slope, which demonstrated that a steady 1:1 complex of LT–MB was produced under the optimized conditions.
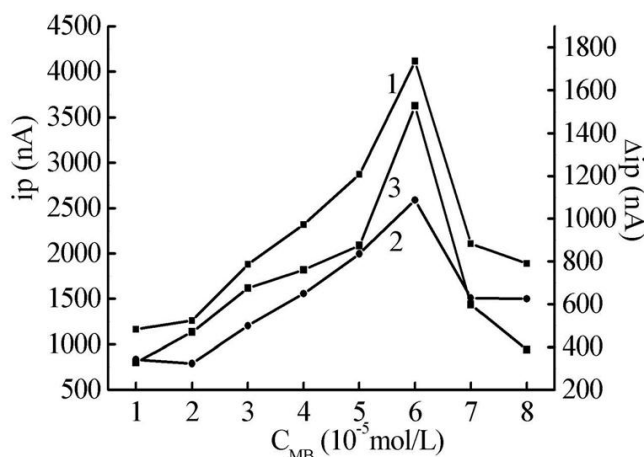

Fig2. *The relationship between ip and C_{MB} (1, 2), and Δip and C_{MB} (3).*

3.2. Optimal of General Procedure

The influences of acidity of buffer solution on the binding reaction of MG with LT was studied according to the Δip value over pH range from 2.0 to 8.0. The results indicated that Δip reached its maximum at the pH 6.0, therefore pH 6.0 was chosen for the following experiments.

The effect of MG concentration (C_{MG}) on Δip value was investigated with 10.0 mg/L LT. The results showed that Δip value reached the max when C_{MG} was 6.0×10^{-5} mol/L. So 6.0×10^{-5} mol/L MG was recommended in this paper.

The maximum value of Δip appeared at 20 min and keep stable for over 2 hours. Consequently, this reaction system gave enough time for routine determination. The effluence of reaction temperature on Δip value was tested at 15, 25, 30, 35, and 40 ºC. The results indicated that there were no obvious diversity among them, so room temperature was used throughout.

3.3. Calibration Curve and Detection Limit

To heighten the sensitivity, differential pulse voltammetry (DPV) technique was selected to establish

the quantitative method for LT. Under the optimal conditions, with MB concentration at 6.0×10^{-5} mol/L, a standard curve for LT determination was obtained between the Δip value and LT concentration. The linear regression equation was obtained as $\Delta ip(\mu A)=1.00C(\mu g/mL)+5.50$ (n=10, r=0.996) with the LT concentration range of 0.8~8.0 mg/L. The relative standard deviation (RSD) for 5.0 mg/L LT determination (n=11) calculated as 2.37 % and the detectability was 0.45 mg/L (3σ).

3.4. Effects of Coexisting Substances

The influences of coexisting substances such as amino acids, bovine albumin (BSA), glucose, metal ions etc. on the detection of 5.0 mg/L LT were tested. As shown in Table 1, most of the cations and amino acids had little influences on the determination of LT. But cyclodextrin (β-CD) showed obvious influence on the interaction, which may be caused by the absorption of it on the surface of electrode.

Table1. *Effect of coexisting substances on the determination of 5.0 mg/L LT*

Tcoexisting substances	concentration (mg/L)	Relative error (%)
L-Glutamic acid	0.5	1.94
L-Tyrosine	0.5	-1.78
L-Arginine	0.5	1.59
glucose	0.5	4.78
BSA	0.5	4.65
β-CD	0.5	3.42
Cu2+	0.5	2.16
Ca2+	0.5	2.05
Mn2+	0.5	-0.60
Sn2+	0.5	-3.85
Mg2+	0.5	-1.72
Zn2+	0.5	2.27

3.5. Sample Determination

LT sodium injection sample with specified amount as 1.00 mL: 1mg was determined by the novel established approach. Table 2 showed the results of the determination and recovery test, which indicated that the approach was suitable for the sample determination.

Table2. *LT injection sample determination results.*

Sample No	Average (n=5) (μg/mL)	RSD (%)	Specified (μg/mL)
20120701	1.04	3.50	1.00

4. CONCLUSION

The electrochemical behaviors of LT–MB complex on glassy carbon electrode was investigated and the interaction mechanism of them was also studied by clcylic voltammertric method. And then a novel determination method for lentinan determination was established on the basis of the decrease of the peak current of MB before and after the interaction with lentinan under the optimal conditions. The values $m=1$ and $\beta_S=2.5\times10^4$ of the LT–MB complex were calculated from the voltammetric data. The novel method can be used for lentinan sample determination with satisfactory results.

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