Construction of an Electrochemical Molecularly Imprinted Sensor Based on L-carnitine Doped Polypyrrole for Vibrio Parahaemolyticus Detection

Jie Liu
Shanghai Ocean University, Shanghai 201306, China
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Abstract: This study addresses the pressing need for rapid detection of pathogenic microorganisms by designing and fabricating an L-carnitine-doped polypyrrole (L-CN/PPy) electrochemical molecularly imprinted sensor, with Vibrio parahaemolyticus as the model target. Employing L-carnitine as a dual-functional dopant and auxiliary recognition element in the electropolymerization process, the sensor not only enhances the conductive properties of polypyrrole but also significantly improves its specific recognition capability towards the target bacteria. The sensor preparation encompasses the electrochemical polymerization of L-CN/PPy composite material, immobilization and elution of template bacteria, followed by bacterial recognition and detection steps. Through systematic optimization of L-CN concentration, polymerization conditions, eluent composition, elution time, and operational parameters, the sensor's performance is maximized. Experimental results confirm the sensor's efficacy in rapidly completing template bacteria elution within just 30 minutes and achieving efficient recognition of Vibrio parahaemolyticus within 20 minutes, demonstrating high sensitivity and selectivity. Moreover, the straightforward and rapid construction method of the sensor introduces a novel technological strategy for practical microbial detection applications, highlighting extensive application prospects.
***Keywords*:** L-carnitine; polypyrrole (PPy); Vibrio parahaemolyticus; molecularly imprinted polymer (MIP)

1. Introduction

L-carnitine, a zwitterionic compound, boasts remarkable biological stability, existing in its ester form under neutral conditions to enhance environmental robustness [1]. Owing to its positively charged quaternary ammonium group, L-carnitine remains unaffected by ambient pH fluctuations. Conducting polymers such as polypyrrole (PPy) have found extensive application in biosensors, where they not only augment sensor conductivity but also facilitate bacterial adsorption through electrostatic interactions between their N-H groups and negatively charged bacterial surfaces. By doping with L-carnitine, this study aims to not only modulate the PPy polymerization process but also enhance bacterial recognition capabilities, thereby boosting the performance of the biosensor.

2. Materials and Methods

2.1 Materials and Reagents

The strain Vibrio parahaemolyticus (VP) utilized in this experiment was obtained from the China Microbial Culture Collection. L-carnitine was purchased from Aladdin, while polypyrrole was sourced from Sinopharm Chemical Reagent Co., Ltd. All other reagents were of analytical grade to ensure accuracy and reliability of the experiments.

2.2 Bacterial Cultivation

The strains were stored at -80°C in 10% (w/v) glycerol broth in cryovials. Vibrio parahaemolyticus was cultivated in 3% sodium chloride alkaline peptone water at 37°C with agitation at 250 rpm for approximately 18 hours [2]. The resulting bacterial suspension was fixed with formaldehyde, followed by centrifugation at 6000 rpm for 5 minutes to discard the supernatant. An equal volume of 1×PBS (pH 7.4) was then added to the tube, and this process was repeated three times to eliminate impurities from the culture medium. Colony counting on nutrient agar plates was performed using standard plate count methodology to ascertain bacterial concentration, establishing a final purified bacterial concentration of 10^8 CFU·mL^-1.

2.3 Preparation of L-CN/PPy/GCE

The sensor fabrication process began with a graphite carbon electrode (GCE) being meticulously polished using 0.03-0.05 μm alumina particles. This was followed by sequential ultrasonic cleaning in ultrapure water, absolute ethanol, and ultrapure water for two minutes each. The electrode then underwent cyclic voltammetry in a specific electrolyte solution to

assess its electrochemical properties, after which it was rinsed, dried, and set aside. A polymer mixture containing 0.05 M polypyrrole (PPy) and 300 μM L-carnitine (LC) in a 0.1 M potassium chloride solution was prepared and the pre-treated GCE was immersed within it. An electrochemical polymerization took place under set conditions (-0.4 V to 0.7 V potential, 50 mV·s^-1 scan rate, 20 cycles), forming the LC/PPy/GCE sensor layer. As a control measure, an identical process was repeated without LC to produce PPy/GCE.

2.4 Optimization of LPBIP Sensor Fabrication Conditions

To attain optimal detection performance for Vibrio parahaemolyticus, the fabrication conditions of the molecularly imprinted biosensor were optimized, including the concentration of L-CN, the number of polymerization cycles, the type of eluent, template removal time, and the rotation speed during elution. Each optimization step controlled for variables, ensuring only one variable was altered per test, with triplicates run to minimize error. For optimizing L-CN concentration and polymerization cycles, ΔR/R ratio changes were monitored, with larger values indicating more favorable conditions. Conversely, for eluent type, elution time, and rotation speed, smaller Rct values signified better conditions.

3. Results and Analysis

3.1 Characterization of LC/PPy/VP

Scanning electron microscopy (SEM) confirmed the successful synthesis of LC/PPy and LC/PPy/VP coatings on the GCE, exhibiting a smooth, uniform surface with embedded bacteria, verifying bacterial immobilization.

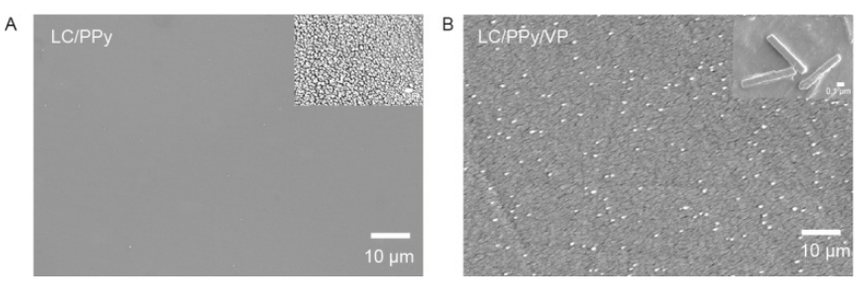

Figure 1. SEM characterization of the electrode following different modifications. (A) LC/PPy/GCE and (B) LC/PPy/VP/GCE.**

Electrochemical assessments validated the films' functionality. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) tests conducted in a 0.1 M KCl solution with 1 mM K3[Fe(CN)6]/K4[Fe(CN)6] revealed significant insights. Comparatively, the LC/PPy/VP-modified electrode displayed reduced peak currents and areas versus LC/PPy, indicative of template bacteria incorporation.

Specifically, the charge transfer resistance (Rct) escalated from 480 Ω for LC/PPy to approximately 50 kΩ upon VP inclusion, evidencing a substantial impedance rise due to decreased film conductivity. This pronounced impedance increment attests to the successful templating of VP within the molecularly imprinted polymer, confirming the sensor's tailored recognition efficacy.

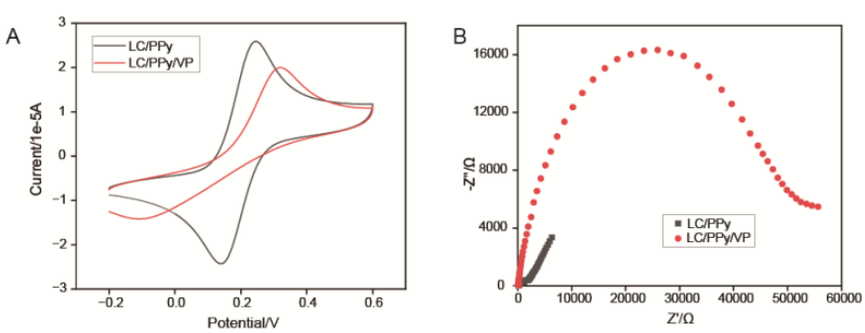

Figure 2. Electrochemical characterization of electrodes after different modifications. (A) CV plots and (B) EIS spectra.

3.2 Construction of the LPBIP Molecularly Imprinted Sensor

The SEM was employed to characterize the electrode interface of the fabricated LPBIP molecularly imprinted sensor

before and after bacterial recognition. As shown in Figure 3, no template bacteria VP are observable on the surface of the GCE after elution. However, following incubation for bacterial recognition (with 250 μL of bacterial suspension for 30 minutes), a multitude of VP bacteria are visibly present on the GCE surface, affirming the successful construction of the LPBIP imprinted sensor.

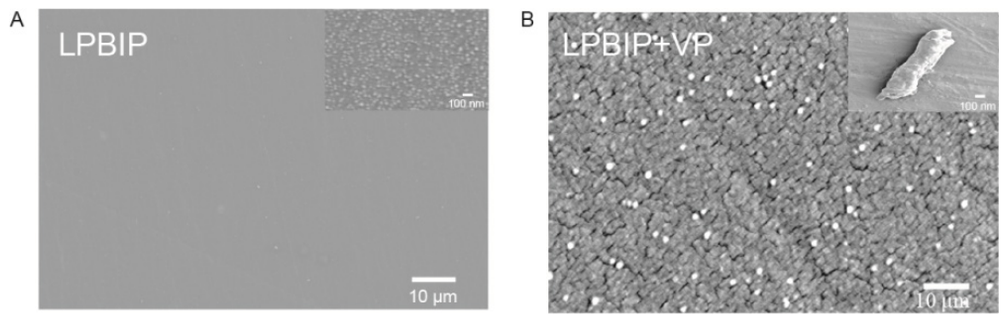

Figure 3. SEM characterization of the LPBIP sensor before and after bacterial recognition. (A) Before recognition and (B) after recognition.**

3.3 Optimization of LPBIP Sensor Conditions

To attain the optimal performance of the LPBIP sensor, conditions such as the concentration of L-CN, the number of polymerization cycles and recognition time during elution were systematically optimized. As illustrated in Figure 4A, the ΔR/R ratio increases with the rise in L-CN concentration from 100 μM to 300 μM, but it decreases when the L-CN concentration further escalates from 300 μM to 500 μM. This decline is speculated to result from the competition between the bipolar ion L-carnitine, which carries a negative charge, and the template bacteria, suggesting that excessively high concentrations of L-carnitine may inversely affect the sensor's efficiency. Consequently, 300 μM is identified as the optimal doping concentration of L-carnitine for maximizing sensor performance.

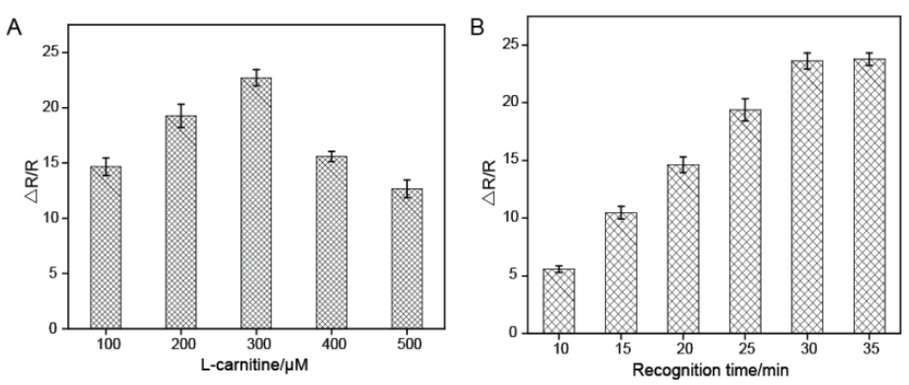

Figure 4. Optimization parameters for LPBIP sensor fabrication. (A) L-CN concentration; (B) Recognition time.

Recognition time of the fabricated sensor is vital for performance evaluation; here, changes in the ΔR/R response serve as a reference. After elution, the LPBIP electrode was incubated in a template solution for 20 minutes (Figure 4B), a significantly shorter time than pure PPy sensors. This expedited recognition is potentially due to L-carnitine's zwitterionic nature, carrying both carboxyl and hydroxyl groups with a positive charge, facilitating faster bacterial recognition through electrostatic and group interaction, thereby reducing recognition time.

4. Conclusion

In this chapter, we have developed a biofouling-resistant molecularly imprinted biosensor based on L-carnitine and polypyrrole for the detection of Vibrio parahaemolyticus. Fabricated through an electrochemical one-step polymerization approach, the sensor leverages L-carnitine's abundance of carboxyl and hydroxyl groups that facilitate affinity interactions with bacterial surfaces, advantageous for both elution and recognition processes. Meanwhile, polypyrrole, as a conductive polymer, undergoes structural modulation during polymerization due to L-carnitine doping. Consequently, our LPBIP sensor requires only 30 minutes for efficient template elution. These preliminary findings lay a crucial foundation for the sensor's

application in real sample testing, highlighting promising prospects in practical usage.

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