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Application of a Disposable Doxorubicin Sensor for Direct Determination of Clinical Drug Concentration in Patient Blood

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A novel simple method for direct detection of clinical drug doxorubicin (DOX) was developed by the disposable sensor. The MWNTs/Polt-L-lysine modified screen printed electrode (SPE) was fabricated to monitor the DOX pharmacokinetic in clinical blood samples. The electrochemical behavior of DOX was observed at MWNTs/Polt-L-lysine SPE by cyclic voltammetry (CV) and square wave voltammetry (SWV). The electrochemical oxidation peak current was greatly increased because of nanocomposites. Under optimized conditions, the oxidation peak current of DOX measured by SWV exhibited a good linear property with the increasing of the concentration in the range of 0.0025 μM to 0.25μM. The detection limit was founded to be 1.0 nM. Furthermore, the disposable DOX SPE was successfully used to monitor the clinical DOX pharmacokinetic with good results. The DOX concentration-time curve was also obtained by the established method. The developed DOX sensor could be successful to monitor the therapeutic drug for clinical individualized treatment.

Keywords: disposable sensor; doxorubicin; modified screen printed electrode; clinical sample determination

1. INTRODUCTION

Doxorubicin (DOX), (2R,4S)-4-(3-Amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyloxy)-2-hydro xyacetyl-1,2,3,4-tetrahydro-2,5,12-trihydroxy-7-methoxynaphthacene-6,11-dione, is an anthracycline antibiotic produced by *Streptomyces peucetius varieta caesius*, which is one of the most important anticancer drug currently in clinical because of its broad spectruman tineoplastic activity [1–4]. Since its discovery, DOX has been nearly recognised as the ‘‘gold standard’’ for the treatment of various cancers including solid breast, ovarian, lung and liver tumours [5–8]. However, the side effects such as

systemic toxicity, cardiotoxicity and drug resistance gradually appear during the therapy, which the clinical application is overshadowed sometimes [9, 10]. Therefore, based on the clinical or medical guide points, monitoring DOX concentrations in patients has become more and more necessary to assess toxicity, side effects, interactions and therapeutic efficiency.

Different analytical methods have been described for determination of DOX [11-15]. In conclusion, there are high performance liquid chromatography (HPLC), LC–MS/M, fluorescence, spectroscopy and electrochemical detection [16-20]. However, these methods have different advantages and disadvantages. Although, HPLC or LC/MS method presents high selectivity and sensitivity, it needs complicated pretreatment and time-consuming, which is not suitable for the clinical rapid determination. Because of low sensitivity and instability, fluorescence and spectroscopy cannot be applied for the factual clinical monitoring. Therefore, the methods that mentioned above seem to be not appropriate for the fast detection of clinical blood DOX concentration. Electrochemical method displays the unique advantage, such as high sensitivity, good specificity, no complex pretreatment to achieve DOX therapeutic drug monitoring.

Recently, the disposable screen-printed electrode (SPE) has attracted more and more interest due to different merits including small size, low cost, fast response, portability and disposability, especially during drug electrochemical determination [21-22]. Hence, the disposable SPE is studied for the clinical drug determination [23-24] in our prophase work. The obtained results revealed the disposable SPE could be used to detect therapeutic drug concentration through electrochemical methods in clinical. Thus, we guessed the SPE could also monitor the anticancer drug DOX to guide the clinical treatment.

In present study, a simple and novel sensor is developed for analysis of clinical DOX concentration based on SPE. Our purpose is to accomplish rapid determination for clinical DOX therapeutic drug monitoring with few complicated pretreatment. Through our knowledge, the research concerning DOX fast detection in the clinical biological samples by using modified SPE has been never reported before, , especially the clinical blood samples.

Based on the superior geometrical and chemical properties, the multi-walled carbon nanotubes (MWCNTs) are always the good catalytical material in the application of electrochemistry since discovery [25-27]. Sensors based on the MWCNTs have shown high sensitivity and good detection limit through promoting electron transfer reaction and enhancing electrochemical signal. Hence, this result offers the theory basis for our following experiment. The literature reported [28-31], the poly-L-lysine (PLL) is an excellent choice to fabricate sensors through its cationic amino-group. During the previous studies, PPL has been found as the linker to immobilize DNA, graphene oxide, carbon nanotubes (CNTs) and other materials on the electrode surface by electro-polymerization with plentiful active amino groups and flexible molecular backbone. However, the attempt of developing DOX SPE modified with MWNTs/PLL is not reported before.

In this work, the clinical doxorubicin blood samples were first examined by the novel DOX SPE modified with MWCNTs/PLL. This proposed sensor, just like Glucose Test Strips, offered a simple and fast way for clinical DOX monitoring with few minutes. Compared with other work in literature, good clinical detection result was obtained.

2. MATERIAL AND METHODS

2.1. Reagents and apparatus

The acidulated MWCNTs (MWCNT-COOH, purity>90%）was purchased from Chengdu Organic Chemicals Co., Lit, Chinese Academy Sciences (Sichuan, China). Poly-L-lysine hydrobromide (PLL, Mw> 300,000) were also employed from Sigma. The 1 mM DOX stock solutions acquired by dissolving with doubled distilled water. And then the stock solution was stored at -20℃ for the continuous experiments. HAc–NaAc buffer solutions (0.1 M, pH 4.5) were employed as the supporting electrolyte. All chemicals and solvents used were of analytical grade.

As Fig.1 displayed, the traditional three electrode system, such as carbon working electrode, Ag/AgCl reference electrode and carbon auxiliary electrode, was replaced by our disposable SPE. Meanwhile, the EC 570 electrochemical workstation (Gaoss Union Technology, Wuhan, China) was obtained to record the voltammogram curves by SPE. Besides, Field Emission Scanning Electron Microscope (FE-SEM) instrument (Quanta 200, FEI Coropration, Holland) was performed for the characterization of modified SPE.

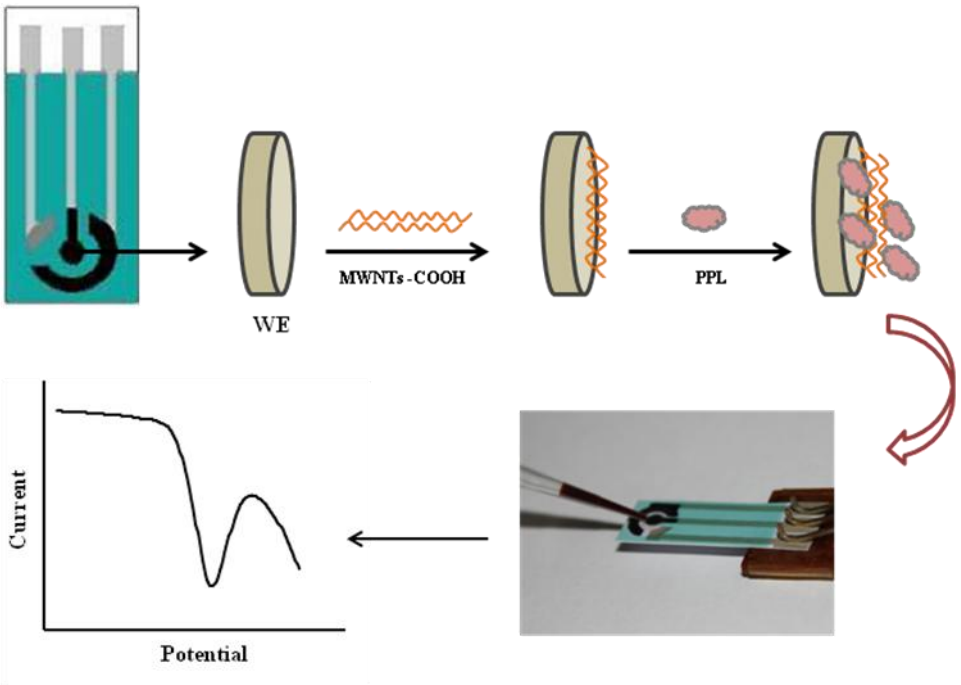

Figure 1. The flow-process diagram of the modified SPE and the real sample analysis

2.2 Preparation of MWCNTs/PLL SPE

The bare screen-printed electrode was covered with MWCNTs/PLL as followings. The 2 mg acidulated MWCNTs and 2mg CTAB were added to 1 mL aqueous solution with ultrasonication for 2 h. Then, the 1.5 μL of MWCNTs solution was fixed on the carbon working electrodes, air-dried for 30 min at room temperature. After that, the MWCNTs /PLL SPE was gotten by dropping 0.5 μL Poly-Lysine (2 mM) on the surface of working electrode. The PLL would fix nanoparticles due to the

amino-group, and increase the stability of the film. As the contrast, bare SPE and MWCNTs SPE were produced and applied to the further investigation. The flow-process diagram of modification was shown in Fig.1.

2.3 Electrochemical analytical procedure

The different amounts of DOX were added to the detection systems and blood samples containing 0.1 mol L^{-1} HAc-NaAc (pH 4.5) buffer solution. The electrochemical behavior curves were recorded by cyclic voltammetric curves (CV) and square wave voltammogram (SWV), respectively. The potential window was in the range of –1.0 to 1.0 V and -1.0 to -0.2 V versus the Ag/AgCl reference electrode. Meanwhile, the influences of pH, accumulation time, scan frequency and selectivity were also discussed to attain the optimal detection condition. The accumulation step was carried out under open-circuit for 3 min. All electrochemical experiments were carried out at room temperature. The CV relevant parameters were set as followings: initial potential -1.0 V, end potential 1.0V, sample interval = 1 mV, scan rate = 100 mV/s. However, the SWV scan parameters were protocoled as: initial potential = -1.0 V, end potential = -0.2V or, step width =5 mV, amplitude =25 mV, scan frequency =25 Hz.

2.4 Determination of real sample

All blood samples were acquired from the clinical volunteers in Wuhan NO.3 Hospital. Based on the previous researches [8, 12], the informed consent was signed with the clinical patient before collecting the blood samples. The female patient (62 kg) was given slow intravenous drip at the dose of 40 mg DOX. As the control group, the blank blood sample was gotten for measuring as 0 h point before the intravenous drip DOX administration. Next, the DOX-containing blood samples were obtained at 0.5 h, 1 h and 2 h after administration. After that, the samples were diluted for the electrochemical analysis without any pretreatment. Every blood sample was diluted from 1.0 mL to 2.0 mL with NaAc-Hac buffer solution（pH 4.5）in electrolytic system cell. Next, SWV was chosen to immediately determinate DOX concentration. As shown in Fig.1, the diluted human whole blood was directly added as a droplet on the SPE surface and scanned under optimal condition by SWV at once.

3. RESULT AND DISCUSSION

3.1 Characterization of bare SPE, MWCNTs SPE and MWCNTs/PLL SPE

The characterization of SPE surface morphology was evaluated by SEM. Thus, the surface topographies of the different modified electrodes were shown as following. Fig.2.A exhibited the SEM image of bare SPE. We could observe that lots of graphite particles were covered on the surface. The modified SPE was different with the bare SPE. The MWCNTs were attached on the surface to increase the electrochemical transfer rate due to the unique high surface area of physical dimension (as seen in Fig.2.B). However, we found the deposited MWCNTs particles could be easily washed away in the experimental process. In order to promote the stability of the MWCNTs SPE, the poly-L-lysine

(PPL) was recommended. From the Fig.2.C, MWCNTs/PPL was fabricated to be a good film by dropping the PPL on the MWCNTs substrate. It was known that there were a lot of NH_2 groups and COOH groups on the chain of PPL and MWCNTs, respectively. So, it was beneficial for the composite film to establish more stabilized interface. In addition, the fixed film greatly increased the effective surface area for capturing DOX and amplifying the electrochemical signal. Thus, the MWCNTs/PLL SPE was attained for successfully detection of DOX in next experiments. The result was consistent with the literatures [32, 33].

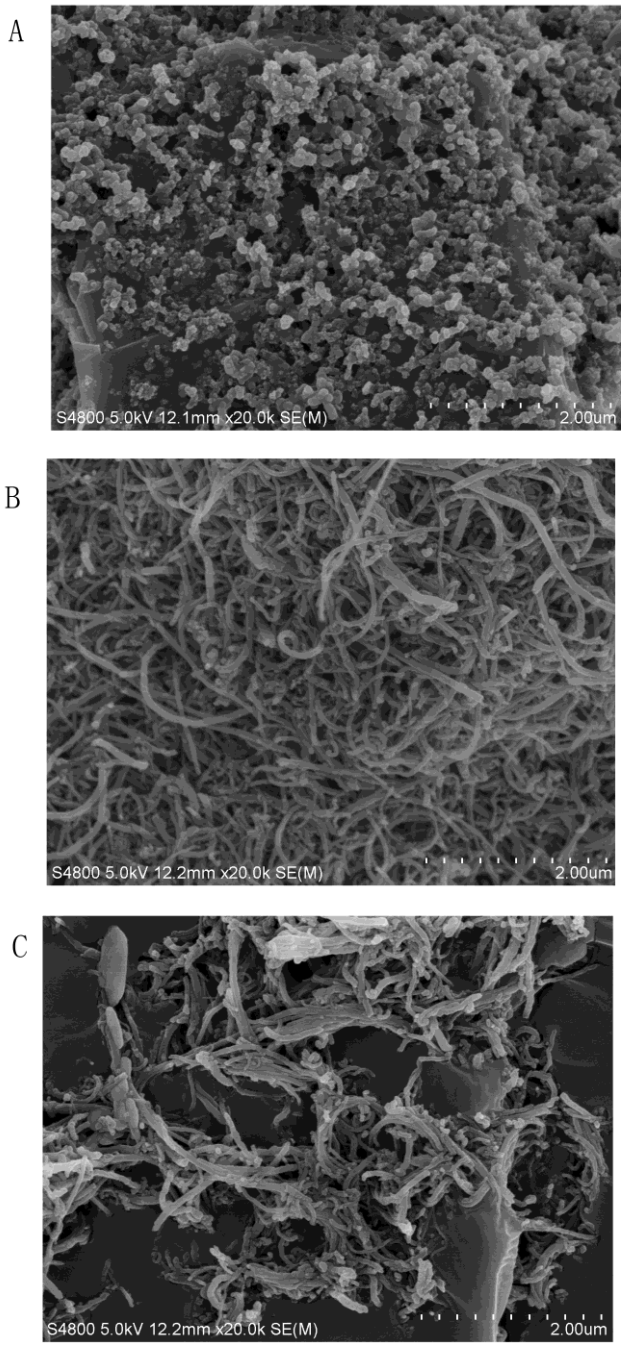

Figure 2. FE-SEM images of a bare SPE (**A**), MWCNTs modified SPE (**B**) and MWCNTs/PPL modified SPE (**C**)

3.2 Electrochemical behavior of DOX at MWCNTs/PPL SPE

The electrochemical behavior of DOX at the different modified SPE was investigated by CV in 0.1M HAc-NaAc buffer. The CV curves of DOX were obtained at bare SPE, MWCNTs SPE and MWCNTs/PPL SPE in Fig.3.A. Due to the structure of DOX, there were two pairs of redox peaks appearing at the bare SPE (curves a) at about -0.63V and 0.38V, respectively. While, curve b in Fig.3.A revealed the redox peak currents were greatly enhanced at MWNTs SPE. This result could be mainly ascribed to the remarkable catalytic effect and nano-size effect of MWCNTs. As previous literatures reported [16, 17], the MWNTs would promote ion-exchange rate and amply electrochemical behavior signal multi-times during the determination. However, the effect of electrochemical signal amplification was decreased because the MWCNTs on the SPE surface were rushed off in the fluid detection system. Consequently, the enhancement about the DOX peak current was greatest at the MWCNTs/PPL SPE (curve c). This result strongly demonstrated the MWCNTs/PPL could effectively catalyze the electrooxidation reaction of DOX which was owe to the large surface area, subtle electronic properties, stability of composite film. The peak potentials of DOX were about -0.69 V and 0.32 V at the MWCNTs/PPL SPE which the peak shapes at -0.69 V was more sensitive rather than at 0.38 V. Besides, the the peak shape at 0.4 V was possibly disturbed in real samples because of urine. These results were similar with reports [19, 27]. Thus, the peak currents of DOX at about -0.69 V was chosen for the further study.

Meanwhile, the SWV behavior of DOX was also explored at different sensors [28]. Fig.3.B exhibited the SWV curves, and an oxidation peak was found at -0.64V. Compared with bare SPE (curve a) and MWCNTs SPE (curve b), the DOX peak current enhanced greatest at the MWCNTs/PPL SPE (curve c). High current could be ascribed to the large surface area and excellent electro conductivity of MWCNTs. But the increase was not obvious in curve b (as seen in Fig.3.B) duo to the unstable MWCNTs film. Afterwards, this phenomenon was improved by the composited film. Curve c confirmed the MWCNTs/PPL SPE could greatly catalyze the electrooxidation of DOX and increase the peak shape by SWV with stable and good results. Thus, the SWV was selected for the sensitive determination by the MWCNTs/PPL SPE.

3.3 The effect of pH

The pH value of supporting electrolyte was the important factor during the real detection. So, the peak current (I_{pa}) of DOX was evaluated with different pH values in the range from 3.5 to 6.5. As observed in Fig.4.A, the peak current of DOX reached a maximum during pH 5.0-5.5. And next, the peak current decreased with the increasing of pH value. Besides, the peak potential E_p of DOX was also discussed. The Fig.4.B indicated that the Ep of DOX shifted to negative values when pH increased. The relationship between peak potential of DOX and pH was as the following equations: E_p(V)=-0.3828-0.0565pH(R=0.9945). Considering the actual determination, the pH 5.5 could be chosen as the optimum pH. When the pH was 5.5, the peak potential of DOX was about -0.703 V.

3.4 The effect of scan frequency

The scan frequency of SWV was requisite condition in detection system. Based on this reason, the influences of different scan frequencies on the 0.05 μM DOX at the MWCNTs/PLL SPE were explored by SWV, for a constant scan increment of 6 mV and pulse amplitude of 25 mV. From Fig.4.C, it could be obviously observed that the current increased when frequency increased in the range from 10 to 25 Hz. While, when the frequency continuously raised, the peak current decreased inversely. Therefore, the optimal frequency was designated as 25 Hz. The result was the same to the literature [28].

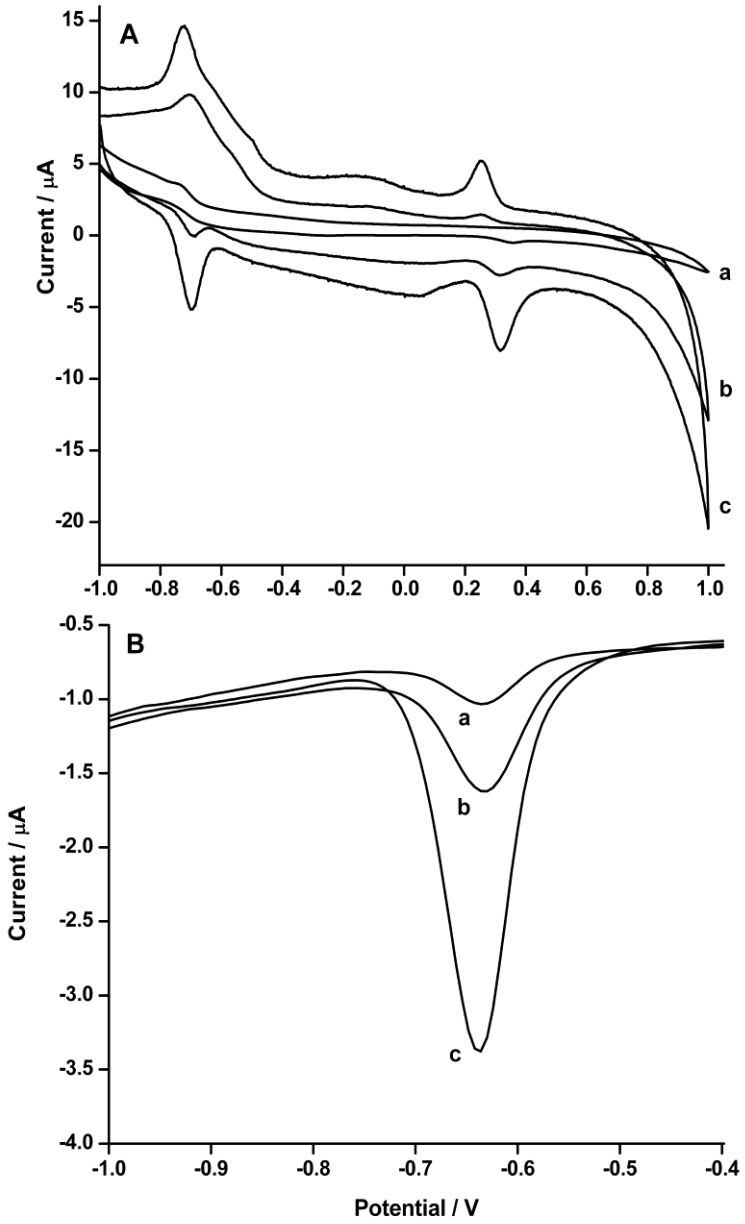

Figure 3 Cyclic voltammogram curves (**A**) of 2μM DOX at bare SPE (a), MWCNTs SPE (b), MWCNTs/PPL SPE (c) in 0.1M HAc-NaAc buffer, scan rate: 100mV/s. Square wave voltammograms (**B**) of 0.1 μ M DOX at bare SPE (a), MWCNTs SPE (b), MWCNTs/PPL SPE (c) in 0.1M HAc-NaAc buffer, scan frequency: 25Hz.

3.5 The effect of accumulation time

According to other reports [19, 26], accumulation time is usually regarded as the important parameter to enhance the detection sensitivity. Thus, the influence of accumulation time on the peak current was studied. As presented in Fig.4.D, the peak current 0.05 μM DOX increased with the increasing of accumulation time. After that, it reached the maximum when accumulation time was more than 3 min. Therefore, the accumulation time of 3 min was decided for further determination of DOX in real samples.

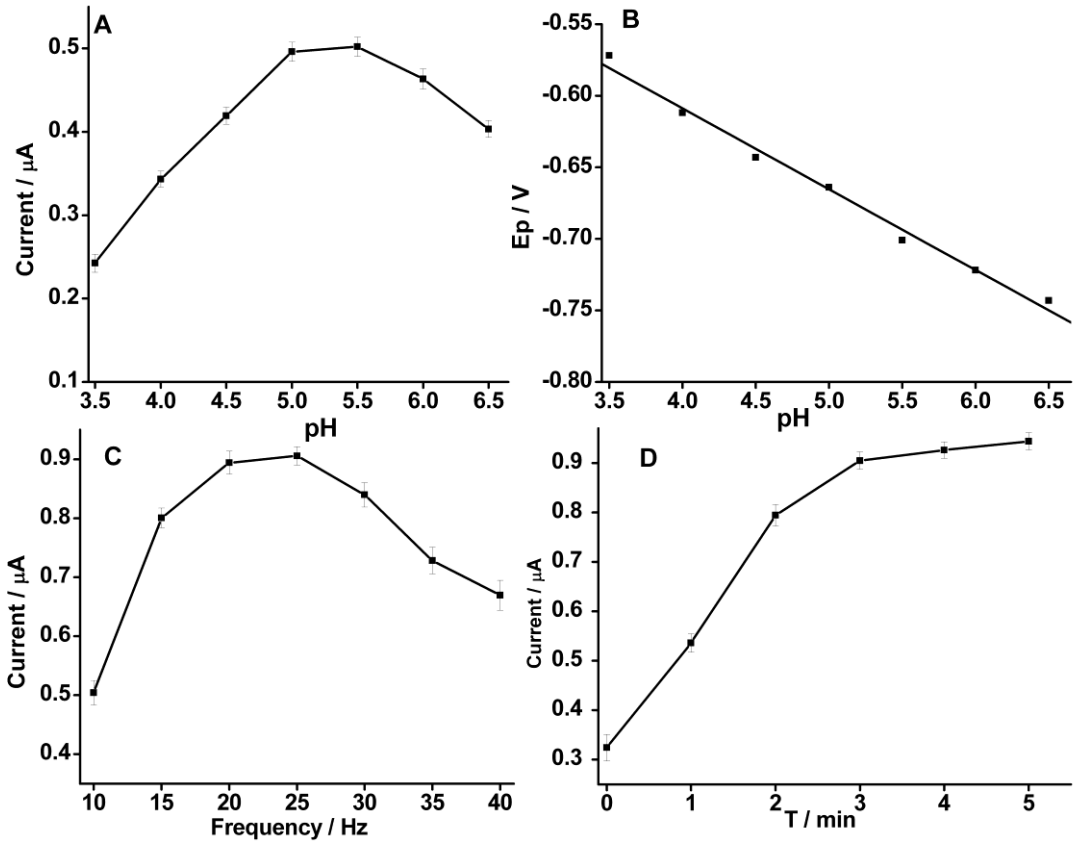

Figure 4. Effects of pH on the electrochemical currents of 0.05μM DOX in 0.1M HAc-NaAc buffer at the MWCNTs/PPL SPE (**A**); the relationship of the peak potential Ep of 0.05μM DOX against pH (**B**); the peak current responses of square wave voltammogram with different frequencies to 0.05μM DOX (**C**); the relationship between accumulation time and peak currents of 0.05μM DOX in 0.1 M pH 4.5 acetic acid buffer (**D**).

3.6 Calibration curve and limit detection

In order to further investigate the electrochemical responses of different concentration DOX, the SWV calibration was performed under the optimized analytical conditions: potential parameters =-1.0 to -0.4V, step width =6 mV; amplitude =25 mV, frequency =25 Hz. The calibration procedure of clinical healthy human blood samples was established as following. The diluted clinical blood sample was obtained by adding the supporting electrolyte. For example, 0.5 mL HAc-NaAc buffer containing different concentration DOX was mixed with 0.5 mL blank blood sample. Finally, the 0, 0.0025,

0.005, 0.01, 0.025, 0.05, 0.1 and 0.25μM DOX-containing blood samples were prepared for the analysis detection. Based on the previous optimal voltammetry procedure, the calibration curves of blood DOX samples were presented in Fig.5. With repeating five times, the SWV calibration curves revealed the peak current was linear with DOX concentration in the range from 0.0025 μM to 0.25 μM. Besides, the linear regression equation was displayed as Ip (μA) = 0.0270c (μM) +0.2597 (R=0.9954) with the detection limit of 1.0 nM. The SWV curve a at MWCNTs/PPL SPE in blank blood sample indicated there was no interference oxidation peak. After the addition of different concentration DOX to the blank blood samples, an obvious oxidation peak was found at about -0.625V. This result demonstrated the DOX determination was not disturbed which was similar to the previous researches [34]. The relative standard deviations (RSD) of 0.05uM DOX on different electrodes were about 5.2% (n=10), indicating good reproducibility. The storage stability of MWCNTs/PPL SPE was also well kept for over three months.

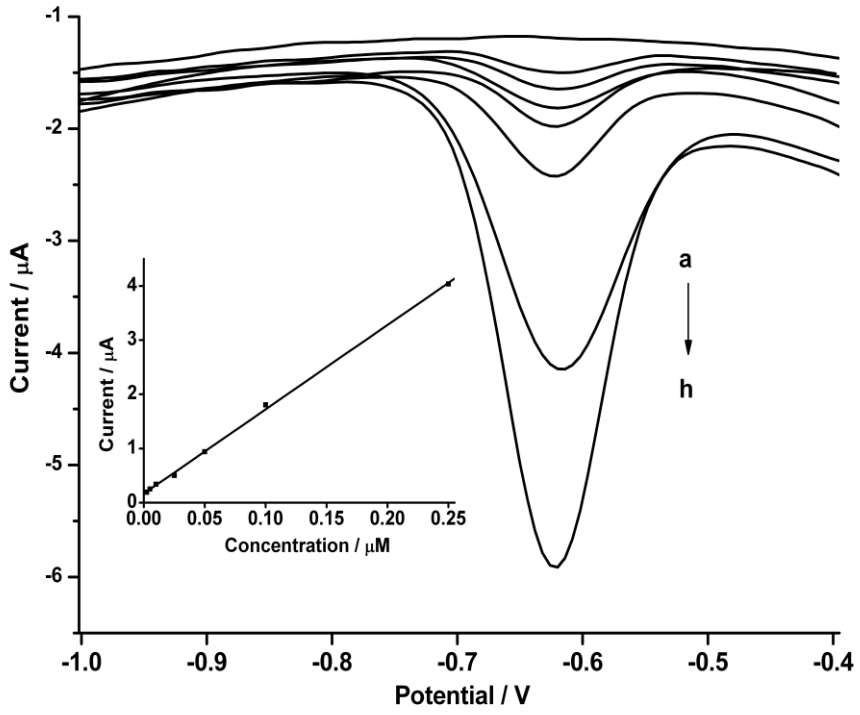

Figure 5. Square wave voltammograms of different concentration DOX in blood sample diluted with 0.1μM HAc-NAc (pH 4.5), frequency 25Hz, DOX concentration from a to h: 0, 0.0025, 0.005, 0.01, 0.025, 0.05, 0.1, 0.25 μM,

Furthermore, the developed sensor in our work was compared with other researches [16-20, 26, 27], as listed in the Table 1. To our knowledge, there was little study about the disposable doxorubicin sensor for monitoring clinical blood samples. It was the first attempt to detect clinical doxorubicin using MWCNTs/PPL SPE. The result showed the fabricated sensor could be successfully used to immediately determinate clinical whole blood samples. In addition, compared with serum samples, the whole blood sample detection in present paper does not need deproteinization, centrifugalization and quantification pretreatments which simplified the clinical analysis process. The results would be

obtained in few minutes. In addition, the established method could also provide many advantages in instantaneous monitoring and measurement for doctors. Most important of all, the fabrication of MWCNTs /PPL sensor was easy to achieve mass production and industrialization. Compared with the materials such as nano-Au, protein anti body and DNA, the MWCNTs and PPL presented the stable physical and chemical properties, which these materials were simple to get and the cost was also relatively lower a lot.

Table 1. Comparison of the linear range and detection samples gotten at the MWCNTs /PPL modified SPE for detection of DOX with other sensors.

Methods	Linear range (nM)	Detection samples	Reference
dsDNA/PAM GCE	——	Drug sample	[16]
Silver-amalgam Hg(Ag)FE	9.2-109.7	Human urine sample	[17]
Solid lipid nanoparticles	79.0-298.0	Cosmetic and tablet	[18]
Magneticgraphene GCE	17.0-12114.0	Plasma	[19]
Layered double hydroxide GCE	10.0-2110.0	Plasma and urine sample	[20]
Cyclodextrin-Graphene GCE	10.0-200.0	Tablet sample	[26]
AuNPs -GMCs GCE	0.1-11.8	Drug and tablet	[27]
MWCNTs/PPL SPE	2.5 -250.0	Clinical whole blood	This work

3.7 Selectivity and reproducibility of MWCNTs /PLL SPE

For inspecting the analytical selectivity in clinic application, the influences of various interferences in biological samples were evaluated. The effect of different substance on the peak current of DOX was measured, containing 100 μM glucose, 100 μM ascorbic acid, 100 μM dopamine and100 μM uric acid, which would affected the real biological sample detection. The oxidation peaks of ascorbic acid, dopamine and uric acid were gained at about 0.2-0.4 V, which the DOX detection was not disturbed.

The recovery and reproducibility were also investigated as Table 2. From Table 2, the average recovery of independent experiments was calculated to be 92.60% to 106.87%. Besides, the relative error (RSD) was also counted about ±5%. The proposed method was demonstrated to have good reproducibility for the determination of DOX.

Table 2. Determination of DOX in clinical blood samples by MWCNTs/PPL SPE

Sample	Added c (nM)	Detected c^{+} (nM)	Recovery (%)	RSD (%)
1	10	9.26	92.60	5.25
2	50	051.12	102.24	4.73
3	100	106.87	106.87	3.37

+ Average of five determinations.

3.8 Clinical real sample analysis

It was the first time to describe the time course of clinical DOX concentration by MWCNTs/PPL sensor in this study. As designed in experimental section 2.5, the DOX concentrations of clinical patient were monitored, and the time–concentration curve was also carried out. The DOX concentration was detected zero at 0 h before intravenous drip administration, which could deduct the possible DOX interference. Then, after giving drug intravenous drip, the DOX-containing blood samples were collected at different time points such as 0.5 h, 1 h, and 2 h. The Fig. 6 revealed the DOX pharmacokinetics curve in clinical patient. The DOX concentration presented gradually attenuation over time, which the monitor result was the sample to the studies [8, 12]. Moreover, the blood concentration of DOX was lower than the detection range after 2 hours. The clinical detected result further suggested the proposed methods could achieve the therapeutic drug monitoring and guide individual treatment.

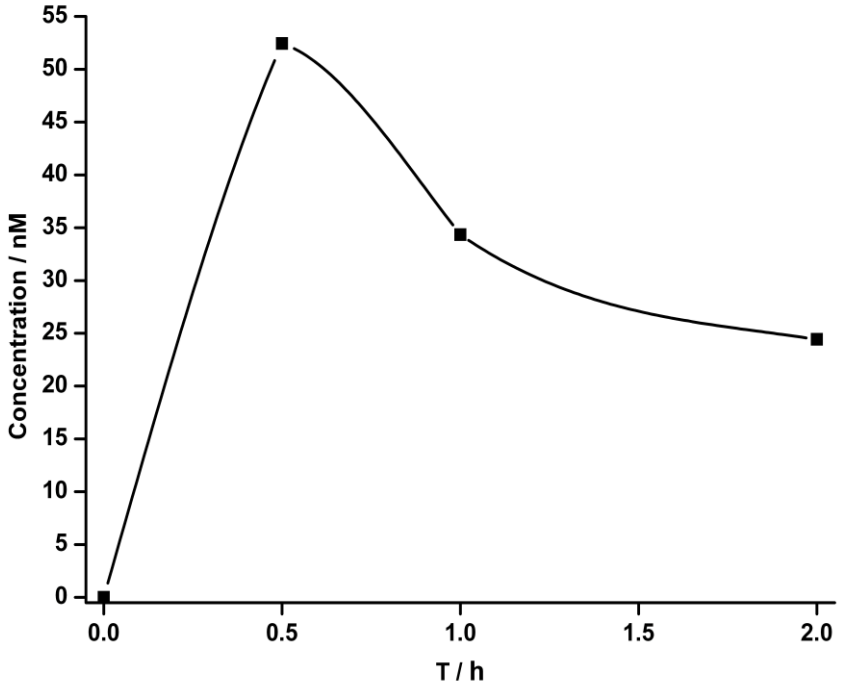

Figure 6. Concentration–time profiles of DOX in clinical patient whole blood after a single-dose administration of 40 mg DOX

4. CONCLUSION

The simple and novel DOX sensor based on the MWCNTs/PPL composites was established and successfully applied to determinate clinical DOX whole blood samples. The modified SPE exhibited well-defined redox response to DOX oxidation. The result exhibited fine stability and efficient electrocatalytic activity. The electrochemical oxidation peak of DOX at modified sensor was attained at about -0.63 V by SWV. The detection results of clinical samples indicated that the proposed sensor showed excellent characteristics, for example, low production cost, easy mass industrialization, high sensitivity, and simple fast analysis procedures. According to the above points, the developed

sensor could provide possibility to produce the clinical medical diagnosis apparatus and instrument for determination of clinical drug. And the DOX sensor might be similar to Glucose Test Strips.

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