

## Application of Sensitive Sensor Modified with MWNTs/Al<sub>2</sub>O<sub>3</sub>/poly-L-lysine Composite for 17β –estradiol Determination in Pregnant Woman Blood

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A novel disposable sensor modified with MWNTs/Al<sub>2</sub>O<sub>3</sub>/poly-L-lysine film was developed for detection of clinical 17β-estradiol (17β-E<sub>2</sub>) urine samples in this study. The electrochemical behavior of 17β-E<sub>2</sub> was greatly increased due to the good electrocatalytic activity of MWNTs/Al<sub>2</sub>O<sub>3</sub>/poly-L-lysine nanocomposites. The optimum experimental conditions such as scan rate, pH, accumulation time were obtained, and the peak potentials of 17β-E<sub>2</sub> was explored at about 530mV. The peak current of 17β-E<sub>2</sub> measured by CV displayed the good linear property with the increasing of the concentrations. Under optimized conditions, the disposable sensor displayed excellent analytical performance with linear ranges of 17β-E<sub>2</sub> from 0.5nM to 50nM. And a very low detection limit of 0.014 nM was attained. Furthermore, the MWNTs/Al<sub>2</sub>O<sub>3</sub>/poly-L-lysine SPE was used to the rapid and successful determination of the hormone in spiked synthetic and real clinical pregnant woman urine samples with wonderful selectivity and sensitivity.

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**Keywords:** Disposable sensor, 17β-estradiol, clinical pregnant woman urine samples, modified screen printed electrode

### 1. INTRODUCTION

As one of most active estrogens, 17β-estradiol (17β-E<sub>2</sub>) was usually abused to fatten animals and increase the profitability, which was considered illegal and harmful to the endocrine function of

animals and humans[1]. In fact, previous reports had been indicated that the high amount accumulation of  $17\beta$ -E<sub>2</sub> would cause the obstruction of normal physiological processes such as sexual abnormalities, endocrine and reproductive disorder, and even cancer development [2,3]. Thus, it was extremely necessary to develop a sensitive, simple and fast analytical method and device for  $17\beta$ -E<sub>2</sub> detection.

Different methods for determination of  $17\beta$ -E<sub>2</sub> were been proposed including HPLC [4,5], fluorimetry[6] and Enzyme-linked immuno-sorbent assay[7]. However, these assays presented complicated treating process, time-consuming, labor intensive and high costs [2]. Consequently, electrochemical analytical method was employed for the rapid response and low cost [8]. While, the disadvantage of bare electrochemical was too low sensitive to determine  $17\beta$ -E<sub>2</sub> at ultra-trace levels. Therefore, some researches have focused on modifying electrode to increase the sensitivity [9-11]. But another challenge for the actual application was the limitation of mass-detection which the pillar electrode could not been produced in mass. Hence, the disposable sensor appeared for the further study.

Recently, the researches about the disposable screen-printed electrode (SPE) for drug determination had been reports[12,13], which exhibited excellent property such as sensitivity, disposability, rapid response, portability, small size and low cost. In addition, disposable SPE was developed fast in different fields including the monitor of blood glucose and water heavy metal [14,15]. In the previous work, the disposable sensor was successfully applied for the drug detection with good results. According the literature, the disposable SPE for detection of  $17\beta$ -E<sub>2</sub> had been never reported [12,13]. Therefore, the method was developed for clinical therapeutic drug monitoring by modified SPE first.

Due to the unique geometrical and chemical properties [16], the multi-walled carbon nanotubes (MWNTs) could promote electron transfer reaction, enhance the detection of electrochemical signal [17]. Sensors modified with MWNTs presented excellent sensitivity and low detection limit. In addition, MWNTs was reported to be successfully used for synthesizing DNA biosensor, which provided a very simple and sensitive method for detecting biorecognition events. In the previous studies, the electrodes fabricated with MWNTs displayed good results for determination of  $17\beta$ -E<sub>2</sub> [17]. Thus, it was good mind to develop sensor with MWNTs. Al<sub>2</sub>O<sub>3</sub> was commonly used as the very promising modifier to catalyze the oxidation of different compounds. Hammani et al. had constructed a cheap and sensitive electrochemical sensor based on Al<sub>2</sub>O<sub>3</sub> supported on activated carbon for detection of phenol, which Al<sub>2</sub>O<sub>3</sub> exhibited good catalytic activity [18]. Furthermore, Arafat M.M established gas sensors through Al<sub>2</sub>O<sub>3</sub> nanoparticles, which showed excellent sensing performance and selective sensitivity for determination [19]. Poly-L-lysine (PLL) contained multiple functional groups including cationic amino-group, which was an excellent alternative to fabricate sensors [20]. Therefore, PLL was applied to immobilize DNA, carbon nanotubes (CNTs) and nanopartical materials to the surface of the electrode through plentiful active amino groups and flexible molecular backbone [8]. This present work aimed to use PLL to functionalize Al<sub>2</sub>O<sub>3</sub> and CNTs for further attaching bioactive molecules.

In this paper, disposable SPE based on MWNTs/Al<sub>2</sub>O<sub>3</sub>/PLL was first developed for determination of  $17\beta$ -E<sub>2</sub>. Due to the good property of composites, the modified sensor could be applied to monitor clinical pregnant woman urine sample.

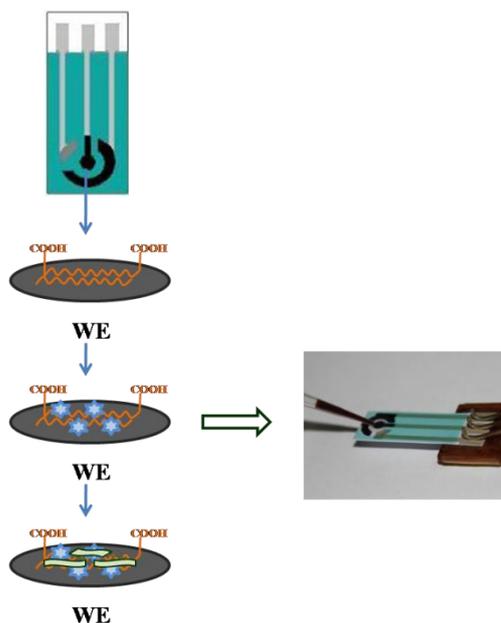
## 2. MATERIAL AND METHODS

### 2.1. Reagents and apparatus

17 $\beta$ -estradiol (17 $\beta$ -E<sub>2</sub>) was employed from Sigma. MWNTs (purity > 90%) was purchased from Chengdu Organic Chemicals Co., Ltd, Chinese Academy Sciences (Sichuan, China). Al<sub>2</sub>O<sub>3</sub> nanoparticle was obtained from Aladdin Chemistry Co., Ltd (Shanghai). 0.01% PLL solution was prepared by dissolving 0.01g poly-L-lysine in doubled distilled water. The stock 17 $\beta$ -E<sub>2</sub> sample (1 $\mu$ M) was obtained by dissolving in ethanol and stored at -20°C for experiments. HAc-NaAc buffer solution (0.1 M, pH 4.5) was employed for supporting electrolyte. Other chemicals and solvents were the analytical grade during the experiment.

The screen-printed electrodes (SPE) contained three electrode system such as carbon working electrode, Ag/AgCl reference electrode and auxiliary electrode, which could be produced in mass instead of traditional pillar electrode. The EC 570 electrochemical workstation (Gaoss Union Technology, Wuhan, China) was used to record the electrochemical behaviors of 17 $\beta$ -E<sub>2</sub> at different SPEs. The characterization images of different modified SPEs were attained by SEM (Quanta 200, FEI Corporation, Holland).

### 2.2 Fabrication of MWNTs/Al<sub>2</sub>O<sub>3</sub>/ PLL SPE



**Figure 1.** The fabrication procedure of modified sensor.

MWNTs/Al<sub>2</sub>O<sub>3</sub>/ PLL SPE was developed as following. First, 2mg/mL MWNTs solution was casted on the carbon working electrode, and then air-dried 30 min at room temperature. Next, 2mg/mL Al<sub>2</sub>O<sub>3</sub> nanoparticles suspension solution was dropped on the surface of MWNTs to get MWNTs/Al<sub>2</sub>O<sub>3</sub> film. Last, MWNTs/Al<sub>2</sub>O<sub>3</sub>/ PLL SPE was fabricated by dropping 0.01% PLL solution to fix the MWNTs/Al<sub>2</sub>O<sub>3</sub> film on the electrode surface through the amino-group, which could improve the

stability of modified SPE. Meanwhile, the MWNTs SPE, Al<sub>2</sub>O<sub>3</sub> SPE and MWNTs/Al<sub>2</sub>O<sub>3</sub> SPE were also obtained for further investigation comparison. The whole fabrication procedure of the modified sensor was displayed as Fig.1.

### 2.3 Analysis procedure

The appropriate amounts of 17 $\beta$ -E<sub>2</sub> were added to the supporting systems such as 0.1 mol.L<sup>-1</sup> HAc-NaAc (pH 4.8) buffer solution and diluted real samples. The cyclic voltammetric curves (CV) was recorded over the potential range from 0.0 to 1.0 V versus the Ag/AgCl reference electrode. Whole electrochemical experiments were processed at room temperature with open-circuit accumulation for 6 min.

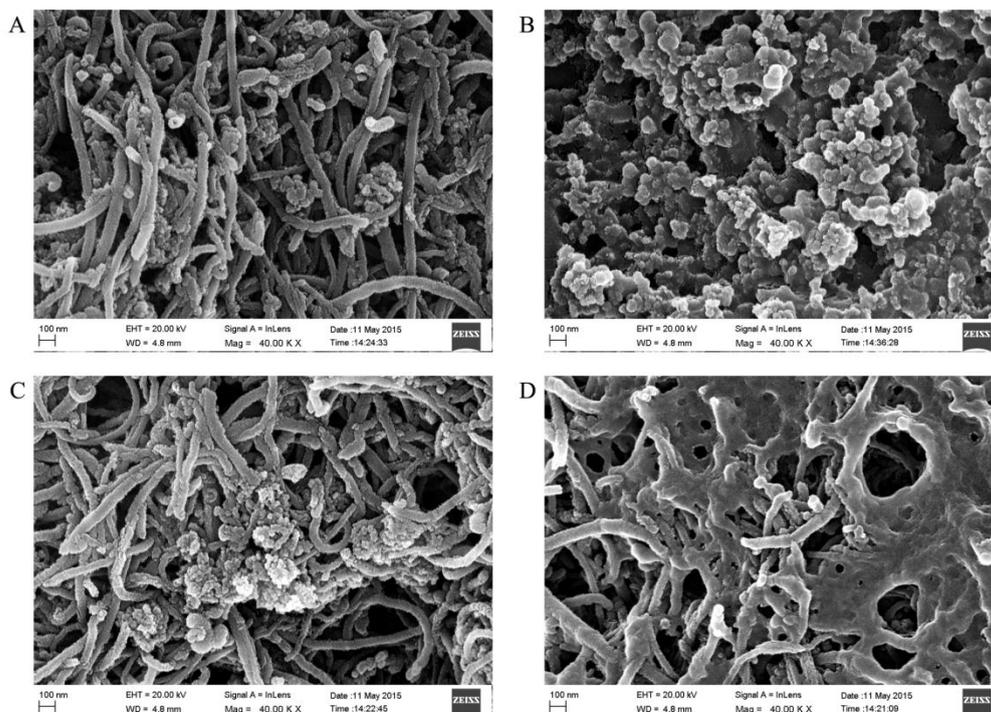
### 2.5 Analysis of real sample

Whole blood samples (2mL) were obtained from three clinical pregnant women after signing informed consent, which analytical samples were detected without any other pretreatments. Each 0.5 mL sample was diluted to 1.0 mL in electrolytic cell with HAc-NaAc buffer solution (0.1M, pH4.8). As displayed in Fig.1, the diluted real sample was electrochemically scanned immediately to CV curve for detection 17 $\beta$ -E<sub>2</sub>.

## 3. RESULT AND DISCUSSION

### 3.1 Characterization of MWNTs/Al<sub>2</sub>O<sub>3</sub>/ PLL SPE

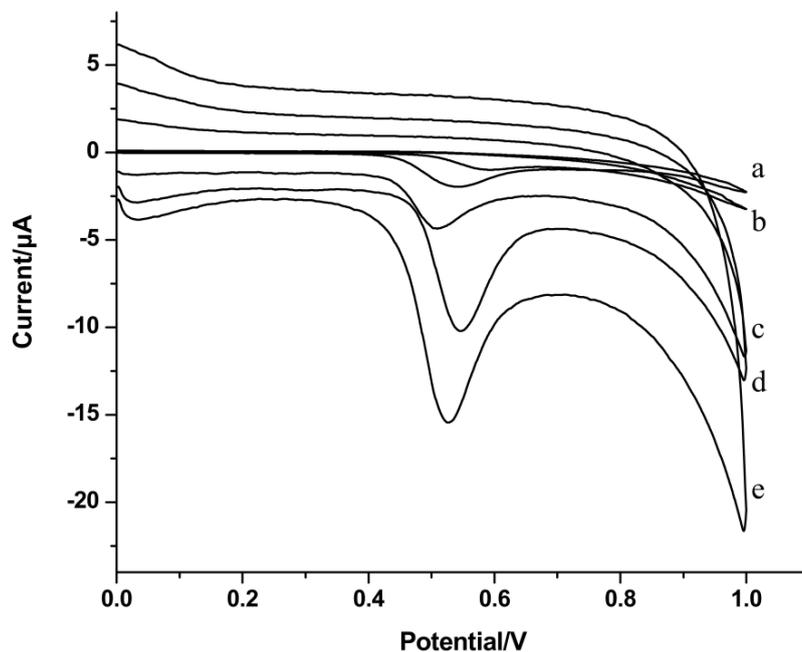
The surface morphology characterizations of modified SPEs were investigated by scanning electron microscopy (SEM), which the results were displayed as Fig.2. It could be found that MWNTs were attached on the sensor surface (Fig.2.A). While, Al<sub>2</sub>O<sub>3</sub> nanoparticles were covered on the surface of SPE in Fig.2.B. As the nano materials, MWNTs and Al<sub>2</sub>O<sub>3</sub> nanoparticles presented excellent property such as enhancing the electrochemical transfer rate and increasing the electrochemical activities. Thus, MWNTs/Al<sub>2</sub>O<sub>3</sub> SPE (Fig.2.C) was obtained and exhibited better electrochemical performance for determination because of the nanoparticle size advantages and electrochemical catalytic. However, the experimental results showed MWNTs film, Al<sub>2</sub>O<sub>3</sub> film and MWNTs/Al<sub>2</sub>O<sub>3</sub> film were unstable and would be easily scoured off by detection system. Therefore, PLL was recommended to solve this problem, which firmly fixed the composite film on SPE surface through plentiful active amino groups. As displayed in Fig.2.D, MWNTs/Al<sub>2</sub>O<sub>3</sub>/ PLL film exhibited the high surface area, which the sensor was good for analyzing 17 $\beta$ -E<sub>2</sub>.



**Figure 2.** SEM images of MWNTs modified SPE (A), Al<sub>2</sub>O<sub>3</sub> modified SPE (B), MWNTs/ Al<sub>2</sub>O<sub>3</sub> modified SPE (C) and MWNTs / Al<sub>2</sub>O<sub>3</sub>/PLL modified SPE (D)

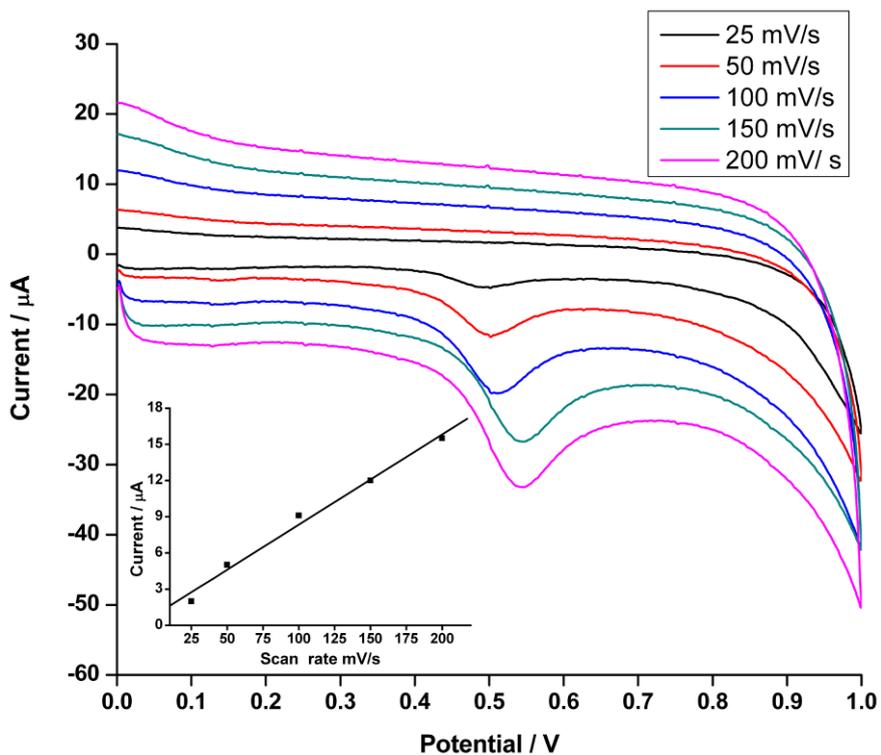
### 3.2 Electrochemical behavior of 17 $\beta$ -E<sub>2</sub> at MWNTs/Al<sub>2</sub>O<sub>3</sub>/ PLL SPE

The characterization behavior of 17 $\beta$ -E<sub>2</sub> was the most important research content which was investigated with CV in HAc-NaAc buffer (0.1 mol.L<sup>-1</sup>, pH 4.8) by different modified SPE. CV curves of 17 $\beta$ -E<sub>2</sub> at different SPEs were respectively presented in Fig.3, including bare SPE, MWNTs SPE, Al<sub>2</sub>O<sub>3</sub> SPE, MWNTs/Al<sub>2</sub>O<sub>3</sub> SPE and MWNTs/Al<sub>2</sub>O<sub>3</sub>/ PLL SPE. It was found only one oxidation peak of 17 $\beta$ -E<sub>2</sub> (curve a) appeared with the potential 0.62V at bare SPE. However, as curve b exhibited, the oxidation peak current of 17 $\beta$ -E<sub>2</sub> at MWNTs SPE was greatly enhanced compared with bare SPE, which the result was the same with Lin's report [17]. Furthermore, the oxidation peak current of 17 $\beta$ -E<sub>2</sub> also increased at Al<sub>2</sub>O<sub>3</sub> SPE (curve c). MWNTs and Al<sub>2</sub>O<sub>3</sub> nanoparticles promoted ion-exchange to amplify the electrochemical signal, which finally improved the detection sensitivity [17-19]. So, we observed that the peak current of 17 $\beta$ -E<sub>2</sub> at MWNTs/Al<sub>2</sub>O<sub>3</sub> SPE further increased because of the conjunction effect (curve d). While MWNTs film, Al<sub>2</sub>O<sub>3</sub> film and MWNTs/Al<sub>2</sub>O<sub>3</sub> film were not stable enough, which could possibly rinse off by detection samples. PLL displayed better biofilm property. Curve e in Fig.3 demonstrated that MWNTs/Al<sub>2</sub>O<sub>3</sub>/PLL SPE effectively catalyzed the electro oxidation of 17 $\beta$ -E<sub>2</sub> and greatest improved the peak shape. The result was mainly thought to the subtle electronic properties, large surface area and film stability of MWNTs/Al<sub>2</sub>O<sub>3</sub>/PLL.



**Figure 3.** CV curves of coexisted 50 nM  $17\beta\text{-E}_2$  at bare SPE (curve a), MWNTs SPE (curve b),  $\text{Al}_2\text{O}_3$  SPE (curve c), MWNTs/ $\text{Al}_2\text{O}_3$  (curve d) and MWNTs/ $\text{Al}_2\text{O}_3$ /PLL SPE (curve e) in 0.1M HAc-NaAc buffer, scan rate: 100mV/s.

### 3.3 The influence of scan rate

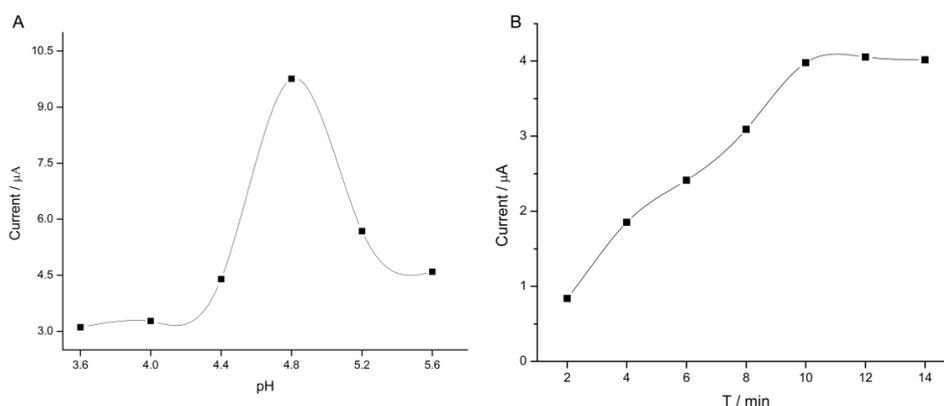


**Figure 4.** Cyclic voltammogram (CV) curves of 50 nM  $17\beta\text{-E}_2$  at MWNTs/ $\text{Al}_2\text{O}_3$ /PLL SPE in HAc-NaAc buffer (0.1 M, pH 4.8) with different scan rates (curves from a to f: 25, 50, 100, 150, 200  $\text{mV s}^{-1}$ ). Inset: The dependence of the peak currents on the scan rates.

The cyclic voltammogram (CV) curves of mixed 50 nM 17 $\beta$ -E<sub>2</sub> at MWNTs/Al<sub>2</sub>O<sub>3</sub>/PLL SPE with different scan rates were recorded in HAc-NaAc buffer (0.1M, pH 4.8). According to the result in Fig. 4, the peak potential of 17 $\beta$ -E<sub>2</sub> shifted to positive value with the increasing scan rate in the range of 25 to 200mV/s. Considering the oxidation peak current and peak shape, the optimized scan rate was selected as 100 mV/s for the detection. Moreover, the oxidation peak current of 17 $\beta$ -E<sub>2</sub> presented a linear relationship with the scan rates as following:  $I_p=0.879+0.074v$ ,  $R=0.9935$ , which suggested that the electrochemical oxidation reaction was controlled by the adsorption process. The peak potential also shifted to the positive value when the scan rate increased.

### 3.3 Influence of pH

CV was applied to investigate the supporting electrolyte pH values from 3.6 to 5.6. However, the relationship between pH value and the peak current was presented in Fig.5. It could be observed that oxidation peak current of 17 $\beta$ -E<sub>2</sub> increased with the increasing pH value, which got a maximum at pH 4.8 and then decreased instead. In conclusion, the result indicated the optimum pH 4.8 was fit for the subsequent experiments. The relationship between corresponding potential of 17 $\beta$ -E<sub>2</sub> and pH was obtained as following equation:  $E_p(V) = -0.805 - 0.058pH$  ( $R=0.9711$ ). The peak potential  $E_p$  shifted to the negative values with increasing pH. When the pH was 4.8, the oxidation peak potential of 17 $\beta$ -E<sub>2</sub> was 0.53 V.



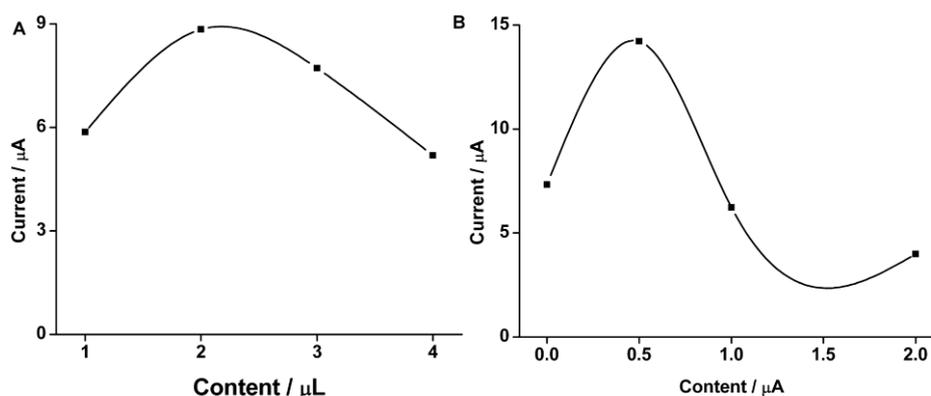
**Figure 5.** Effects of pH (A) and accumulation time (B) on the electrochemical response of 50 nM 17 $\beta$ -E<sub>2</sub> at MWNTs/Al<sub>2</sub>O<sub>3</sub>/PLL SPE in 0.1 M HAc-NaAc buffer (pH 4.8).

### 3.4 Influence of accumulation time

The electrochemical oxidation of 17 $\beta$ -E<sub>2</sub> was controlled by the adsorption process. Therefore, accumulation time was the important factor for the determination sensitivity. The effect of accumulation time on the oxidation peak current was investigated by CV in Fig.6. The current of 50 nM 17 $\beta$ -E<sub>2</sub> increased with the increasing accumulation time, which the maximum value was obtained at about 10 min. After that the peak current kept stable when accumulation time increased. Thus, the accumulation time of 10 min was chosen for further 17 $\beta$ -E<sub>2</sub> detection in real samples.

### 3.5 Influence content of MWNTs/Al<sub>2</sub>O<sub>3</sub>/PLL

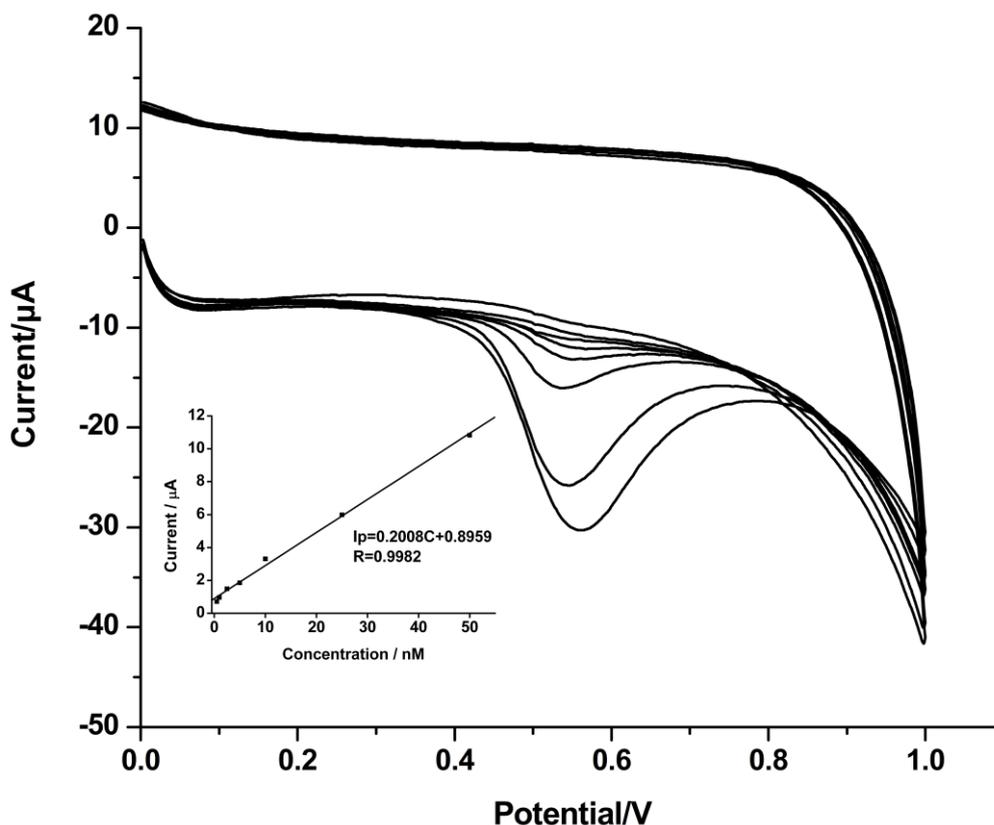
As the important factor for the electrochemical response, the modifier content of MWNTs/Al<sub>2</sub>O<sub>3</sub>/PLL was investigated. It was obtained that the response current of 17 $\beta$ -E<sub>2</sub> reached maximum when MWNTs and Al<sub>2</sub>O<sub>3</sub> was added to 2  $\mu$ L. However, the peak current would decrease with further more modified composition (Fig. 6A). Meanwhile, PLL was also discussed, and the optimal content 0.5  $\mu$ L was selected (Fig. 6B). The result suggested that the larger background current would lead low sensitivity and poor detection for 17 $\beta$ -E<sub>2</sub>. Thus, 2  $\mu$ L MWNTs/ Al<sub>2</sub>O<sub>3</sub> and 0.5  $\mu$ L PLL were screened as the best content for the sensor fabrication.



**Figure 6.** Relationship between modifier amounts and oxidation peak currents of 17 $\beta$ -E<sub>2</sub> (50 nM) in HAc-NaAc buffer (0.1 M, pH 4.8), including MWNTs/Al<sub>2</sub>O<sub>3</sub> (A) and PLL (B).

### 3.6 Calibration curve and limit detection

Under the optimized conditions, the analytical performances and calibration curves of the proposed method upon the different concentrations of 17 $\beta$ -E<sub>2</sub> were examined by CV. As presented in Fig.7, the CV peak currents of 17 $\beta$ -E<sub>2</sub> at MWNTs/Al<sub>2</sub>O<sub>3</sub>/PLL SPE increased with the increasing concentration from 0.05nM to 50nM, which the linear relationship (Insert of Fig.7) between the oxidation peak current intensity ( $|I_p|$ ) and the concentration ( $c$ ) was obtained with the following regression equation:  $|I|$  ( $\mu$ A) = 0.2008 $c$  ( $\mu$ M) + 0.8959 (R=0.9982). According to the three times the standard deviation of the blank sample measurement, the limit detection of 17 $\beta$ -E<sub>2</sub> was calculated to be 0.014nM. The repeatability and stability of developed SPE were also investigated, which the relative standard deviations (RSD) of six successive measurements for 0.1nM 17 $\beta$ -E<sub>2</sub> by CV was 3.1%. While, different proposed sensors ( $n=5$ ) were applied to determinate 0.1 nM 17 $\beta$ -E<sub>2</sub> with excellent reproducibility of RSD 4.8%. Furthermore, the storage stability of MWNTs/Al<sub>2</sub>O<sub>3</sub>/PLL SPE was examined through detecting 0.1 nM 17 $\beta$ -E<sub>2</sub>. the response current of the developed sensor was maintained 92.7% with storing in the room temperature over one month, which demonstrated the good stability of the modified sensor.



**Figure 7.** CV curves of different concentrations of 17β-E<sub>2</sub> in HAc-NaAc buffer (0.1 M, pH 4.8), scan rate: 100mV/s. Concentration: 0.5 nM, 1 nM, 2.5 nM, 5 nM, 10 nM, 25nM and 50 nM.

### 3.7 Specificity and interferences

To explore the specificity of MWNTs/Al<sub>2</sub>O<sub>3</sub>/PLL SPE, the influences of various common interferences on the 17β-E<sub>2</sub> detection were investigated. Different excess amount of the interferences including cations, anions, 100 μM ascorbic acid (AA) and 100 μM uric acid (UA) were added to the analytical sample solutions which contained 0.5nM 17β-E<sub>2</sub>. According to our previous studies, the peak potentials of AA and UA were about 0.1-0.2V, which the 17β-E<sub>2</sub> determination would not disturb because of large peak potential separation, especially uric acid. The examining results exhibited that a relative error of largest amount was lower than ±5%. Thus, the developed method presented good selectivity for the 17β-E<sub>2</sub> monitoring.

### 3.8 Determination of 17β-E<sub>2</sub> in injection and clinical blood samples

In order to investigate the practical application of the prepared sensor, the standard addition method was proposed to measure the content of 17β-E<sub>2</sub> pharmaceutical dosage. Different amounts of 17β-E<sub>2</sub> injections were dissolved with 0.1 mol.L<sup>-1</sup> HAc-NaAc (pH 4.8) buffer solution. The determination results were obtained as Table 1. In addition, the recovery and RSD of five independent experiments at MWNTs/Al<sub>2</sub>O<sub>3</sub>/PLL SPEs were calculated to be 102.40% and 3.6%, respectively.

**Table 1.** Application of MWNTs/Al<sub>2</sub>O<sub>3</sub>/PLL SPE for detecting 17β-E<sub>2</sub> in injection and clinical blood samples

| Sample        | Content (nM)   | Standard add (nM) | Total detected (nM) | Average recovery (%) | Average RSD (%) |
|---------------|----------------|-------------------|---------------------|----------------------|-----------------|
| Injection     | 0.500          | 0.250             | 0.768               | 102.40               | 3.6             |
|               | — <sup>a</sup> | 1.0               | 0.959               | 95.94                | 8.9             |
| Blood samples | — <sup>a</sup> | 2.5               | 2.479               | 99.18                | 3.5             |
|               | — <sup>a</sup> | 5.0               | 5.146               | 102.26               | 3.3             |

—<sup>a</sup> below the limit detection

At the same time, the clinical blood samples were diluted 2 times with the supporting electrolyte for detecting 17β-E<sub>2</sub> without any other pretreatment. As shown in Table 1, the average recoveries were obtained from 95.94% to 102.26% after adding different concentration 17β-E<sub>2</sub> into blood samples. Moreover, average RSD was also less than 10%, which revealed the modified sensor could be successfully applied for the real biological monitor. Compared with previously reported sensor [10,14,15,17,20-23], Table 2 presented the analytical performance of fabricated sensor. Although the detection range of MWNTs/Al<sub>2</sub>O<sub>3</sub>/PLL SPE was not low like other sensors, the developed sensor could be successfully used to detect clinical blood samples. While, other sensors couldn't achieve, which was only used for the milk, water and PBS. To our knowledge, it was the first report about the modified SPE for detection of clinical 17β-E<sub>2</sub> whole blood samples. The proposed sensor could be mass production in future, which this simple method could provide fast, instantaneous measurement and monitoring for accurate monitor.

**Table 2.** Comparison of the detection samples and linear range obtained at the MWNTs/Al<sub>2</sub>O<sub>3</sub>/PLL SPE for detection of 17β-E<sub>2</sub> with other sensors.

| Methods  | Linear range (nM) | Detection samples    | Reference |
|--|-------------------|----------------------|-----------|
| CPE/GNR/FS-Au-CA                               | 100-5000          | Milk samples         | 10        |
| α-Fe <sub>2</sub> O <sub>3</sub> -NG-AuNRs/ITO | 0.005-1           | Milk                 | 14        |
| p-Aminothiophenol/ AuNPs/CE                    | 0.0036 - 3.6      | Water                | 15        |
| Pt/MWNTs/GCE                                   | 500-15000         | PBS                  | 17        |
| PPOMC / GCE                                    | 10-2000           | Female blood serums  | 20        |
| AuNPs/CoS/GCE                                  | 0.001-1           | Urine samples        | 22        |
| Magnetic nanoparticles                         | 7-735             | Milk                 | 23        |
| MWNTs/Al <sub>2</sub> O <sub>3</sub> /PLL      | 10.0 -200.0       | Clinical whole blood | This work |

#### 4. CONCLUSION

In this work, MWNTs/Al<sub>2</sub>O<sub>3</sub>/PLL SPE was performed and successfully applied for determination of 17β-E<sub>2</sub> in clinical whole blood. The MWNTs/Al<sub>2</sub>O<sub>3</sub>/PLL presented excellent redox response to with good specificity, repeatability and stability. The 17β-E<sub>2</sub> electrochemical oxidation

peak was found to be about 0.53V by CV. Furthermore, a good linear relationship was also obtained between the oxidation peak current and concentration. Practical assays of 17 $\beta$ -E<sub>2</sub> blood samples from clinical volunteers indicated the prepared sensor exhibited various advantages including high sensitivity, rapid simple analysis procedures, easy industrial realization and low production cost. Therefore, this work could provide much potential for the environment detection and clinical accurate monitor.

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