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Electrochemical Determination of Theophylline Pharmacokinetic under the Effect of Roxithromycin in Rats by the MWNTs/Au/poly-L-lysine Modified Sensor

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In this work, a novel and simple method for pharmacokinetic of theophylline in rats was described with modified sensor based on MWNTs/Au/poly-L-lysine. The modified screen-printed electrode exhibited excellent electrocatalytic activity towards the oxidation of theophylline in real biological samples. The optimum experimental conditions were explored by square wave voltammetry (SWV). The peak current of theophylline was proportional to the concentration in the range of 10 μM to 200 μM with the detection limit 2.0 μM. Compared with the HPLC method, the newly proposed method also exhibited good results in the determination of theophylline pharmacokinetic under the effect of roxithromycin. The drug concentration-time curve of theophylline was also attained at different time points: 10min, 20min, 30min, 60min, 90min, 120min, 240min and 480min. The results strongly showed that the established sensor could be applied to the real-time monitoring such as the clinical drug therapeutic drug monitoring, especially in the case of high concentration thepohylline intoxication.

Keywords: determination, theophylline, roxithromycin, pharmacokinetic, modified screen-printed electrode, the drug concentration-time curve

1. INTRODUCTION

Electrochemical sensors and biosensors have been developed rapidly in pharmaceutical determination and environmental monitor, especially the disposable screen-printed sensors [1-3]. The merits of screen-printed sensors are fast, sensitive, selective, accurate, miniaturizable and low-cost like

the Glucose Test Strips, which could lead to the realization of the therapeutic drug monitoring in clinical[2, 4]. Based on the above knowledge, we have prepared the disposable sensor with chemical modification to detect theophylline pharmacokinetic in rats.

The modification of bare electrode with mediators provides the significant advantages in the evolution of electrochemical sensors. Compared to inert bare electrodes, the modified sensors could shuttle electrons fast and promote the oxidation reaction [5-10]. The nanostructured materials have attracted considerable interests, particularly carbon nanotubes [6-8, 11, 12] and nano-Au [1, 13-15], which have been studied in vast research areas owing to their unique geometrical and chemical properties. The previous literatures have reported that the multi-walled carbon nanotubes (MWNTs) can provide an important and feasible platform for electroanalysis [6, 7]. Depending on their atomic structure, MWNTs have behaved electrically as the semiconductor to promote charge-transfer reactions [8, 11]. The super performance of modified electrode with MWNTs for application in pharmaceutical analytical sensing is well documented [11, 12]. And recently, gold nanoparticles (nano-Au) have been also extensively used for the development of electrochemical sensors because of the nano-structure and fascinating properties, such as conductivity, excellent adsorption ability and good biocompatibility particularly in the design of modified electrochemical sensors [14, 16, 17]. The nano-Au adsorbed on the surface of composites could be easily constructed by some strategies such as polymer entrapment [17, 18]. Due to the presence of multiple functional groups such as the cationic amino-group [19, 20], poly-L-lysine (PLL) has been already considered as an excellent alternative to fabricate sensors, too. Due to the flexible molecular backbone and plentiful active amino groups, PLL could be used to fix carbon nanotubes (CNTs), graphene oxide, many other materials, and even DNA on the electrode surface by electro-polymerization [21-24]. As the linker, researchers directly used PLL to functionalize metal materials and CNTs through the covalent amide group for further attaching bioactive molecules.

In this work, the MWNT-COOH and nano-Au were casted onto the surface of screen-printed working electrode to fabricate the MWNTs/Au SPE. Then, the PLL well immobilized the MWNTs/Au to the surface of SPE to obtain the MWNTs/Au/PLL film by abundant NH_2 groups, which would adsorb carboxylic functionalized MWNTs through electrostatic attraction. The composite film combined the advantages of the materials for electrochemical determination.

In clinical practical treatment, the theophylline (TP), the bronchodilator drug, is widely used in the treatment of chronic obstructive pulmonary disease（COPD）and severe asthmas [25-27]. While, the aminophylline is more frequently used for the treatment instead of TP in clinical, which contains the double salt of TP and ethylenediamine. And it would release the therapeutic part of TP fast in vivio [28, 29]. So the real therapeutic part of aminophylline is still TP. The researches have documented that the safe and blood therapeutic level of TP is 5 to 20 μg/mL [13, 31]. However, when the blood concentration is more than 20 μg/mL, the patient might suffer from fever, insomnia, dehydration, anorexia, arrhythmia, and heartburn tachycardia [15, 31-34]. Even more, the clinical cases have showed that some patients would die with respiratory arrest and cardiac arrest if the concentration of TP is 40 μg/mL or higher [31, 34], especially in drug combination such as with antibiotics, for example the roxithromycin [15, 30]. For avoiding the above situation, the clinical drug guideline points that it is urgently required to real-time monitor.

Many analytical methods have been reported for detection of TP or aminophylline, for example, high performance liquid chromatography (HPLC) [35], spectroscopy [36], chemiluminescence [37], LC/MS [38] and electrochemical detection [13, 15, 31]. Nevertheless, some of these methods are not appropriate for the rapid monitoring of biological TP samples. Specifically, the sensitivity and instability of spectroscopy and chemiluminescence methods are not accurate enough during the clinical actual monitoring or biological determination. Because of complicated pretreatment and time-consuming, HPLC or LC/MS method may delay the drug treatment in clinical emergency situation. However, electrochemical method showed the unique merits, for example, high sensitivity and specificity, no pretreatment and fast detection in few minutes. Consequently, screen-printed electrochemical sensor provides a low-cost, simple and rapid strategy for determination of TP in the pharmaceutical formulations and biological blood samples.

To our best knowledge, no research has been published about TP pharmacokinetic under the effect of roxithromycin in rats by screen-printed modified sensor. Thus, initially the preparation of MWNTs/Au/PPL modified sensor for detection of TP was described in this paper. In addition, the affect of roxithromycin on the TP pharmacokinetic in rat was first studied by developed method. The proposed sensor offered a new and simple way for clinical therapeutic drug detection of TP in animals.

2. MATERIAL AND METHODS

2.1. Apparatus and reagents

The measurements were performed with EC 570 electrochemical workstation (Gaoss Union Technology, Wuhan, China). Based on our previous research, the fabricated screen-printed three-electrodes were acted as the working, auxiliary and reference electrodes, just like strip-based electrochemical sensor. FE-SEM instrument (Quanta 200, FEI Coropration, Holland) and UV-Vis spectroscopic experiment were employed to describe the characterization of modified SPE.

The buffer solution and other solutions were freshly obtained with double distilled water. TP and other reagents were analytical grade which were attained from Sigma. The stock solutions of TP (1mM) was prepared with PBS buffer solution (0.1 M, pH 7.5), and stored at -20℃ prior to use. The aminophylline tables and roxithromycin capsule were purchased from Wuhan University Zhongnan Hosptial (Wuhan, China). The MWNTs (purity 95%) were bought from Chengdu Organic Chemicals Co., Lit, Chinese Academy Sciences (Sichuan, China). In addition, other materials, such as $HAuCl_4 \cdot 3H_2O$ and Poly-L-lysine hydrobromide (PLL, MW> 300,000), were obtained from Sigma.

2.2 Synthesis of nano-Au

According to the literature [1, 16, 17], the nano-Au was synthesized by the standard method with a little modification. Briefly, $HAuCl_4 \cdot 3H_2O$ solution (0.01%, 100 mL) was stirred in a three round-bottom flask. And then, when the reaction temperature reached the boiling point (about 100 ℃), 2.0 ml 1% citric sodium (wt %) was immediately mixed with the solution, finally constantly stirred

and boiled for 10 min. During the next reaction process, the temperature was maintained at 90 ℃. Due to colorimetric character of synthesized gold, when the color of solution gradually became brownish red, it was time that the nano-Au was achieved. After that, the UV-Vis spectroscopic experiment was further performed.

2.3 Preparation of MWNTs /Au/ Ploy-l-lysine SPE

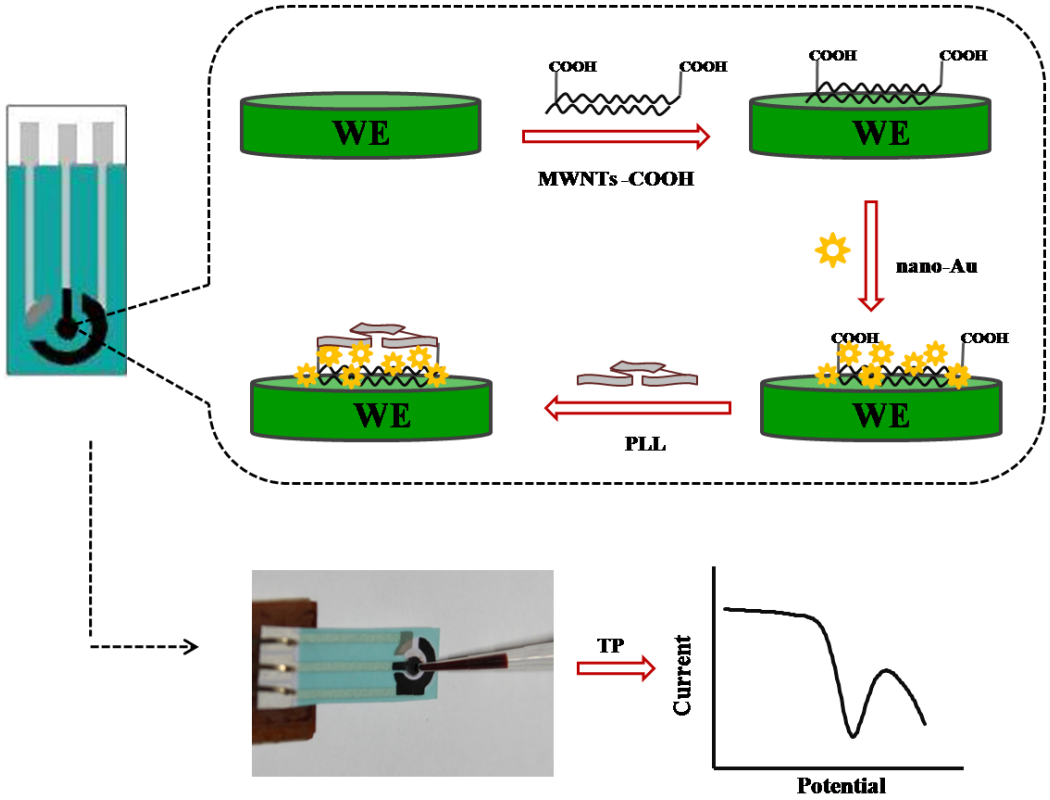

Figure 1. The fabrication procedure of the theophylline sensor.

The MWNTs were acidulated by HNO_3 and H_2SO_4 solution, coated with carboxyl groups. The MWNTs /Au/PLL screen-printed electrodes were prepared as following: dropping 2.0 μL MWNTs-COOH (2mg/mL), 1.5 μL synched nano-Au and 0.5 μL (2 mM) Poly-Lysine on the surface of working electrode, respectively. Because of the amino-group, PLL could make the nano-Au adsorb to the surface of MWNTs, and further increase the stability of the composited film. For comparison; MWNTs SPE and MWNTs/Au SPE were prepared to carry for the further investigation. The fabrication procedure of the modified sensor was displayed as Fig.1.

2.4 Real sample and treatment

The male SD rats (250–300g) were purchased from the A3 animal experimental centre of Wuhan University (SCXK 2008-0004). The ten rats were fasted for 12 h, after that, orally administered aminophylline tables which were dissolved with water at a dose of 20 mg/ kg. Next, five of them were

orally administered roxithromycin with 50 mg/kg. The rats which were only given aminophylline were called controlled group, the other rats were ordered experimental group. The blank blood samples were taken before the administration. And then blood samples of each rat were taken at different time points: 10 min, 20 min, 30 min, 60 min, 90 min, 120 min, 240 min, and 480 min after administration [39]. All blood samples were diluted with equal amount PBS buffer solution for the direct electrochemical assays by no pretreatment. As exhibited in Fig.1, the diluted blood samples was directly dropped on the SPE surface, and then immediately scanned with electrochemical square-wave voltammogram (SWV).

2.5 Analysis procedure

The appropriate amounts of TP were added to the sample solutions containing 0.1 M PBS (pH7.5) buffer solution. The cyclic voltammogram (CV)，differential pulse voltammogram (DPV) and square wave voltammogram (SWV) were respectively obtained to optimize the detection method by scanning the potential from 0 V to 1.3 V versus the Ag/AgCl reference electrode. And then, SWV was recorded from 0.1 V to 1.3 V with the parameters of amplitude 25 mV, step width 6 mV, frequency 20 Hz and quiet time 5 s.

According to the reports [35, 40], HPLC analysis method is frequently applied for monitoring TP. And based on Chinese pharmacopoeia, the standard method for determination of TP is also HPLC analysis. Thus, we also chose the HPLC method to detect the TP pharmacokinetic as reference. Based on the research, the HPLC condition was set with little modification as following: the chromatographic analysis was performed on a liquid chromatographic system equipped with a LC-10Avp plus HPLC module (Shimadzu, Japan). Chromatographic separation was achieved on the Agilent C18 column (250 · 4.6 mm, DI. 5 μm). The mobile phase for the analytical column was methanol–20 mM PBS (pH 7.0) (v/v 1:9). And before analysis, the mobile phases were vacuum-filtered through a 0.22 μm nylon filter. The chromatograms were monitored by UV detection at a wave-length of 274 nm.

3. RESULT AND DISCUSSION

3.1 Characterization of modified sensor

Through the scanning electron microscopy (SEM) and the UV-Vis spectroscopic, the surface morphology characterizations of screen-printed sensors were investigated, which the results were shown in the Fig.2. Compared with the previous reports [13], the nano-Au also appeared an absorption peak at about 525 nm (Fig.2 A) due to the characteristic absorption peak. In addition, the SEM images exhibited MWNTs (B), MWNTs-Au (C) and MWNTs/Au/PLL (D) film. The disposed MWNTs were covered on the sensor surface (Fig.2 B) to form the porous structure which was the same with studies [9, 11]. The spaghetti-like MWNTs could promote electron transfer rate and improve electrochemical

signal because of their remarkable catalytic structure. Fig.2 C showed the nano-Au was coated on the surface of carboxylic multi-walled carbon nanotubes as the capping agent. The experimental dates indicated MWNTs/Au composites presented better electrochemical performance. This situation was attributed to the electrochemical catalytic, nanoparticle size advantages, and because of the larger surface area [13] of nano-Au and MWNTs. However, the MWNTs film and MWNTs/Au film were not stable and would fall off in determination system. Based on the research [14, 15], some materials like chitosan, PLL and other polymer were recommended. In order to eliminating this problem, the PLL was chosen.

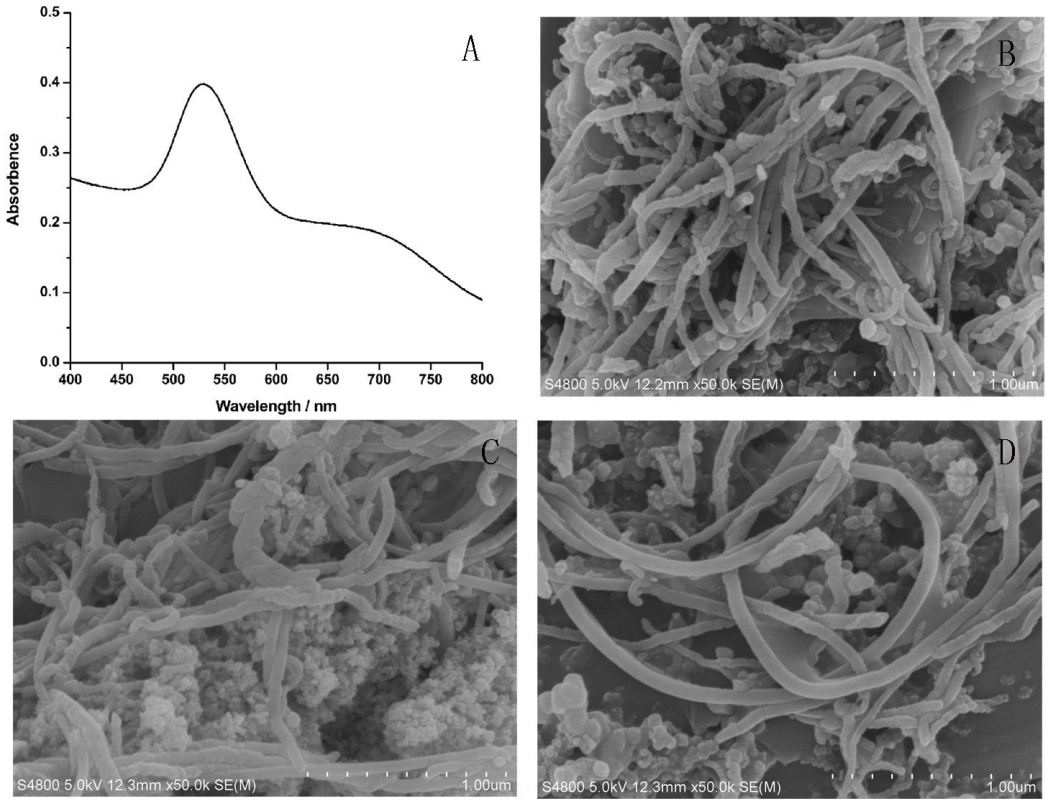

Figure 2. UA-vis absorption spectra of nano-Au (**A**), SEM images of MWNTs modified SPE (**B**), MWNTs/Au modified SPE (**C**), MWNTs /Au/PLL modified SPE (**D**)

Through abundant NH_2 groups, the PLL was adsorbed onto MWNTs/Au film successfully, which was favorable to the building of stabilized interface (Fig.2 D). The MWNTs/Au/PLL SPE was more sensitive for determination of TP during the following experiments.

3.2 Compare of analytical methods and electrochemical behavior of TP at modified SPE

First of all, the cyclic voltammogram (CV)， differential pulse voltammogram (DPV) and square wave voltammogram (SWV) were attained to explore the electrochemical behaviors of (100 μM) TP at bare screen-printed electrode. As displayed in figure, the bare disposable sensors presented

none electrochemical response (dotted line) in the blank PBS buffer with CV, DPV and SWV (Fig.3 A-C) methods. However, one irreversible oxidation peak (solid line) of TP appeared. Different with the literature [13], SWV method responded most sensitively in all of three methods, not the DPV method. These results demonstrated that the disposable sensor could be applied to detect TP by SWV method.

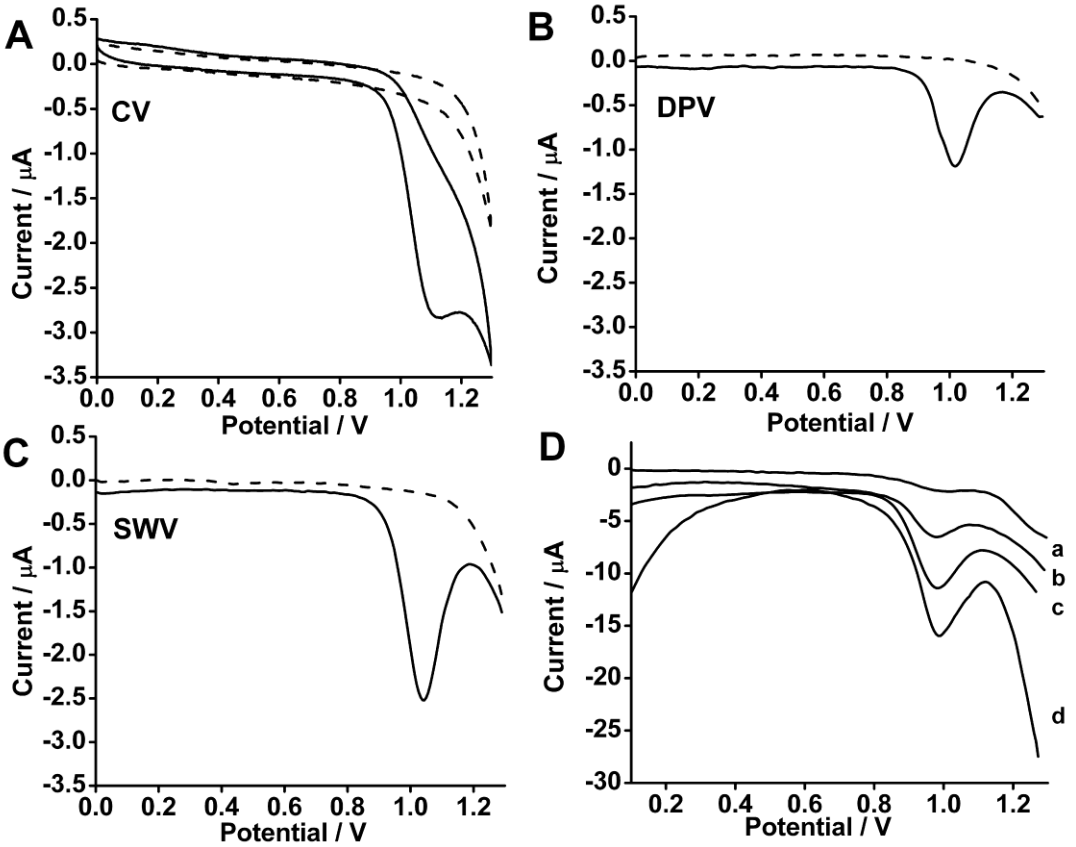

Figure 3.A Cyclic voltammogram (CV) curves of blank sample (dotted line) and 100 μM theophylline (solid line) at bare SPE in 0.1 M PBS buffer, scan rate: 100mV/s, **B** Differential pulse voltammogram (DPV) curves of blank sample (dotted line) and 100 μM theophylline (solid line) at bare SPE in 0.1 M PBS buffer, **C** Square wave voltammogram (SWV) curves of blank sample (dotted line) and 100 μM theophylline (solid line) at bare SPE in 0.1 M PBS buffer, scan frequency: 20Hz. **D** Square wave voltammograms of 50 μM theophylline at bare SPE (curve a), MWNTs SPE (curve b), MWNTs/Au SPE (curve c) and MWNTs /Au/PLL SPE (curve d) in 0.1 M PBS buffer, scan frequency: 20 Hz.

Then, the detail electrochemical behavior of TP at MWNTs/Au/PLL SPE was described with SWV in 0.1 M PBS buffer (pH 7.5). It could be observed the obvious oxidation peak (curve d) at about 0.98V was gotten at MWNTs/Au/PLL SPE (Fig.3 D), which was the same with results in references [41, 42]. On the contrary, only a small and wide anodic peak (curve a) at about 1.08V was obtained with bare SPE. As shown in reference [9], the high surface area and excellent electro conductivity of MWNTs was responsible for electrochemical signal increasing, which the oxidation peak (curve b) enhanced obviously at MWNTs SPE. The nano-Au improved the response peak current of TP further

by MWNTs/Au composites film (curve c). However, the enhancement of current response was not stable enough because the film would be washed off by the biological sample fluid. For the purpose of increasing the stability and sensitivity, as described in studies [22-24], the PLL played an important role to immobilize the composites through the plentiful active amino groups combining with the carboxyl groups of MWNTs. The curve d suggested that the MWNTs /Au/ PLL film was more stable and sensitive to accelerate electron transfer and catalyze the oxidation during the determination of TP. Hence, the MWNTs /Au/ PLL SPE was appropriate for the later study.

3.3 Effect of scan rate

The influence of scan rate on the oxidation peak current and potential of TP at the MWNTs/Au/ PLL SPE was evaluated by cyclic voltammetry. From Fig.4, the oxidation peak current of TP increased linearly with the square root of scan rate in the range of 10–300 mVs^{-1}. Moreover, the equation was obtained as Ip (μA) = 15.58021 + 3.37731 $v^{1/2}$ (mV s^{-1}), with the correlation coefficient of R=0.98855, which demonstrated that the oxidation process of TP on MWNTs/Au/ PLL SPE was diffusion-controlled (Fig. 4, inset).

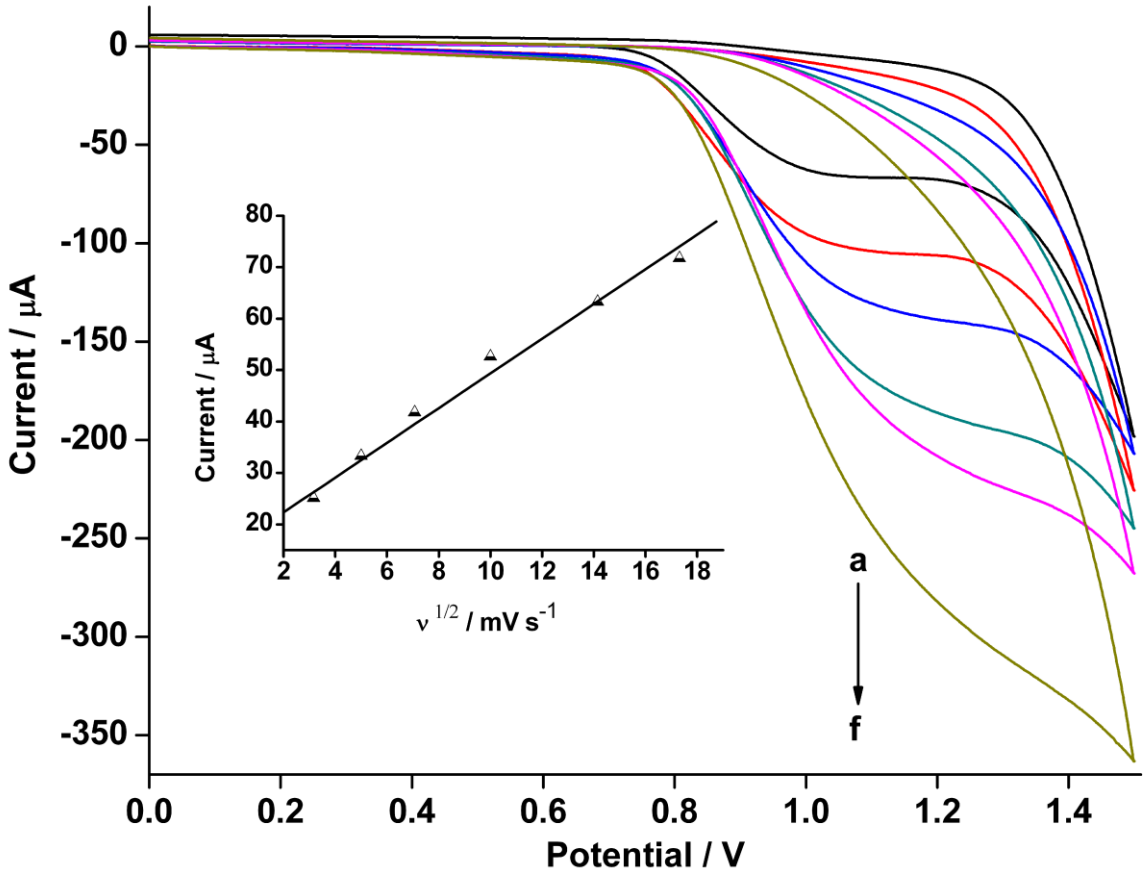

Figure 4. Cyclic voltammogram (CV) curves of 500 μM theophylline at MWNTs /Au/PLL SPE in 0.1 M PBS buffer at different scan rates (curve a to curve f contained 10, 25, 50, 100, 200, 300 mV s^{-1}. Inset: The dependence of the peak currents on the square root of the scan rates.

The oxidation peak potential Ep of theophylline also changed linearly with the logarithm of the scan rate (log *v*) from 10 to 300 mVs^{-1}. The potential moved positive shift based on the equation of E*p* = 0.789+ 0.166 log *v*, R= 0.99498. Based on the Laviron's theory, the value of αn_α was calculated as 0.90. As for a totally irreversible electrode reaction process, was assumed to be as 0.5. According to the mentioned result, the n_α was measured to be about 2, indicating 2 e^- to take part in the irreversible oxidation of redox-active theophylline

3.4 Effect of pH

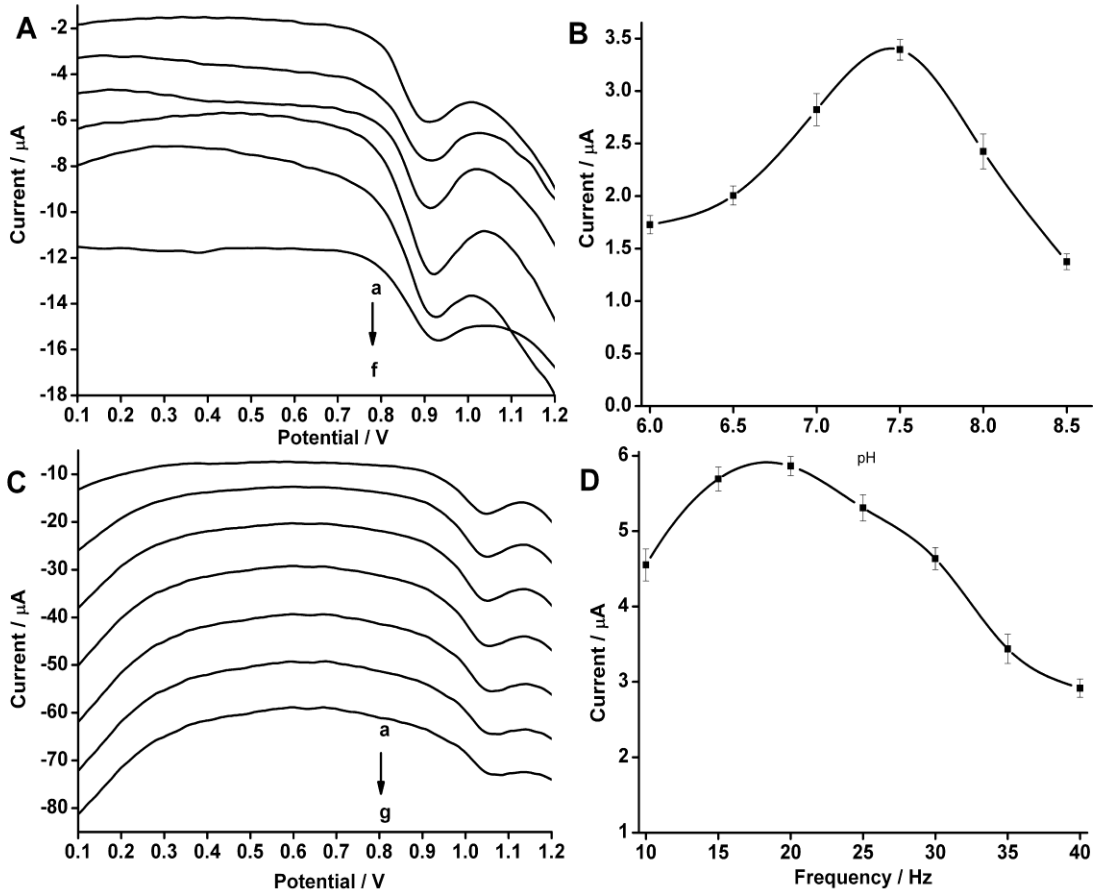

Figure 5 Square wave voltammogram curves of 50 μM theophylline at different pH (curve a to curve f: 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5) in 0.1 M PBS buffer (**A**) and the currents of theophylline response to pH (**B**); SWV curves of 50 μM theophylline at different frequency: (curve a to curve g: 10, 15, 20, 25, 30, 35 and 40 Hz) (**C**) and the currents of theophylline response to frequency (**D**).

The effect of pH values on the behavior of TP at MWNTs/Au/ PLL SPE was investigated by the experiment date and it aimed to optimize the electrocatalytic response of the modified electrode for the TP. SWV curves of modified sensor in 50 μM TP at different pH values were recorded as Fig.5 A and Fig.5 B. Just like reference [41], the variation tendency of pH *vs.* peak was the same. The peak current increased along with the increasing of pH value from 6.0 to 7.5. However, when the pH further increased, the oxidation peak current decreased. The pH 7.5 was the suitable condition for the

experiment. Therefore, the pH 7.5 was chosen as the optimum pH value for the biological sample determination.

3.5 Effect of the scan frequency

The scan frequency was one of most important parameters in SWV method. The study have discussed the linear sweep voltammograms (LSV) [4], indicating that the SWV parameter could affect the concentration curve detection. Consequently, the effect of different scan frequencies for detection of 50 μM TP was also screened (other parameters: constant scan increment = 6 mV and pulse amplitude 25 mV). From the Fig.5 C and Fig.5 D, as scan frequency increased in the range of 10-20 Hz, the oxidation peak currents increased continuously. Nevertheless, the high increasing frequency of 20-40 Hz led to opposite changes in peak currents. However, the result was different with the report [4], which might be attributed to the different modified electrode or the detection sample. Other sensor was used to detect two or more substance, that theophylline, isoproterenol or acetaminophen would produce multiple interference. In this work, the detection object was only the theophylline. So, the interference factor was less. Thus, the scan frequency of 20 Hz was more appropriate to the real monitoring by consideration.

3.6 Calibration and real sample analysis

In order to survey the concentrations of TP in the rat whole blood, the samples were treated through diluting 0.5 mL whole blood to 1.0 mL sample with 0.1M PBS (pH 7.5) for the electrochemical detection. And then, based on the optimized procedures mentioned above, the calibration curves of blood TP samples at MWNTs/Au/PLL SPE were attained by SWV (Fig.6). The oxidation peak currents of TP were linear to the concentrations in the range of 10 μM to 200 μM, with the linear regression equation as Ip (μA) = 0.0095c (μM) +0.0802 (R=0.9966). Furthermore, the detection limit of biological blood samples was calculated as 2.0 μM. Compared with the RNA sensor [44], the sensitivity of developed sensor was higher. And the fabricated sensor in this study was more gotten in production. Besides, as described in the Table1, the recoveries were found to be 96.68%-105.24%, and the RSD was less than 5%. The result was also acceptable in actual determination like other sensor.

Table 1. Determination of TP in rat blood samples by MWNTs/Au/poly-L-lysine.

Sample	Added c (μM)	Detected c $^{+}$ (μM)	Recovery (%)	RSD (%)
1	25.00	24.17	96.68	3.31
2	50.00	52.62	105.24	3.67
3	100.00	102.83	102.83	2.39

+ Average of five determinations.

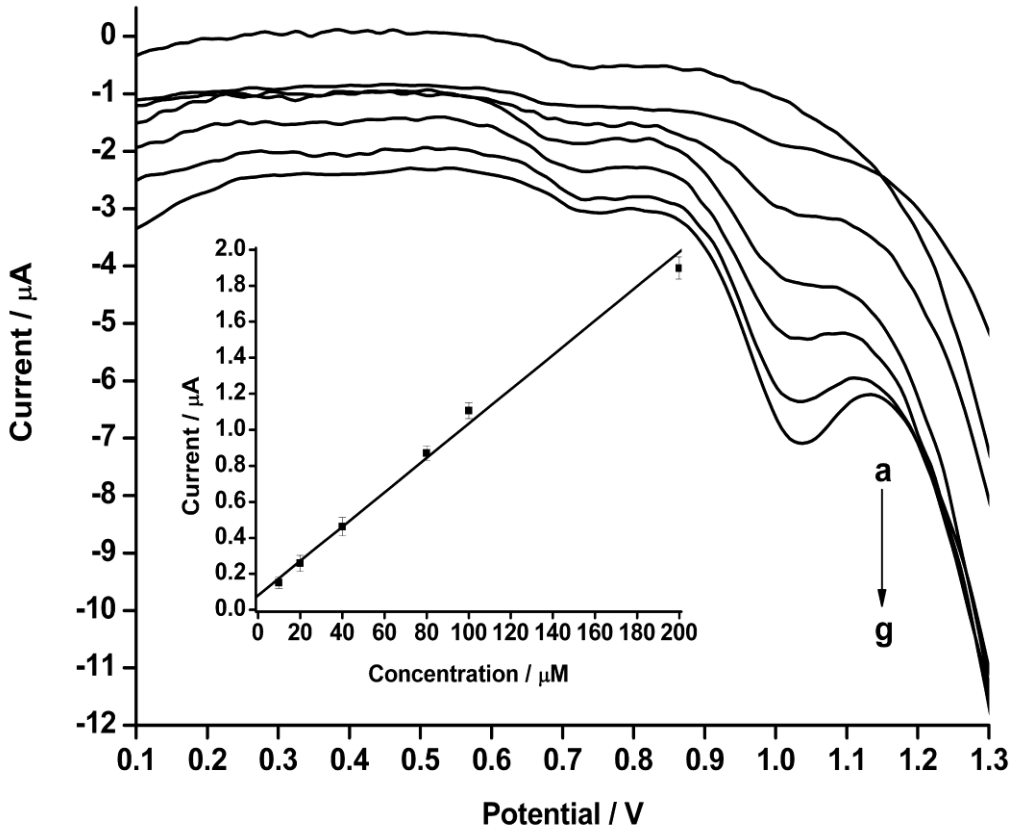

Figure 6. SWV curves of different concentration TP in blood samples (curve a to curve g: 0, 10, 20, 40, 80, 100, 200μM) under optical condition at the MWNTs/Au/PLL SPE. Insert: the calibration curve of TP (n=3).

In brief, the established method had good accuracy and repeatability. And these date again revealed that the proposed sensor was sufficient for practical detection of biological samples. As seen in fig.6, a peak at nearly 0.7 appeared at MWNTs/Au/PLL SPE in blank blood sample. Based on the report, this might attribute to the xanthine. The following interference experiment would be carried out.

Furthermore，as seen in Table 2, the developed sensor in this experiment was compared with other reported sensors[1, 4. 11, 13-15, 43-44]. It was the first time to apply the modified SPE to monitor theophylline metabolism in rats. The results turned out that the MWNTs/Au/PLL SPE successfully monitored the biological samples compared with other sensors.

Table 2. Comparison of the MWNTs/Au/PLL SPE for the determination of TP with other sensors

Methods	Linear range (μM)	Detection samples	Reference
AT-AuNps GCE	0.04-40.0	Human blood serum	[1]
1,4-BBFT/IL GPE	12.0-1200.0	Tea and blood serum	[4]
MWCNT-PE GCE	2.0-150.0	Tablet and urine samples	[11]
$MnOx/NH_2$-IL/Chit GCE	1.0-120.0	Table sample	[13]
GNP-CHIT-IL hybrid/r-GO GCE	0.05-2.0	Tea and tablet	[14]

GNP/l-cys/Gr/Nafion GCE	0.04-60.0	Tea and tablet	[15]
RNA AuNP	0.1-10	Serum	[43]
RNA GO	1-100	Serum	[44]
MWNTs /Au/PLL SPE	10.0 -200.0	Rat whole blood	This work

3.7 Reproducibility, stability of MWNTs/Au/PLL SPE and interference experiment.

The reproducibility of constructed sensor was evaluated for the response to 50 μM TP which was expressed as the relative standard deviation (R.S.D.) of 5.78% for n = 8. Different with other sensors [11, 13, 14, 43, 44], our sensor presented a good stockpile for a long time. The stability of modified sensor was investigated by recording the response to 50μM TP over 30 days. After stored at 4 ℃ refrigerator for 30 days, the peak currents at modified sensors still kept about 95.4% (n=8). The fabricated sensor showed good reproducibility and excellent stability. Other sensors such as RNA, CNTs etc modified glassy carbon electrode [14, 15, 43, 44], could not be stored in quantity for weeks. While, the MWNTs/Au/PLL SPE could achieve this goal.

Furthermore, the selectivity of established sensor was studied in biological sample. The interference experiment was performed as Fig.7. The curve a was SWV curve of blank blood sample, and an obvious oxidation peak emerged at about 0.73 V, which was attributed to the xanthine. This result was the same to the literature [1], which reported the xanthine was the main interferent in blood sample. In order eliminate the interference, 50 μM TP was added to blood samples. The obvious oxidation peak of TP appeared at about 1.0 V (curve b), which was not disturbed by the xanthine. In addition, the curve c was obtained to further investigate the effect of xanthine. After adding 500 μM xanthine, the results suggested that the oxidation peak of TP still maintained stable, and the change of peak current less than ±5% within the margin of error. In summary, the proposed SPE had good selectivity for the determination of TP in biological blood samples.

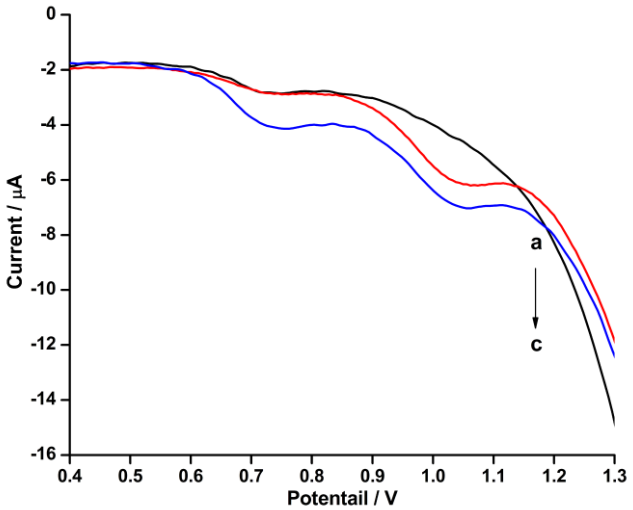

Figure 7. Evaluation of selectivity of MWNTs /Au/PLL SPE. SWV curves of blank blood sample (curve a), 50 μM added theophylline blood sample (curve b) and 500μM added xanthine blood sample.

3.8 Application to determination of TP pharmacokinetic in rats

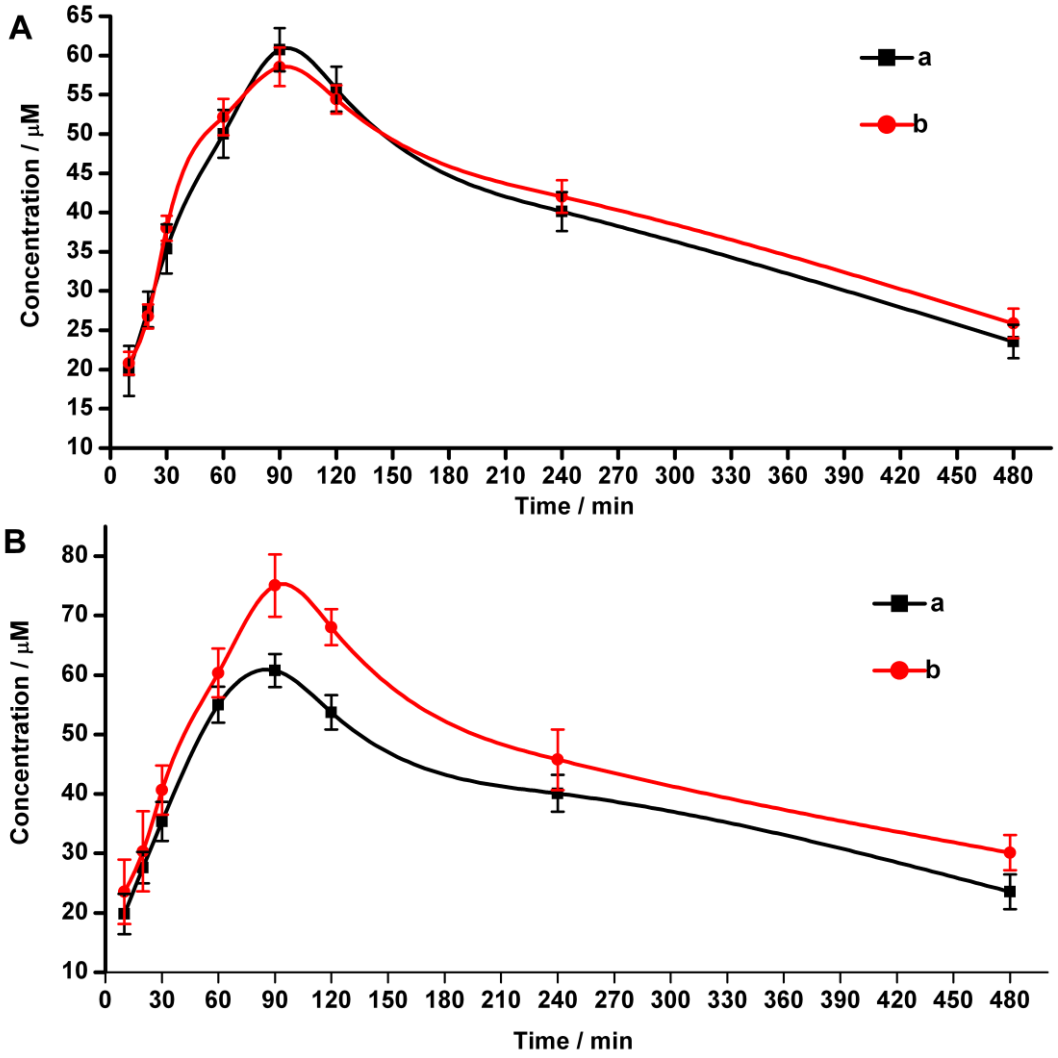

Figure 8.A The rat blood sample concentration-time course of theophylline after administration at different time points determined by proposed method (curve a) and HPLC method (curve b); **B** Comparison of the concentration-time courses of theophylline pharmacokinetic between controlled group rats and experimental group rats.

The developed method was applied to describe TP pharmacokinetic under the effect of roxithromycin in rats. It was first time to present the TP pharmacokinetic using modified sensor different with reports. Based on the experiment section，the blood samples were taken at different time points including 10 min, 20 min, 30 min, 60 min, 90 min, 120 min, 240 min and 480 min after a single oral 20 mg/ kg TP. The concentration–time profile of TP pharmacokinetic was shown in Fig.8.A (curve a) by modified sensor.

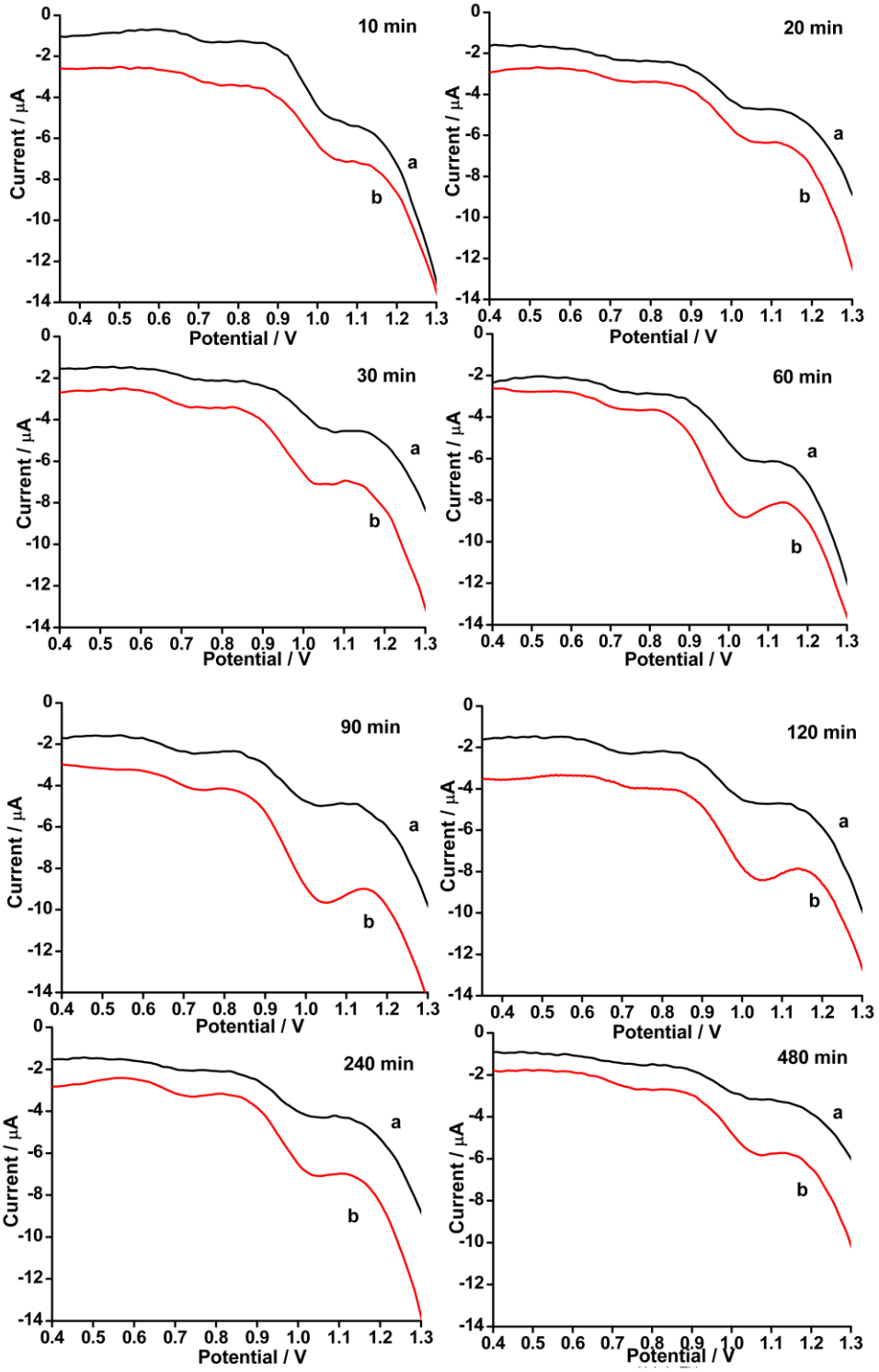

Figure 9. Some SWV curves of controlled group (curve a) and experimental group (curve b) at different time points.

Besides, HPLC method was also employed to attest the proposed method in this work (Fig.8.A curve b). HPLC method was the common recognition method in experiments [35, 40]. The results of proposed method and HPLC method represented the the metabolic tendency of TP concentration.

From figure 8.A, we could observe the curves were similar. The maximum drug concentration time detected by developed sensor and HPLC were both about 90 min. The TP reached the maximum concentration about 60μM with RSD less than 5% by two methods. These results were in agreement with the reports [39, 40], and the concentrations of TP gradually declined after 120 min. In summary, it proved again that the established sensor was accurate, reliable and effective during the practical determination. Furthermore, compared with the HPLC method, the TP pharmacokinetic monitor could be accomplished in 5 minutes by the disposable modified sensor without any pretreatment. It not only simplified the TP process, but also achieved whenever time point detection as quickly as few minutes, rather than some hours by HPLC.

For exploring the influence of the roxithromycin on the pharmacokinetic of TP in rats quickly, we employed the proposed method to constantly detect drug concentration at different time points after orally administering roxithromycin with 50 mg/kg, such as 10 min, 20 min, 30 min, 60 min, 90 min, 120 min, 240 min and 480 min. There was little literature about the pharmacokinetic of TP giving by sensor detection. The experimental results were reported and compared as in Fig.8.B. The curve a was the concentration–time profile that the rats were just orally given TP (curve a: controlled group), while the curve b presented the concentration–time tendency of experimental group rats (curve b). The roxithromycin would reduce the metabolic rate, and increase the concentration of TP in blood. The TP pharmacokinetic parameters of two group rats were also different. The maximum drug concentrations (C_{max}) of controlled group and experimental group rats were about 60 μM and 75μM, respectively. The maximum drug concentration time (t_{max}) was nearly the same to be about 90 min. However, the half-life period ($t_{1/2}$) of TP was prolonged from about 300 min to 330 min after orally administering roxithromycin. The results again demonstrated that the established method could be used for practical analysis of TP pharmacokinetic, even in the monitor of various drug combinations. Some SWV curves of controlled group and experimental group at different time points were shown in Fig.9.

4. CONCLUSION

To our best knowledge, few researches had published about disposable sensor for detection of the influence of roxithromycin on the TP pharmacokinetic in rats. In present study, the novel and simple method was suggested to monitor the TP concentration by sensor modified with MWNTs/Au/PLL composites film. It was the first time to study the TP pharmacokinetic situation after administering roxithromycin using proposed sensor. The experimental results revealed that the MWNTs/Au/PLL SPE performed well-defined redox response to TP with good sensitivity and high selectivity. By SWV, the electrochemical oxidation peak of TP was obtained at about 1.0 V, and the oxidation peak currents were increasing linearly with concentrations. Compared with HPLC, the established method displayed excellent characteristics including simple operation, fast detection procedures and good repeatability. Moreover, it didn't need deproteinization, centrifugalization and quantification pretreatments. Based on this key point, the fabricated sensor was successfully applied to directly determinate the blood TP pharmacokinetic process influenced by roxithromycin. The ideal

results indicated the present method was reliable, and the modified SPE could act as Glucose Test Strips to achieve rapid monitor for the intoxication effect of TP accumulation in clinical.

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