

An Amperometric Sensor for Sunset Yellow FCF Detection Based on Molecularly Imprinted Polypyrrole

Jinfeng Xu, Yi Zhang, Hao Zhou, Mingwei Wang, Peidong Xu, Juankun Zhang*

Key Laboratory of Industrial Fermentation Microbiology, Ministry of Education, College of Biotechnology, Tianjin Key Lab of Industrial Microbiology, College of Biotechnology, Tianjin University of Science and Technology, Tianjin 300457, People's Republic of China
Email: *zhangjk@tust.edu.cn

Received 2012

ABSTRACT

An electrochemical method for fast detecting the concentration of sunset yellow FCF in wine samples was developed in this study. The sensor based on imprinted films which fabricated by electropolymerization of pyrrole on a glassy carbon electrode in the presence of sunset yellow FCF as the template. Comparing to the polypyrrole non-imprinted modified (NIP) electrode, the polypyrrole molecularly imprinted polymer (MIP) electrode improved the electrochemical performance of the sensor significantly. The peak current at about 0.26 V was linear with the concentration of sunset yellow FCF from 0.4 to 2 μM and from 2 to 8 μM . It can be used for more than 10 times to maintain a stable response result. The sensor had the good selectivity on sunset yellow FCF, amaranth and tartrazine, which the selection factors were 1.00, 0.80 and 0.85, respectively.

Keywords: Electrochemical Sensor; Polypyrrole; Sunset Yellow FCF; Molecularly Imprinted Polymers

1. Introduction

Sunset yellow FCF is a synthetic azo dye, which has been widely added into foods and drinks for its high stability to light, oxygen and pH and low production cost [1]. However, studies have shown that sunset yellow FCF can cause many adverse health effects such as high genotoxicity and cytostaticity [2]. In China, sunset yellow FCF is permitted and the value of acceptable daily intake (ADI) is between 0 and 2.5 mg·kg⁻¹ according to the hygienic standards for uses of food additive (GB 2760-1996). Until now, several methods have been applied for the detection of sunset yellow FCF, such as spectrophotometry [3], HPLC [4,5] and LC/MS [6]. However, the detecting process of these methods was too complicated to proceed, can't meet the daily food scene fast detection requirements. Recently, numerous electrochemistry methods are applied to sunset yellow FCF detection via modified working electrode such as multi-walled carbon nanotubes film-modified electrode [1,7] and Platinum wire-coated electrode [8], which have high sensitivity but lower selectivity. A sensor based on MIPs as the merits of high sensitivity and label free evoked much attention. Nevertheless, a sensor based on MIPs for sunset yellow FCF detection has not been reported. Herein, an amperometric sensor for sunset yellow FCF measuring was developed in this paper.

2. Experimental

2.1. Chemicals and Apparatus

Pyrrole (98%), sunset yellow FCF, amaranth, tartrazine were purchased from Sangon Biotech (Shanghai) Co.,Ltd. All reagents were analytical grade and were used as received, without further purification. The wine was bought on the market.

*Corresponding author.

All experiments were performed through an electrochemical analyzer LK2005A (Tianjin, China) connecting to a computer and carrying out with a conventional three-electrode cell with glassy carbon (GC) electrode ($\phi = 4\text{mm}$) which was covered by non-MIP or MIP polypyrrole film as the working electrode, while an Ag/AgCl/saturated KCl electrode and a platinum wire served as the reference electrode and the auxiliary electrode respectively.

2.2. Preparation of Electrodes Based on MIP and NIP Films

A GC electrode was polished carefully with aqueous alumina slurry (0.5 μm) and repeatedly rinsed with distilled water. The electrolyte solution was prepared with PBS(pH7.0) containing 0.02 M sunset yellow FCF, 0.05 M pyrrole and 0.1 M KCl. The electropolymerization was performed through cyclic voltammetry of +0.4 V to -1.0 V, with a scan rate of 50 mV $\cdot\text{s}^{-1}$ and 10 consecutive scans. Then, the embedded sunset yellow FCF molecules were removed from the PPy film by overoxidized process at +1.3 V in the 0.1 M NaOH solution for 600 s. And, the molecularly imprinted film electrode was activated to obtained MIP film electrodes in 0.2 M PBS (pH6.0) containing 0.1 M KCl by cyclic potential scan. As a control, the non-imprinted PPy-GC electrode was prepared and treated in exactly the same way except that the template molecules were omitted from the electropolymerization stage.

2.3. Electroanalytical Measurements

There are twice current measurements before and after incubation of sunset yellow FCF, which were performed using square wave voltammetry (SWV) in 0.2 M PBS(pH7.0) solution con-

taining 4mM $\text{Fe}(\text{CN})_6^{3-/4-}$, between 0.6V and 0.0V. After the first current measurement, the imprinted or non-imprinted PPy-GC electrode was dipped into a 0.2 M PBS(pH6.0) containing prepared concentration of sunset yellow FCF to adsorb for 10 min, then was washed with distilled water carefully to remove the possible adsorptive substance on the electrode surface, and then was transferred to the first detection electrochemical cell using the same measurements (SWV). All measurements were performed at room temperature.

2.4. Analysis of Real Samples

50.00 mL samples of wine were boiled to eliminate CO_2 and ethanol, adjusted pH to 6.0 using 2.5 M NaOH after cooling, and then the wine was transferred to a 50mL flask to volume with distilled water. Standard curve regression method was used to determine the content of the sunset yellow FCF. The treated wine samples were then spiked with appropriate amount of sunset yellow FCF for recovery experiments.

2.5. The Structure of Three Analogue

To investigate the selectivity of the MIP and NIP electrode in this research, three same concentration of analogue were detected to evaluate the detection response. The structure of sunset yellow FCF, amaranth and tartrazine were shown in the **Figure 1**.

3. Results and Discussion

3.1. Electropolymerization of MIP and NIP Polypyrrole

The thickness of polymer film can easily be adjusted by controlling the scan rates and the number of cycles during electropolymerization process [9]. We developed series of experiments in which electrodes were fabricated with different cycles of 5, 10 and 15 to optimize the number of CV cycles to use to form the 'sensing' layer of the electrode. The results shown that the highest current response of the MIP electrodes to sunset yellow FCF was obtained by applying 10 cycles. **Figure 2** showed that one cathodic peak was observed at -0.77 V at the first negative scan, and the current decreased significantly with the increase of cyclic scan times and were stable after 8-cycle scans. The other cathodic peak was observed at -0.07 V. After the first negative scan and was constant. The reductive peak appeared at 0.06 V and peak current was constant also. As shown in the inset of **Figure 2**, there was not a cathodic peak at -0.77 V, which could correspond to the reduction of sunset yellow FCF molecules. The results indicated that the formation of PPy films on the surface of GC electrode hindered the

monomer further accessing to the GC electrode surface, which was the same as Xie's [10] study and the sunset yellow FCF and the PPy backbone could unified together stably. It was because that the positive characteristic of the Ppy backbone and the negative charge of sunset yellow FCF molecules were strongly absorbed onto the electrode surface through hydrogen bonding and electrostatic interactions.

3.2. Electrochemical Behavior of Sunset Yellow FCF on the Imprinted PPy-GC Electrode

The electrochemical behavior of sunset yellow FCF was investigated by MIP and NIP electrode. The catalytic effect of the MIP electrode to different concentrations of sunset yellow FCF were investigated in pH 7.0 PBS by SWV. Sunset yellow FCF gave an oxidation peak response at about 0.250V at the NIP electrode, while an anodic peak appeared at about 0.275V with the use of MIP electrode and the peak current decreased with the increased concentration of sunset yellow FCF. The enhanced peak current response and a shift in the oxidation potential of sunset yellow FCF at about 0.025V in the anodic direction were the clear evidences of the catalytic effect of the MIP electrode towards the oxidation of sunset yellow FCF.

The calibration curve for the SWV peak current versus sunset yellow FCF was observed at MIP electrode under optimum experimental conditions. There were two linear regions in the curve which were 0.002mM to 0.008mM with the correlation coefficient of 0.987 and 0.0004 mM to 0.002mM with the correlation coefficient of 0.993.

3.3. Optimization of Experimental Parameters

1) Concentration Selection of Pyrrole and Sunset Yellow FCF

The concentrations of monomer (pyrrole) and template (sunset yellow FCF) during polymerization determine the analytical behavior of the sensor. The monomer concentration should be proportional to the thickness of the deposit and amount of imprinted molecule (template) in the polymeric matrix [9]. So it is necessary to select the proper concentration of sunset yellow FCF and pyrrole to both make the response of sunset yellow FCF highest and obtain not a signal for pyrrole. The electrochemical performances of several imprinted PPy-GC electrodes which were produced in different solutions of sunset yellow FCF and pyrrole, were investigated in 0.2M PBS pH 7.0 containing 1.7×10^{-5} M sunset yellow FCF at the MIP electrode. The response of the MIP electrode to sunset yellow FCF samples were shown in **Table 1**. The highest current response was obtained at 50mM pyrrole and 20mM sunset yellow FCF (serial number1), which were chosen as the optimum concentrations.

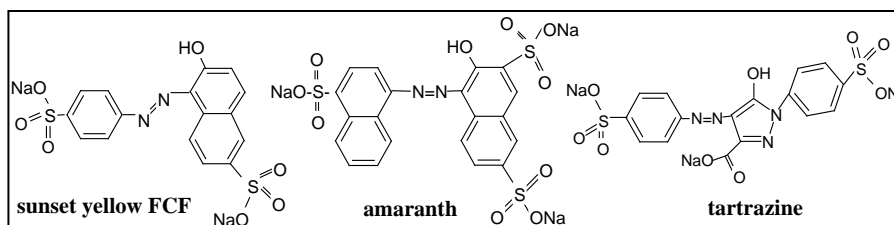


Figure 1. The structure of sunset yellow FCF, amaranth and tartrazine.

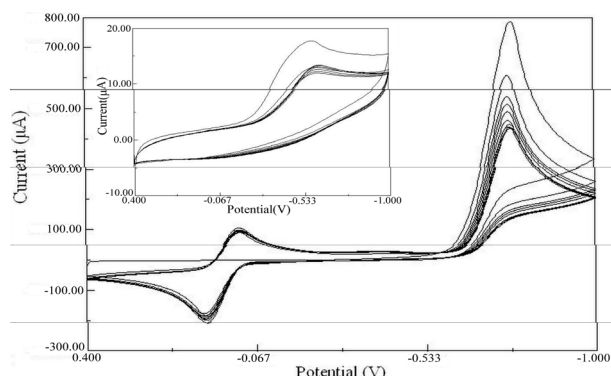


Figure 2. Cyclic voltammograms of the electropolymerization for imprinted PPy-GC electrode. Inset was cyclic voltammograms of the electropolymerization process for nonimprinted PPy-GC electrode. Scan rate: $50 \text{ mV} \cdot \text{s}^{-1}$, sweep cycle: 10.

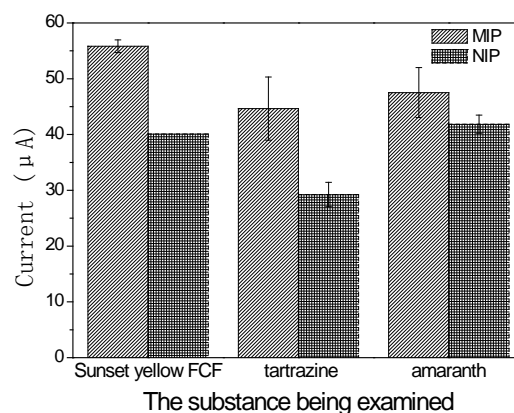


Figure 3. Selectivity of the imprinted and non-imprinted PPy-GC electrode for sunset yellow FCF, amaranth and tartrazine.

Table 1. The Response of Different MIP Electrodes to Sunset Yellow FCF Samples.

Serial Number	Concentration of pyrrole(mM)	Concentration of sunset yellow FCF (mM)	Wave current value before enrichment (μA) ^a	Wave current value after enrichment (μA) ^a	Wave current value of sunset yellow FCF in the sample ^a
1	100	10	120.875 ± 10.605	70.423 ± 18.592	50.451 ± 7.993
2	50	10	128.364 ± 9.498	66.363 ± 6.388	62.000 ± 5.820
3	30	10	128.052 ± 1.406	75.915 ± 6.685	49.180 ± 4.671
4	20	10	117.126 ± 1.641	52.256 ± 4.341	64.870 ± 2.700
5	10	10	144.131 ± 3.868	76.057 ± 2.066	68.074 ± 1.802
6	10	20	147.133 ± 6.405	80.081 ± 8.53	67.052 ± 5.600
7	30	20	147.979 ± 7.627	84.065 ± 10.680	63.913 ± 3.953
8	50	20	142.711 ± 3.844	66.420 ± 7.917	80.679 ± 0.702

a . Average of three measurements \pm standard deviation.

2) Selection of Incubation Time

To select the proper incubation time, the prepared Ppy-GC electrode was incubated in the 0.2 M PBS containing $1.7 \times 10^{-5} \text{ M}$ sunset yellow FCF for different time of 0~20 min. The results shown that the square wave voltammetric responses were decreased rapidly with the time increasing in the first 4 min, then maintained constantly after 8 min. Thus, we chosed 10 min to be the the optimum incubation time for the determination of sunset yellow FCF in all experiments reported.

3) Selectivity of the Imprinted PPy-GC Electrode

The prepared imprinted and non-imprinted PPy-GC electrode were incubated into three structure analogues solution of 0.005 mM to study the selectivity of the sensor. The results were shown in **Figure 3**, indicated that the imprinted Ppy-GC electrode had higher recognition selectivity to sunset yellow FCF than to amaranth and tartrazine on the same conditions. The choose factors of the sensor for sunset yellow FCF, amaranth and tartrazine were 1.00、0.80 and 0.85 respectively. The results also indicated that the MIP PPy-GC electrode had a better detection performance than non-MIP PPy-GC electrode. It was because that the imprinted PPy films for the formation of delicate imprinted sites and the stabilization of imprinted sites which can significantly increases selectivity of electrochemical biosensors through their size, shape and functional group dis-

tribution [14]. Therefore, the imprinted Ppy-GC which we had prepared could apply to the detection of sunset yellow FCF in wine sample.

4) Analysis of Commercial Samples and Recovery Experiment

Through the research methods above, the content of sunset yellow FCF in the wine sample was determine. It was found that there was $8.81 \times 10^{-2} \text{ mM}$ sunset yellow FCF in treated wine sample, the recovery was 24.4% which was very low. The reason might be that the polypyrrole films had the positive charge after activation treatment to absorb charged material in the sample which competed the binding sites of sunset yellow FCF. So when detecting the content of sunset yellow FCF, it was needed to do some special treatments to the wine sample to shield the interference of electronic material.

4. Conclusion

In this work, the molecularly imprinted PPy films modified glassy carbon electrode was prepared by electrochemical polymerization of pyrrole with the cyclic voltammetry in the presence of template of sunset yellow FCF molecules. The results demonstrated that the electrochemical sensor could significantly improve the sensitivity and selectivity of sunset yellow FCF

analysis. Therefore, the electrochemical sensor could be potentially exploited for detecting the residual analysis of sunset yellow FCF in the wine samples.

5. Acknowledgements

The authors thank the Ministry of Science and Technology of the People's Republic of China (2009GJA10047) to support the work.

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