

## Determination of Vanillin in Infant Formula Using Poly Valine Modified Electrode

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A poly valine modified electrode was prepared by cyclic voltammetry. The electrochemical behavior of vanillin at the modified electrode was studied in detail. A new method for measuring vanillin was established. It was found that in pH 7.0 phosphate buffer solution, the oxidation peak current is linearly proportional to its concentration of vanillin in a range of  $9.60 \times 10^{-8} \sim 6.61 \times 10^{-5} \text{ mol L}^{-1}$ , with a limit of detection of  $1.00 \times 10^{-8} \text{ mol L}^{-1}$ . This method is simple, highly sensitive and shows good reproducibility. The method has been demonstrated in the determination of vanillin in infant formula samples.

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**Keywords:** vanillin, infant formula, valine, electrochemical determination

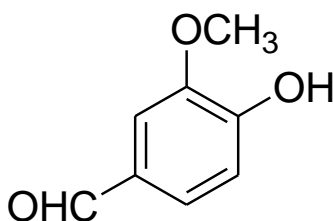
### 1. INTRODUCTION

Vanillin is 3- methoxy -4- hydroxy benzaldehyde. Chemical structure of vanillin is shown in figure 1. It is the *world's biggest* product of *synthetic spices*, which is widely used in producing confectionery, beverage and medicine as fragrance agent and flavoring agent [1]. However, excessive ingestion of vanillin can trigger allergic reactions [2], migraine headaches [3] and it can also affect the function of liver and kidney [4]. In china, according to the “national food safety standards for use of food additives” provisions of GB2760-2011 requirements, all covered by the use of 0 to 6 months of infant formula foods may not add any edible spices. Because the infant body organs of immature, metabolic detoxification ability is rather poor, eat food additives food will increase their daily metabolic burden [5]. Hence, it is necessary to detect and control the content of vanillin.

At present, a variety of techniques have been applied to the analysis of vanillin, such as high performance liquid chromatography (HPLC) [6, 7], gas chromatography (GC) [8], capillary electrophoresis (CE) [9]. Moreover, vanillin is an electro-active compound and electrochemical

technique could be used for the determination of vanillin. Thus, procedures based on electroanalytical methods for the detection of vanillin in food samples have been reported [4, 10-15].

These electroanalytical methods have advantages of being inexpensive and operating simply. Amino acid as the modifying material can be aggregated onto a glassy carbon electrode surface by covalent bond, has chemical stability, good reproducibility, and can be used for determination of electrochemical active material. Poly valine modified electrode was prepared and used for the electrochemical determination of vanillin in this paper. The electrode exhibited a good electrocatalytic activity for the oxidation of vanillin and it was applied for the first time to the determination of infant formula samples. The new method was developed for the determination of vanillin, which can provides technical support for infant food quality and safety monitoring and inspection.



**Figure 1.** Chemical structure of vanillin

## 2. EXPERIMENTAL PART

### 2.1. Apparatu sand Chemicals

Vanillin was brought from Aladdin Reagents (Shanghai) Co., LTD; Valine, disodium hydrogen phosphates and citric acid were brought from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). All chemicals were used as received without any further purification. Infant formula foods were brought from local market (Heze, China). Disodium hydrogen phosphates - citric acid buffer solutions were prepared by mixing the stock solutions of 0.2 mol L<sup>-1</sup> disodium hydrogen phosphates and 0.1 mol L<sup>-1</sup> citric acid. All aqueous solutions were prepared in double distilled water

All the electrochemical experiments were carried out by a CHI660C electrochemical workstation with three electrodes system (CH Instruments, China); KQ-100DB type ultrasonic cleaning equipment (Kunshan, China) and 79-1 type magnetic stirrer was used (Jiangsu, China). All experiments were performed at room temperature.

### 2.2 Preparation of samples

Infant formula milk powders were used as the sample matrix. Ten grams of each sample was dissolved with 16 ml of double distilled water and 4 mL of acetonitrile. Then 1 mL of saturated copper sulphate solution was added to precipitate protein [16]. The sample was extracted with ultrasonic cleaning equipment for 10 minutes. The supernatants were filtered and the filtrate diluted to 50 mL with disodium hydrogen phosphates - citric acid buffer solution (pH=7.0).

### 2.3 Preparation of poly valine modified electrode

A glassy carbon electrode ( $\Phi=3.8$  mm) was polished with metallographic sandpaper (grit 1000) and wet alumina powder ( $0.05\mu\text{m}$ ). Next, the electrode was flushed with distilled water. Then, to flush thoroughly, the electrode was rinsed ultrasonically with 1:1  $\text{HNO}_3$ , absolute ethanol, and distilled water, respectively. After rinsing, the prepared GCE as working electrode, a Ag/AgCl electrode as reference electrode, and a platinum as counter electrode, the poly valine modified electrode was prepared by immersing the GCE into an aqueous solution prepared by mixing 10.00 mL of disodium hydrogen phosphates - citric acid buffer solution (pH 6.0), 3.50 mL of  $0.01 \text{ mol L}^{-1}$  valine, and 6.50 mL of water and swept in a potential range from -1.5 to 2.2V for 10 cycles at a scan rate of  $100 \text{ mV s}^{-1}$  without stirring; then the surface of modified electrode was washed with double distilled water to be used.

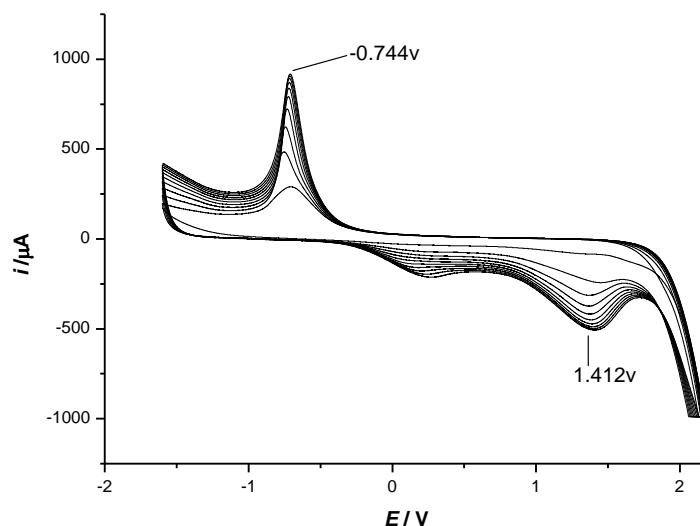
### 2.4 Method for determination of vanillin

To a 50-ml *electrochemical cell*, 20 ml of disodium hydrogen phosphates - citric acid buffer solution (pH 7.0) and a certain amount of standard solution of vanillin or infant formula milk powders samples was added. After stirring for 50 s, it was swept in a certain potential. After the end of each scan, the modified electrode was placed in the blank solution and scanned until the peaks disappeared to be used again.

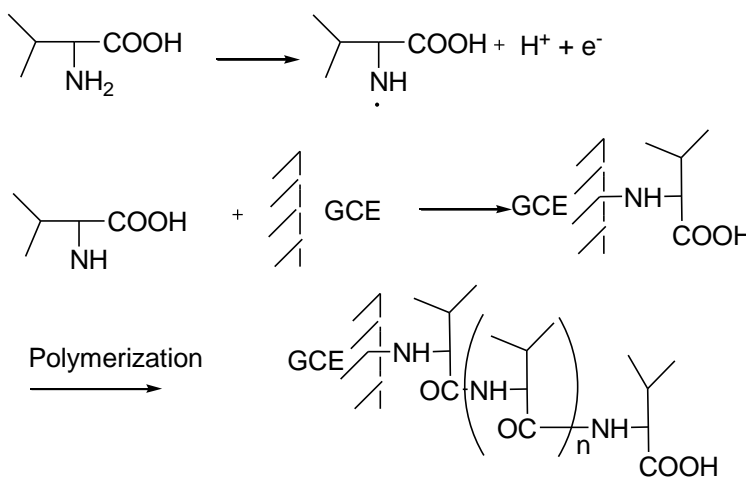
## 3. RESULTS AND DISCUSSION

### 3.1 Electrochemical polymerization conditions

In this paper, the effects of preparation conditions, such as potential range, electropolymerization cycles, scan rate and pH of solution, are discussed to enhance the performance of the modified electrode. The results show that the optimum polymerization potential was chosen as -1.5 V-2.2 V. During the polymerization experiment of valine, the results show that the valine modified electrode was prepared at a scan rate of  $100 \text{ mV s}^{-1}$  gives the largest peak responses to vanillin. It was also found that the peak responses of vanillin increased with the number of scan cycles and reached maximum at 10 cycles and then stabilized. The disodium hydrogen phosphates - citric acid buffer solution (pH 6.0) which gives the largest peak responses to vanillin was chosen in polymerization experiment of valine. Figure 2 shows the cyclic voltammograms of valine under the above optimized polymerization conditions. The valine monomer is oxidized first at a higher positive potential to form  $\alpha$ -amino free radical, which could be linked on an electrode surface and then formed polyvaline films[17,18] (figure 3).



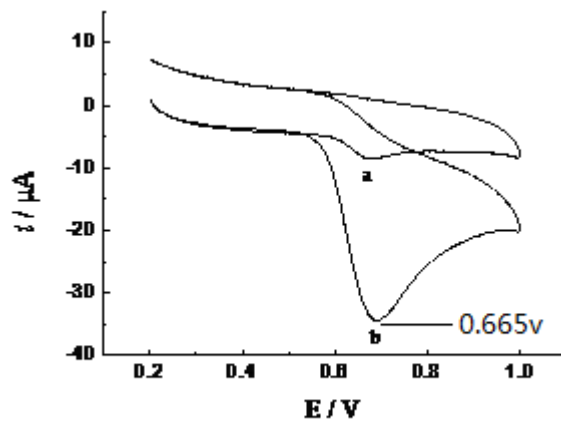
**Figure 2.** Cyclic voltammograms of valine on the glass carbon electrode



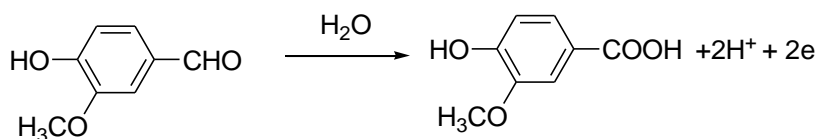
**Figure 3.** The electro-polymerization of valine on the GCE.

### 3.2 The electrochemical characteristics of vanillin at the poly valine modified electrode

Figure 3 shows cyclic voltammetry curve of vanillin at bare electrodes (a) and poly valine modified electrode (b) under the optimum polymerization conditions. As can be seen from the Figure 3, the response of vanillin at the bare electrode was very small while the peak current at poly valine modified electrode increased sharply. The oxidation peak current was 8.11 times compared with the bare electrode. There is only one oxidation peak of vanillin at poly valine modified electrode and so the reaction is irreversible reaction. I put forward suppose of the reaction mechanism of vanillin on the surface of GC electrode, and this could be described as follows in Figure 5 .



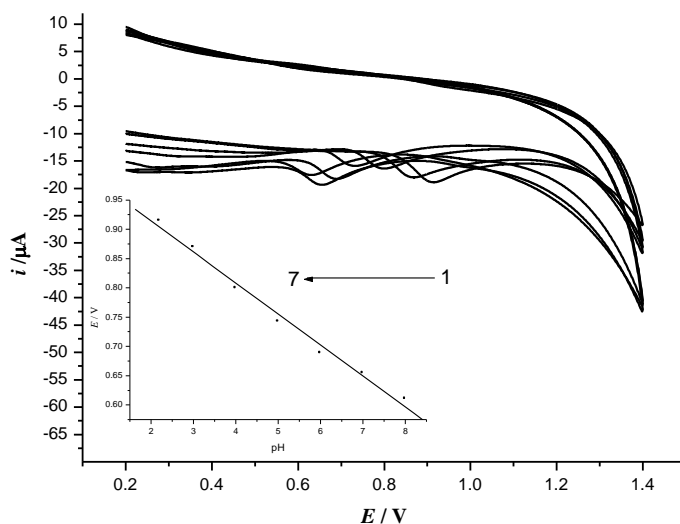
**Figure 4.** Cyclic voltammograms of vanillin ( $1.20 \times 10^{-5}$  mol/ L) at the bare electrode (a) and the valine modified electrode (b) (pH7.0). Scan rate: 120mV/s



**Figure 5.** Oxidation reaction of vanillin

### 3.3 The best condition for the determination of vanillin

#### 3.3.1 Effect of pH on the oxidation of vanillin



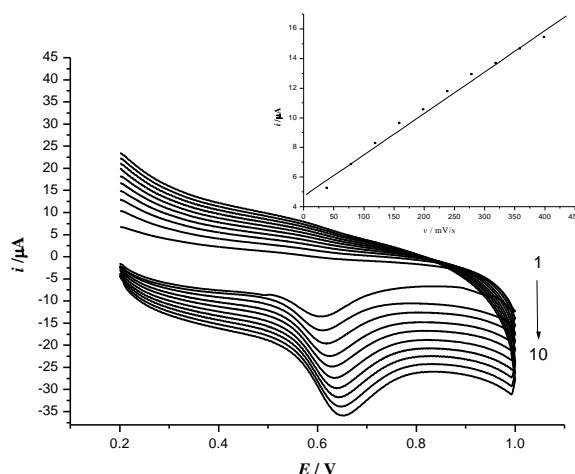
**Figure 4.** Effect of pH on the potential for the oxidation of vanillin ( $1.20 \times 10^{-5}$  mol/L) in the citric acid-phosphate buffer (2.2~8.0). Insert is the oxidation peak potential of vanillin versus pH value

The effect of the medium's pH from 2.2 to 8.0 on the electrochemical signal was analyzed. The oxidation peak negatively shifted with increasing pH value of the solution (Fig.4), which indicates that

the reaction involves a transfer process of protons. There is a linear relationship between  $E_{pa}$  and pH and the regression equation is expressed as  $E_{pa}=1.01-0.051\text{pH}$ ,  $r=0.9964$ . It also can be seen from Fig. 4 that the oxidation peak current of vanillin increased slightly with increase in the solution pH until it reaches 7.0. Therefore, pH 7.0 was used in this study.

### 3.3.2 Effect of scan rate and stirring time

When all other conditions remained unchanged and only changed the scanning rate, the results showed that the oxidation peak current increased as scan rate is increased (Fig. 5).



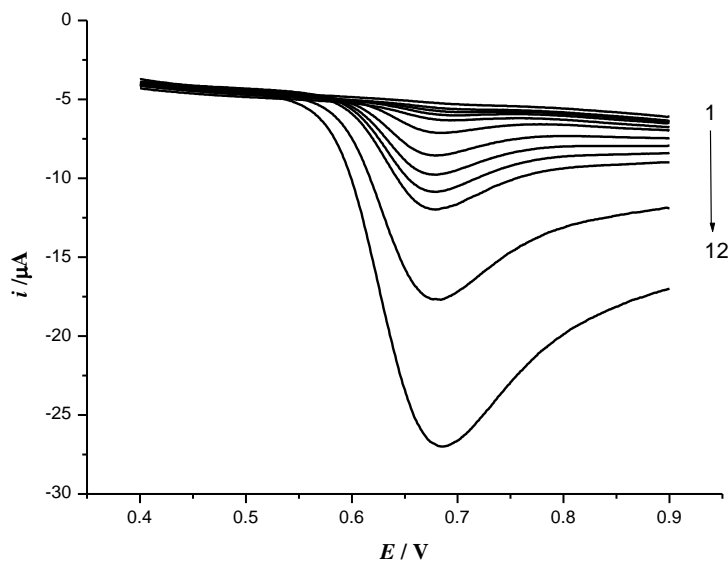
**Figure 5.** Cyclic voltammograms of vanillin ( $1.20 \times 10^{-5} \text{ mol L}^{-1}$ ) at different scanning rates. Inset is the plot of oxidation vanillin peak currents versus scan rates. 1-10: 40, 80, 120, 160, 200, 240, 280, 320, 360, 400  $\text{mV s}^{-1}$

There is a linear relationship between the oxidation peak current and the scan rate in the  $40 \sim 400 \text{ mV s}^{-1}$ . The regression equation is expressed as  $i_{pa} = 4.71 \times 10^{-6} + 2.79 \times 10^{-8} v$ ,  $r=0.9944$  which shows that the oxidation of vanillin was adsorption controlled. The peak shape is good and has high sensitivity at scan rate of  $120 \text{ mV s}^{-1}$ , so  $120 \text{ mV s}^{-1}$  was chosen in this experiment. In addition, with the stirring time increasing, the oxidation peak current increased first and then stabilized, so 50 s was chosen in this experiment.

### 3.4 Linear range and detection limit

Under the optimized conditions, the linear range and detection limit were investigated by differential pulse voltammetry (DPV). Figure 6 showed the differential pulse voltammograms of various concentrations of vanillin at the poly valine modified electrode. From the figure 6, we can see that the oxidation peak current of vanillin increased linearly with the increase of the concentration of

vanillin in a ranging from  $9.60 \times 10^{-8} \sim 6.61 \times 10^{-5} \text{ mol L}^{-1}$ , and the linear equation and correlation coefficient can be expressed as  $i_{pa} = 0.04c + 1.07 \times 10^{-6}$ ,  $r = 0.9972$ . The detection limit of a standard solution was estimated to be  $1.00 \times 10^{-8} \text{ mol L}^{-1}$  with DPV.



**Figure 5.** Curves of differential pulse voltammetry of vanillin at different concentrations. 1-12:  $8.00 \times 10^{-8}$ ,  $1.20 \times 10^{-7}$ ,  $4.80 \times 10^{-7}$ ,  $9.60 \times 10^{-7}$ ,  $1.20 \times 10^{-6}$ ,  $2.40 \times 10^{-6}$ ,  $4.80 \times 10^{-6}$ ,  $7.20 \times 10^{-6}$ ,  $9.60 \times 10^{-6}$ ,  $1.20 \times 10^{-5}$ ,  $3.53 \times 10^{-5}$ ,  $6.61 \times 10^{-5} \text{ mol L}^{-1}$

**Table 1.** Comparison of different electrodes for vanillin determination.

Electrode	Linear range (mol/L)	Detection limit (mol/L)	References
AuPd Nanoparticles-graphene composite modified electrode	$0.1 \times 10^{-6} - 7 \times 10^{-6}$ and $10 \times 10^{-6} - 40 \times 10^{-6}$	$2.0 \times 10^{-8}$	4
PVC-graphite composite electrode	$6.60 \times 10^{-4} \sim 9.20 \times 10^{-3}$	$2.90 \times 10^{-4}$	10
GCE	$5.00 \times 10^{-5} \sim 3.00 \times 10^{-4}$	$1.60 \times 10^{-7}$	11
Graphite electrode	$5.00 \times 10^{-6} \sim 4.00 \times 10^{-4}$	$4.00 \times 10^{-7}$	12
graphene/GCE	$6.00 \times 10^{-7} - 4.80 \times 10^{-5}$	$5.60 \times 10^{-8}$	13
CDAb/Au-AgNPs/GCE	$0.2 \times 10^{-6} - 50 \times 10^{-6}$	$0.04 \times 10^{-6}$	14
Lysine/CPE	$1.00 \times 10^{-5} \sim 100 \times 10^{-3}$	$2.88 \times 10^{-6}$	15
Valine/GCE	$9.60 \times 10^{-8} \sim 6.61 \times 10^{-5}$	$1.00 \times 10^{-8}$	This work

In addition, the comparison of poly valine modified electrode with other modified electrodes for vanillin determination was listed in Table 1. Compared with other modified electrodes, the proposed method was better or comparable to these previous reported methods. These results indicated that poly valine modified electrode was an appropriate platform for the determination of vanillin.

### 3.5 Stability, reproducibility and selectivity of poly valine modified electrode

To test the stability of the poly valine modified electrode, the storage stability of the poly valine electrode was evaluated by storing the electrode in air for two weeks. It was found that the electrode retained 96% of its initial current response after stored in air for two weeks indicating good stability of poly valine modified electrode. In addition, to test the reproducibility of poly valine modified electrode, six modified electrodes were fabricated based on the same procedure and used for the determination of  $1.20 \times 10^{-5}$  mol L<sup>-1</sup> vanillin standard solution. The relative standard deviation (RSD) of the peak currents was 5.3% (n = 6). This indicates that the electrode had good reproducibility.

The selectivity of poly valine modified electrode was also investigated by cyclic voltammetry. The results show that the 100 times of K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>, Fe<sup>3+</sup>, Cu<sup>2+</sup>, Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, sucrose, glucose, fructose, citric acid or ascorbic acid had no obvious influence on the results of the determination of vanillin within the  $\pm 5$  % error.

### 3.6 Determination of recovery

The samples were pre-treated according to the method of preparation of samples in section 2.2 and analyzed using the method for the determination of vanillin in section 2.4. The present method was used for the determination of vanillin in real samples of infant formula. The results are listed in Table 2. From the table 2 we can see that the proposed method can be successfully applied for the determination of vanillin.

**Table 2.** Determination of vanillin in infant formula

Samples		Found in Samples (mol/L)	Added (mol/L)	Total found (mol/L)	Recovery (%)	RSD (%)
Infant formula	1	$4.80 \times 10^{-7}$	$4.00 \times 10^{-7}$	$8.70 \times 10^{-5}$	97.5	2.54
	2	$3.60 \times 10^{-6}$	$4.00 \times 10^{-6}$	$7.40 \times 10^{-5}$	95.0	3.12
	3	$5.20 \times 10^{-6}$	$4.00 \times 10^{-5}$	$9.40 \times 10^{-5}$	105	3.56
0 to 6 months of infant formula foods	1	-	$6.00 \times 10^{-7}$	$5.90 \times 10^{-5}$	98.3	4.27
	2	-	$2.0 \times 10^{-6}$	$1.92 \times 10^{-5}$	96.0	4.16
	3	-	$2.00 \times 10^{-5}$	$1.97 \times 10^{-5}$	98.5	2.79

## 4. CONCLUSION

The obtained poly valine modified electrode has strong electrocatalysis activity toward the oxidation of vanillin, the electrode also exhibits good stability and reproducibility, and it can be



applied to the determination of vanillin in real samples. The method is simple, rapid, sensitive and provides a safe and effective new method for the detection of vanillin in food.

#### ACKNOWLEDGEMENT

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