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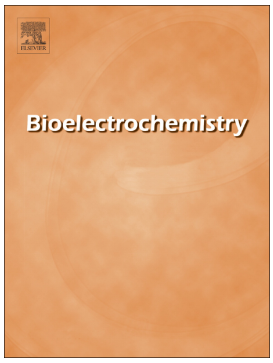

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Ag-4-ATP-MWCNT electrode modified with dsDNA as label-free electrochemical sensor for the detection of daunorubicin anticancer drug

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Abstract

This paper describes the synthesis of Ag-4-ATP-MWCNT nanocomposite and its use as a modifier of working electrode. The surface of the electrochemical Ag-4-ATP-MWCNT electrode was modified with a double-stranded DNA (dsDNA) to detect daunorubicin-DNA interactions. Differential pulse voltammetry was applied to develop an electroanalytical procedure for the determination of daunorubicin and evaluate its interaction with dsDNA immobilized on the electrode surface. After the optimization of operational parameters, the linear dependence of the peak current on the daunorubicin concentration was observed in the range of 0.10×10^{-8} to 1.00×10^{-5} mol L^{-1}, with the detection and quantification limits of 3.00×10^{-10} and 1.00×10^{-9} mol L^{-1}, respectively. The proposed biosensor was successfully applied to validate its capability for the determination of daunorubicin in human serum and urine samples.

Keywords: Ag-4-ATP-MWCNT, dsDNA biosensor, daunorubicin anticancer drug, voltammetry.

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1. Introduction

Biosensors are analytical devices that combine a biological part with a physicochemical detector and are used for the detection of an analyte [1].

Biosensors have been utilized to detect a variety of biomolecular complexes, such as oligonucleotides [2–5], antibody-antigen interactions [6, 7], hormone-receptor interactions [8], enzyme-substrate interactions [9, 10], and lectin-glycoprotein interactions [11].

These biosensors can be fabricated by modifying the surface of electrodes with biological compounds such as DNA, enzymes, and antibodies [12, 13].

DNA biosensors have attracted enormous attention due to their wide applications such as gene analysis, clinical diagnostics, and even forensic applications [14, 15].

The interaction mechanism of drugs with DNA has been investigated during the past decades [16, 17]. Bard et al investigated the interaction between DNA and chemical compounds, like metal ions and their complexes, water, organic molecules and proteins and this interaction is reversible [18]. Some compounds like carcinogens and drugs interact with DNA which results in irreversible damage and so the harmful effects of these drugs should be investigated potentially. These researches are valuable, since they are considered as an immediate response to harmful substances or as important clues in targeted development of novel chemotherapeutic.

Daunorubicin (DNR), which is also known as daunomycin, is one of new approved anthracycline anticancer drugs intercalated with the DNA molecule and inhibiting the synthesis of nucleic acid. It consists of a weakly basic amino sugar, daunosamine, linked via a glycosidic bond to the red-pigmented tetracyclic moiety daunomycinone [19]. This four-ring structure is the chromophore of the anthracycline molecule, and aqueous solutions of DNR are red. The anthraquinone ring also has the ability to intercalate between DNA base pairs [20, 21]. DNR is indicated for remission induction in acute non-lymphocytic leukemia of adults and for remission induction in acute lymphocytic leukemia of children and adults [22-26].

Currently, nanocomposites are attracting enormous attention as an integrated miniaturized device using biological element [27].

Carbon nanotubes (CNTs) have been widely used as electrode modifying materials because they give unique advantages such as improvement of electronic properties, a large surface area to volume ratio and rapid electrode kinetics [28].

Presently, modification of an electrode by noble metal nanoparticles, particularly silver nanoparticles, is one of the effective methods to improve the conductivity of the

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electrode. In addition, these are nanoparticles applied widely in biomarker detection [29], antibacterial studies [30], oxygen reduction reaction [31] and Application in capacitance [32]. Therefore, silver nanoparticles are widely applied to modify the electrode surface.

In this work, Ag-4-ATP-MWCNT nanocomposite was prepared for modifying carbon paste electrode. Then modified electrode surface was coated with dsDNA. We also demonstrate the simple, rapid, low-cost and sensitive electrochemical biosensor which was proposed for the determination of DNR in human serum and urine samples. Finally, experimental and operational parameters were optimized for the determination of DNR at the dsDNA-coated Ag-4-ATP-MWCNT carbon paste electrode. The capability of the biosensor was then validated by the determination of DNR in human serum and urine samples.

Experimental section

2.1. Apparatus

The electrochemical measurements were performed with an Autolabpotentiostat/galvanostat (PGSTAT 101, Eco Chemie, The Netherlands). The experimental conditions were controlled with Nova 1.11 software. A three-electrode system was used including a platinum wire as the auxiliary electrode, an Ag/AgCl/KCl (saturated) as the reference electrode, and an Ag NPs/Carbon nanotube paste electrode (Ag-4-ATP-MWCNTPE) as the working electrode. pHs were measured by a Metrohm 827 pH meter (Herisau, Switzerland). Product X-ray diffraction data were recorded by a RigakuDmax C III, X-ray diffractometer using Ni-filtered Cu Kα radiation. The morphology and composition of nanocomposite were analyzed with field emission scanning electron microscope (CARL ZEISS-AURIGA 60 microscope, Jena, Germany), which was equipped with an energy-dispersive X-ray analyzer (EDX) and transmission electron microscope (LEO912-AB, LEO, Massachusetts, U.S.A).

1.2. Reagents and materials

Salmon sperm double-stranded DNA (dsDNA) and DNR were purchased from Sigma (St. Louis, USA). Stock solution (1.0 mg mL^{-1}) of the DNA was prepared in Tris–HCl (TE) buffer (pH=7.4) and kept frozen. Multi-walled carbon nanotubes >98% on acarbon basis, O.D. × L 6-13 nm × 2.5-20 μm (MWCNT) were purchased from Sigma (St. Louis, USA). Pure graphite powder, acetic acid, phosphoric acid and other materials were purchased from Merck (Darmstadt, Germany).

2.3. Synthesis of MWCNT-COCl

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2.0 g of MWCNTs was dispersed in 40 mL of HNO_3 6.0 mol L^{-1} and refluxed with stirring for 24 h. The solid was filtered, washed with distilled water and dried at 80 °C, at this stage, MWCNTs were carboxylated. Subsequently, carboxylic acid groups were converted to acyl chloride. 200 mg of MWCNT-COOH was dispersed in a solution of thionyl chloride, stirred for 24 h at 70 °C. The solid was then separated by filtration, washed with anhydrous tetrahydrofuran and dried [33].

2.4. Synthesis of Ag nanoparticles

Ag nanoparticles were prepared by dropwise addition of 0.01 M aqueous $NaBH_4$ solution to an equal volume of ammoniacal aqueous 0.5 mM $AgNO_3$ and 2.0 mM 4-aminothiophenol (4-ATP in toluene) under vigorous stirring. The prepared Ag–4-ATP nanoparticles were filtered and washed thoroughly several times with water and ethanol, followed by redispersion in dimethylformamide (DMF) [34].

2.5. Synthesis of Ag-4-ATP-MWCNT nanocomposites

100 mg of MWCNT-COCl and 40 mL of DMF were dispersed for 20 min at room temperature by theultrasonic bath. To achieve Ag decoration, the dispersed mixture and silver nanoparticles synthesized according to the procedure mentioned in section 2.4, were stirred for 48 h at 80 °C. After completion of the reaction, solid product was separated by centrifugation. The product was washed several times with deionized water and methanol, followed by drying for 48 h at 50 °C [35].

2.6. Preparation of Ag-4-ATP-MWCNT nanocomposite paste electrode

Graphite powder and Ag-4-ATP-MWCNT nanocomposite (90/10%) wetted with paraffin oil was carefully hand-mixed in a mortar. In this way, Ag-4-ATP-MWCNT modified carbon paste electrode (Ag-4-ATP-MWCNTPE) was prepared. The paste was then packed into one end of a glass tube (3.4 mm (i.d.)) and the electrical contact was provided via a copper wire connected to the paste in the inner hole of the tube. The electrode surface was slowly polished by rubbing it on a piece of glossy paper until it was shiny.

2.7. DNA immobilization

The dsDNA was immobilized on an Ag-4-ATP-MWCNTPE using a potential of +0.50 V for 240 s in 10 mL of 0.50 mol L^{-1} acetate buffer solution (pH 4.80) containing 8 mg L^{-1} of the dsDNA with 200 rpm stirring. The electrode was then rinsed with distilled water [36].

2.8. Cyclic voltammetry of $K_3[Fe(CN)_6]$

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The cyclic voltammogram of 2.0 mmol L^{-1} K_3 $[Fe(CN)_6]$ in 0.01 mol L^{-1} PBS and 0.50 mol L^{-1} KNO_3 was recorded from -200 to 500 mV using a scan rate of 100 mV s^{-1}.

2.9. Adsorptive stripping differential pulse voltammetry at dsDNA coated Ag-4-ATP-MWCNTE

The dsDNA-modified Ag-4-ATP-MWCNTPE was plunged into a 0.50 M acetate buffer (pH 5.50) containing 2.5 µM DNR and stirred for 120 s by applying a potential of -0.4 Volt. In this stage, DNR was accumulated on surface electrode and the electrode was rinsed with 0.50 M acetate buffer (pH 5.50) for 5 s. The accumulated DNR was then measured using differential pulse voltammetry. Operating optimized conditions for DPV studies were: step potential of 5.0 mV; modulation amplitude of 25.0 mV; modulation time of 0.05 s and interval time of 0.5 s.

3. Results and Discussions

3.1. Characterization of Ag-4-ATP-MWCNT nanocomposites

The compositions and morphologies of Ag-4-ATP-MWCNT were characterized by XRD, FESEM-EDS and TEM.

3.1.1. XRD analysis

The composites structural characteristics were investigated by X-ray powder diffraction (XRD) (Figure 1). XRD analysis of the Ag-4-ATP-MWCNT nanocomposite indicates high-intensity diffraction peaks at 2Θ = 37.7, 44.0, 64.2, and 79° related to index as (1 1 1), (2 0 0), (2 2 0), and (3 1 1) diffraction for Ag (JCPDS (Joint Committee on Powder Diffraction Standards) No. 04-0783), respectively. In addition, Bragg diffraction peak at about 26◦ (2Θ) is the characteristic of multi-walled carbon nanotubes [37]. The XRD results revealed that the composites were formed of Ag and MWCNTs.

Fig. 1

3.1.2. FESEM–EDS analysis

The FESEM images of Ag-4-ATP-MWCNT are shown in Fig. 2. It can be seen in the figure that MWCNT was distributed reticularly with an average diameter of 20–40 nm and decorated by Ag nanoparticle, which implies the successful synthesis of Ag-4-ATP-MWCNT nanocomposites. To further confirm the presence of Ag nanoparticles on MWCNTs, energy dispersive spectroscopy (EDS) was performed. Therefore, the results show the presence of Ag on MWCNT surfaces.

Fig. 2

3.1.3. TEM analysis

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As shown in the images of transmission electron microscopy (TEM) (Figure 3), silver nanoparticles with as ice of about 20-40 nm was coated on MWNTs. As can be seen, the Ag nanoparticles as well, were connected to the MWCNTs. The MWCNTs are about 20 nm in diameter and compatible with the supplier's data sheet. The Ag nanoparticles shape is spherical and the particle sizes are approximately 20–40 nm.

Fig. 3

3.2. Cyclic voltammetry study on the immobilization of DNA onto Ag-4-ATP-MWCNTPE

For the study of DNA immobilization on the Ag-4-ATP-MWCNTPE surface, cyclic voltammetry (CV) of $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ redox couple, was employed as the indicator. The operation of this indicator is based on the electrostatic repulsion of the redox couple and the negatively charged DNA phosphate backbone [38]. As shown in Fig. 4, at the bare CPE, the voltammogram of $K_3[Fe(CN)_6]$ was characterized by a pair of well-defined peaks with a cathodic peak potential (E_{pc}) of 117 mV and an anodic peak potential (Epa) of 339 mV and the peak-to-peak potential separation (ΔEp) of 222 mV (Curve b in Fig. 4). A slight increase was seen in the current of the MWCNT modified electrodes (curve 4c). Compared with the MWCNT modified electrode, the Ag NPs modified electrode showed higher current (curve 4d). In addition, there is further increase when Ag-4-ATP-MWCNT nanocomposites were used for modifying the electrode (Curve e in Fig. 4). After the immobilization of dsDNA on the Ag-4-ATP-MWCNTPE surface, a decrease in the ΔEp was observed indicating the decrease of the electrochemical reversibility of the $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ redox couple at the DNA-coated electrode (Curve an in Fig. 4). Simultaneously, both the cathodic and anodic peak currents decreased. This decrease can be described as follows: after the immobilization of dsDNA on the Ag-4-ATP-MWCNTPE, the negatively charged phosphate groups on dsDNA resist the access of the $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ redox couple to the electrode surface as a result of electrostatic repulsion [39]. Therefore, the difference between the CV profiles for these electrodes shows the immobilization of the dsDNA on the surface of the electrodes.

Fig. 4

3.3. Interaction between DNR and the dsDNA in solution

Fig. 5A shows DP voltammograms of DNR in acetate buffer (0. 50 mol L^{-1}, pH 5.50) recorded in the presence and absence of the dsDNA. In the presence of DNA, the anodic current was reduced due to the formation of DNR -DNA complex. Given that the diffusion rate of this complex is small, adecrease in the anodic current of DNR is attributed to the decrease in the unbound DNR concentration [40]. Interaction of DNR with dsDNA solution was found to be intercalation [41]. A decrease in the DNR oxidation current (about one quarter) in the presence of DNA is observed due to DNR

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being involved in DNA interaction. The peaks corresponding to the oxidation of the DNA bases, for the guanine and adenine groups at the potential of + 0.84 and + 1.16 V [42], respectively were not detectable. This implies that double-helical DNA structure was not changed and there was no damage to the dsDNA.

3.4. Electrochemical behavior of DNR at the surface of dsDNA-coated Ag-4-ATP-MWCNTPE

After the accumulation of DNR at bare CPE (Curve a in Fig.5B), MWCNTPE (Curve b in Fig.5B), Ag nanoparticle modified carbon paste electrode (Curve c in Fig.5B), Ag-4-ATP-MWCNTPE (Curve d in Fig.5B), and dsDNA-coated Ag-4-ATP-MWCNTPE (Curve e in Fig. 5B) and stirring of 0.50 µM DNR in 0. 50 mol L^{-1} acetate buffer (pH 5.50) for 120 s at close circuit condition (potential -0.4 V), the electrode was washed with distilled water and transferred into blank of 0.50 mol L^{-1} phosphate buffer (pH 5.50) as the supporting electrolyte, then DP voltammograms were recorded (Fig. 5). As shown in Fig. 5e, the largest anodic signal is in dsDNA-coated Ag-4-ATP-MWCNTPE, which implies that there is a strong interaction between DNR and DNA layer immobilized on the electrode surface.

Fig. 5

3.5. Optimization of experimental and operational parameters

To obtain excellent analytical performance, experimental parameters including immobilization time, accumulation pH, the effect of accumulation time, stripping pH and stripping medium concentration were optimized.

3.5.1. Immobilization time

The effect of the dsDNA immobilization time on I_{pa} of the accumulated DNR was examined applying DPV. According to results (Fig. 6), the amount of DNA adsorbed increased with an increase in the immobilization time and began to saturate at approximately 240 s. Thus, the immobilization time of 240 s was used in subsequent studies.

Fig. 6

3.5.2. Accumulation pH

The electrochemical oxidation of DNR was also studied using DPV for a range of pH (3.00-11.00). According to results in Fig. 7, the peak currents increased up to pH = 5.50 and then remained constant.

Fig. 7

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3.5.3. Effect of accumulation time

The interaction of DNR with the dsDNA is dependent on the accumulation time. The dependence of the I_{pa} of DNR on the time of accumulation was studied in acetate buffer pH 5.50 at a dsDNA-coated Ag-4-ATP-MWCNTPE. For the first 150 s, an increase was observed while at longer accumulation times, a leveling-off was observed (Fig. 8). Therefore, accumulation was performed for 120 s in all experiments.

Fig. 8

3.5.4. Stripping pH

Given that proton takes part in the oxidation of DNR, the pH value of solution will greatly influence the I_{pa} [43]. Accordingly, the effect of stripping pH on the response of the biosensor was evaluated in phosphate buffer 0.50 mol L^{-1} and pH values ranging from 3.00 to 9.00. The results demonstrated a maximum of I_{pa} when the pH of the buffer solution was 5.50 (Fig. 9). Therefore, in subsequent experiments, the stripping was performed in in 0.50 mol L^{-1} acetate buffer with pH 5.50.

Fig. 9

3.5.5. Stripping medium concentration

The effect of the concentration of acetate buffer (pH 5.50) used as supporting electrolyte in stripping medium, was investigated for the highest oxidation signal of DNR. The results (Fig. 10) showed that in the buffer concentration range of 0.10–1.00 mol L^{-1}, the greatest I_{pa} was related to 0.50 M concentration. Hence, an acetate buffer of 0.50 mol L^{-1} was selected as the stripping medium for further studies.

Fig. 10

3.6. Analytical performance

Differential pulse voltammetry (DPV) was used to determine the concentration of DNR. The plot of peak current vs. DNR concentration was drawn up. The DNR concentration dependence over the range of 0.10×10^{-8}-1.00×10^{-5} mol L^{-1} was linear and the correlation coefficient was 0.9995 (Fig. 11). Reproducibility was assessed by five replicate measurements (after accumulation on dsDNA-coated Ag-4-ATP-MWCNTPE) at 0.5×10^{-6} mol L^{-1} of DNR; the corresponding RSDs were calculated to be 5.12 %. Theoretical LOD and LOQ (calculated as three and ten times of the standard deviation of the blank, respectively) were found to be 3.0×10^{-10} and 1.0×10^{-9}mol L^{-1}, respectively.

Fig. 11

3.6.1. Inferences of coexisting compounds

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The effects of some possible interfering compounds such as folic acid, glucose, urea and ascorbic acid, on the DNR measurement, were examined. A given species were considered to interfere if it resulted in a ±5% variation of the DNR current. The obtained results are reported in Table 1.

Table 1

3.6.2. Application of the dsDNA-coated Ag-4-ATP-MWCNTPE

To study the applicability of the electrochemical sensor for practical analysis, two real samples including human urine and blood serum were analyzed. According to results in Table 2, the recovery of the spiked amounts is in the range of 97.0-100.4%, which shows that the fabricated sensor based on Ag-4-ATP-MWCNT nanocomposite is a reliable device for analysis of DNR in biological samples.

Table 2, Fig. 12, Fig. 13

4. Conclusions

An electrochemical biosensor was suggested for the determination of DNR based on its interaction with dsDNA. The interaction between DNR and the dsDNA in solution was investigated by DPV at Ag-4-ATP-MWCNTPE. The mode of interaction was found to be intercalation. In addition, the oxidation of DNR at the Ag-4-ATP-MWCNTPE coated with the dsDNA was investigated. A significant increase in I_{pa} alongside a positive potential shift at the modified electrode relative to unmodified Ag-4-ATP-MWCNTPE showed the pre-concentration of DNR at the electrode surface due to interaction with the dsDNA. After optimizing the main experimental parameters, the performance of this fabricated DNA-coated Ag-4-ATP-MWCNTPE was evaluated from ananalytical perspective. As real samples, human serum and urine samples were successfully analyzed the determination of DNR with the proposed biosensor. Finally, it is believed that the label-free DNA-coated Ag-4-ATP-MWCNTPE possesses the advantages of rapidity, sensitivity, thesimplicity of operation, low cost and is applicable to biological samples.

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Figure Caption:

Fig. 1: XRD patterns of Ag-4-ATP-MWCNT nanocomposites.

Fig. 2: (A) FESEM micrographs of Ag-4-ATP-MWCNT nanocomposites. (B) EDX of Ag-4-ATP-MWCNT nanocomposites.

Fig. 3: TEM micrographs of Ag-4-ATP-MWCNT nanocomposites.

Fig. 4: Cyclic voltammograms of (a) dsDNA-coated Ag-4-ATP-MWCNTPE (b) bare CPE, (c) MWCNTPE (d) Ag nanoparticles modified CPE and (e) Ag-4-ATP-MWCNTPE in 0.05 mol L^{-1} PBS containing 2.0 mmol L^{-1} $K_3Fe(CN)_6$ and 0.50 mol L^{-1} KNO_3. Conditions: potential range 0.20 to 0.50 V at a scan rate of 100 mV s^{-1}.

Fig. 5:: A) DP voltammograms of 0.50×10^{-6}mol L^{-1} DNR at Ag-4-ATP-MWCNTPE in 0.50 mol L^{-1} acetate buffer of pH 4.80 in the absence of the dsDNA (a) and in the presence of 12 mg mL^{-1} of the dsDNA (b) Incubation time, 5 min. **(B)** DP voltammograms (DPV conditions: step potential of 5.0 mV; modulation amplitude of 25.0 mV; modulation time of 0.05 s and interval time of 0.5 s.) obtained after the accumulation of DNR at bare CPE (a) MWCNTPE (b) Ag nanoparticles CPE (c) Ag-4-ATP-MWCNTPE (d) and dsDNA-coated Ag-4-ATP-MWCNTPE (e). (Conditions: DNR concentration 0.50×10^{-6}mol L^{-1}, DNA immobilization time:240 s, accumulation medium 0.050 mol L^{-1} phosphate buffer pH 7.00, accumulation time 120 s, stripping medium 0.05 mol L^{-1} phosphate buffer pH 4.00).

Fig. 6: Effect of immobilization time on the peak current of DNR accumulated at dsDNA coated Ag-4-ATP-MWCNTPE (Composition of solution conditions similar to Fig. 5 except immobilization time).

Fig. 7: Effect of accumulation pH on the peak current of DNR accumulated at dsDNA coated Ag-4-ATP-MWCNTPE (Composition of solution conditions similar to Fig. 5 except accumulation pH).

Fig. 8: Effect of accumulation time on the current peak of DNR accumulated at dsDNA coated Ag-4-ATP-MWCNTPE Composition of solution conditions similar to Fig. 5 except accumulation time)

Fig. 9: Effect of stripping pH on the peak current of DNR accumulated at dsDNA coated Ag-4-ATP-MWCNTPE Composition of solution conditions similar to Fig. 5 except stripping pH)

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Fig. 10: Effect of stripping medium concentration on the peak current of DNR accumulated at dsDNA coated Ag-4-ATP-MWCNTPE (Composition of solution and conditions similar to Fig. 5 except stripping medium cocentration)

Fig. 11: Differential pulse voltammograms (DPV conditions: step potential of 5.0 mV; modulation amplitude of 25.0 mV; modulation time of 0.05 s and interval time of 0.5 s.) obtained at dsDNA-coated Ag-4-ATP-MWCNTPE for DNR at different concentrations (Composition of solution and conditions similar to Fig. 5).

Fig.12: Urine sample voltamogram (DPV conditions: step potential of 5.0 mV; modulation amplitude of 25.0 mV; modulation time of 0.05 s and interval time of 0.5 s.) (a) without the spiked amounts (Composition of solution and conditions similar to Fig. 5 except DNR concentration). and (b) with the spiked 0.20×10^{-8} mol L^{-1} DNR (Composition of solution and conditions similar to Fig. 5 except DNR concentration) (c) with the spiked 0.10×10^{-6}mol L^{-1} DNR (Composition of solution and conditions similar to Fig. 5 except DNR concentration)

Fig.13: Human serum sample voltamogram (DPV conditions: step potential of 5.0 mV; modulation amplitude of 25.0 mV; modulation time of 0.05 s and interval time of 0.5 s.) (a) without the spiked amounts Composition of solution and conditions similar to Fig. 5 except DNR concentration) and (b) with the spiked 0.50×10^{-8} mol L^{-1} DNR Composition of solution conditions similar to Fig. 5 except DNR concentration). (c) with the spiked 2.0×10^{-6} mol L^{-1} DNR (Composition of solution and conditions similar to Fig. 5 except DNR concentration)

Table 1. Effect of interfering compounds in electrochemical determination of DNR

Table 2. Analytical performance of biosensor on human serum and urine real samples

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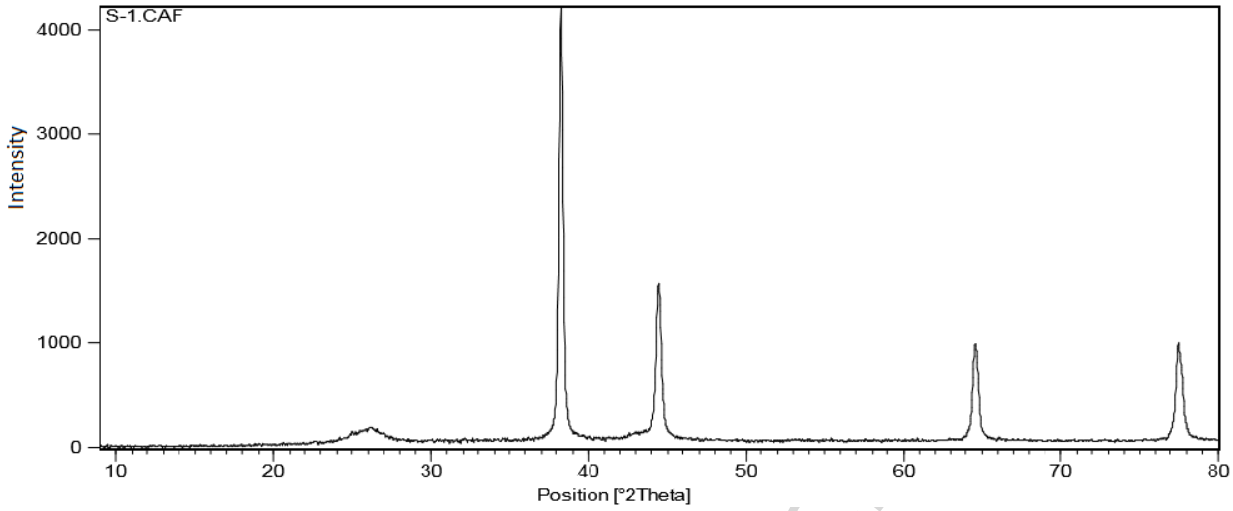

Fig. 1

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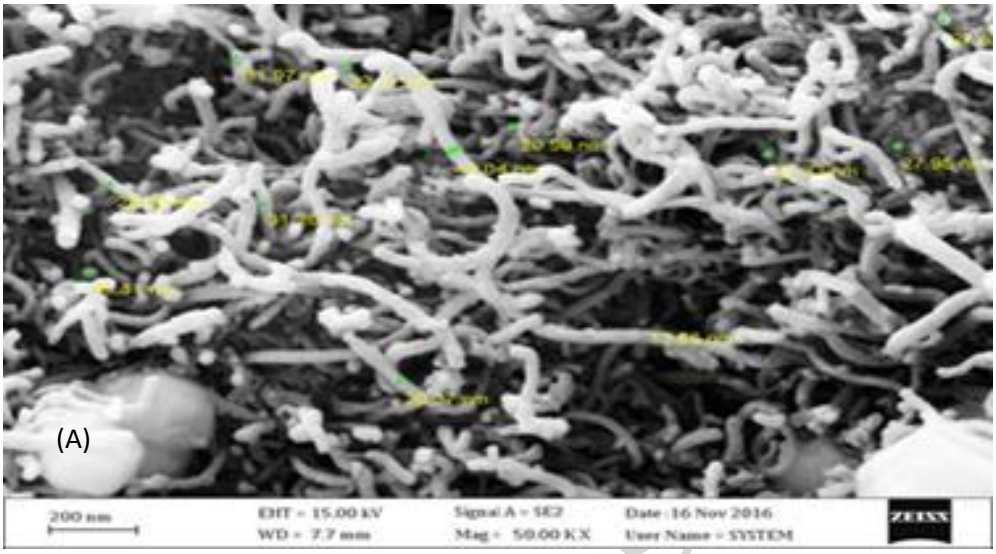

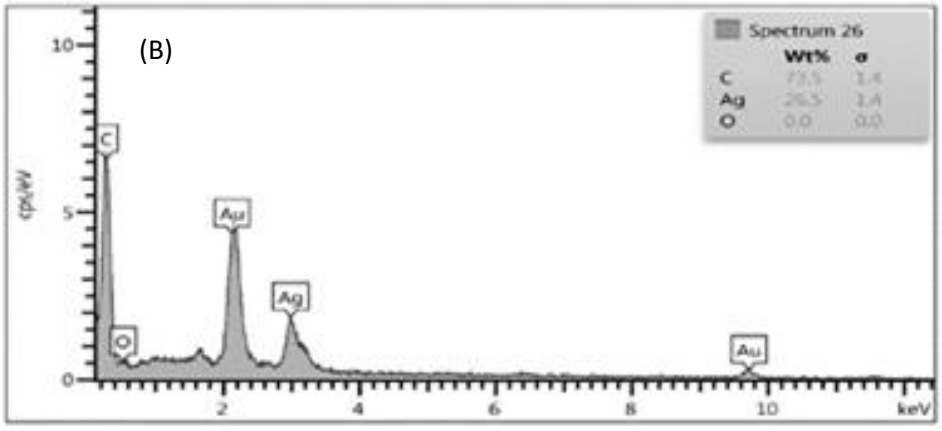

Fig. 2

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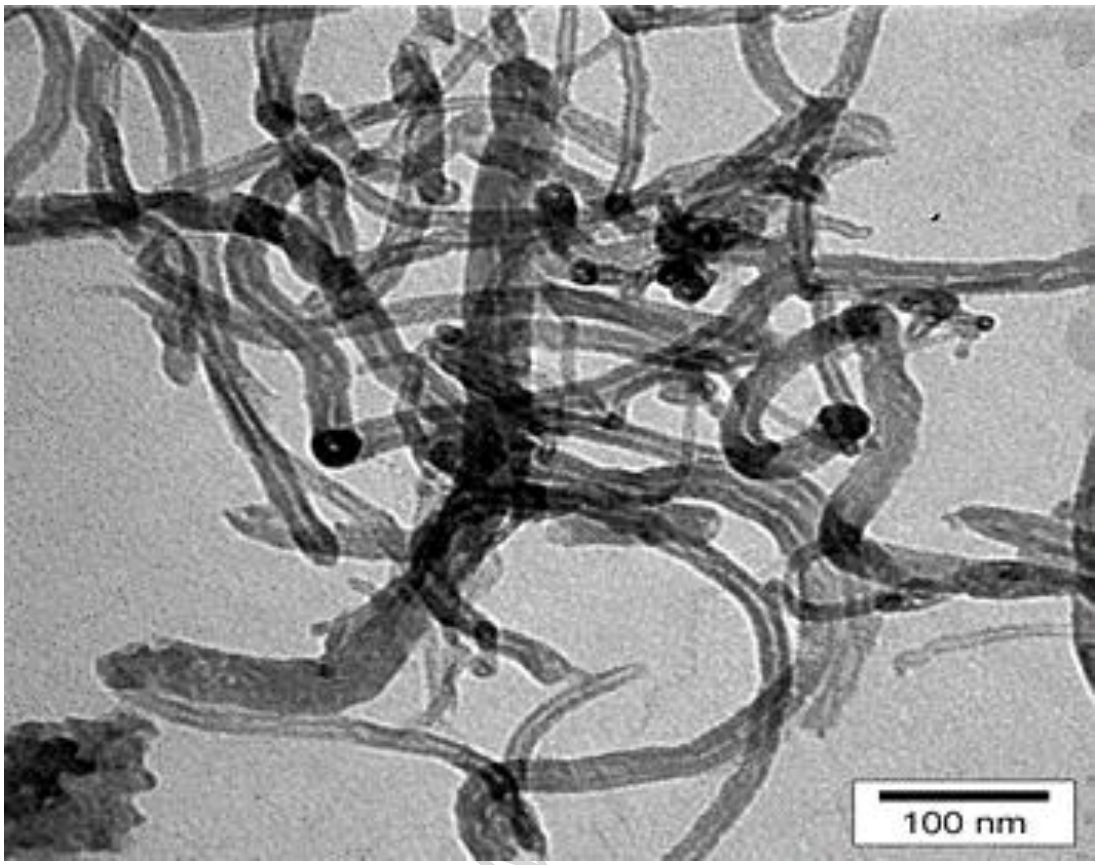

Fig. 3

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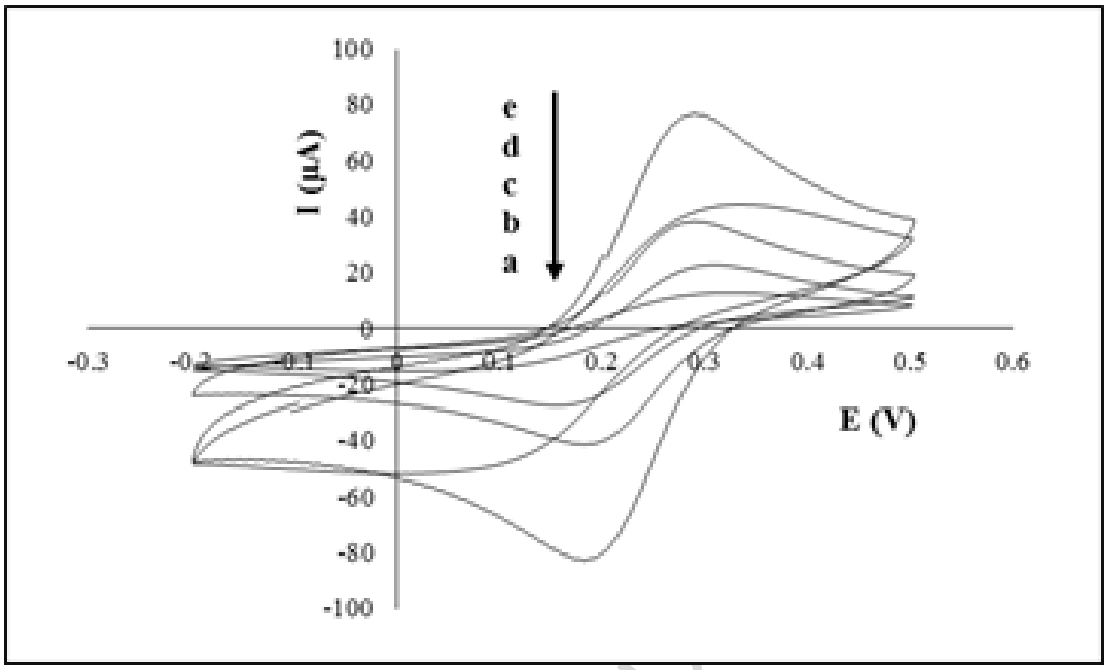

Fig. 4

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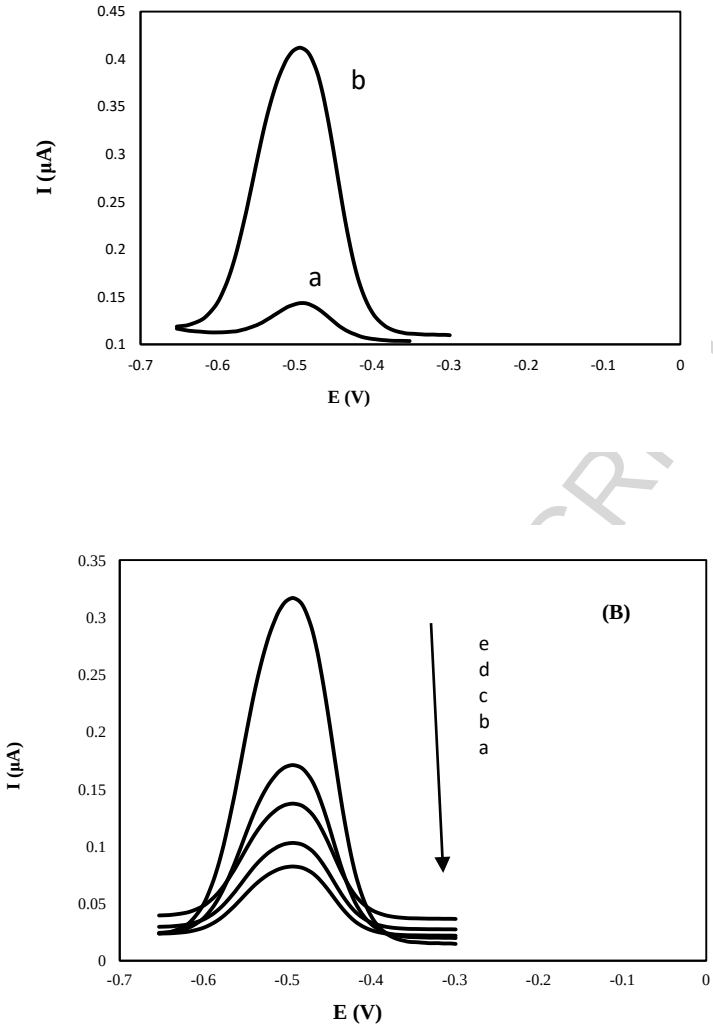

Fig. 5

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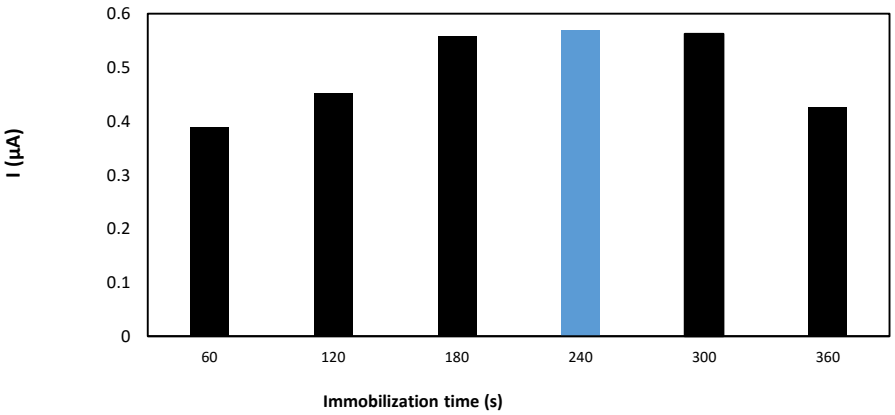

Fig. 6

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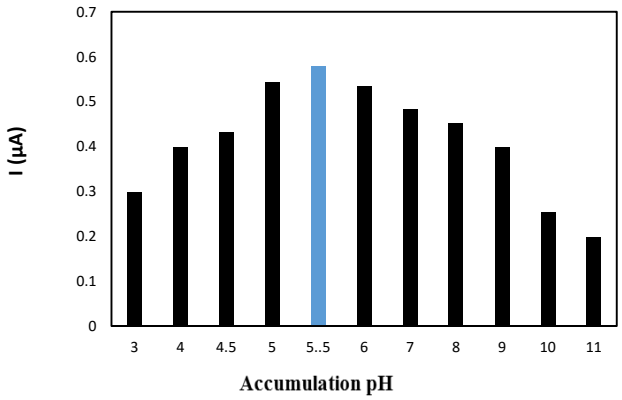

Fig. 7

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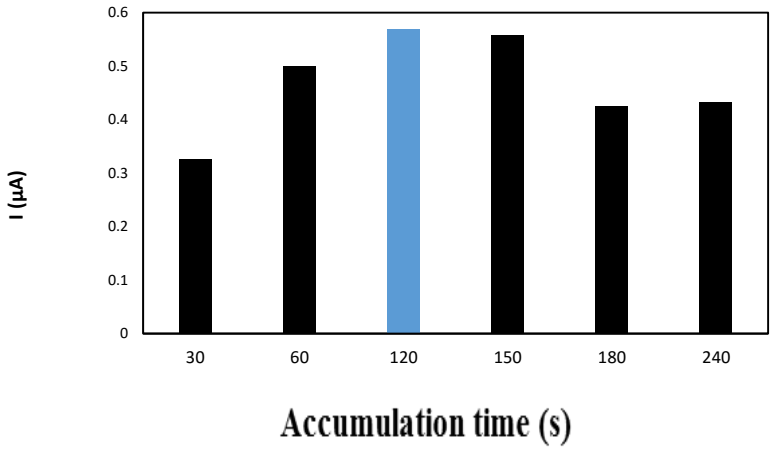

Fig. 8

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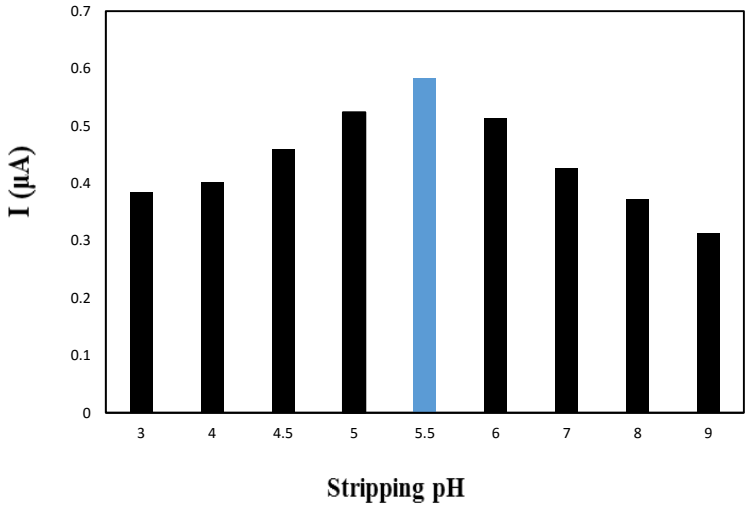

Fig. 9

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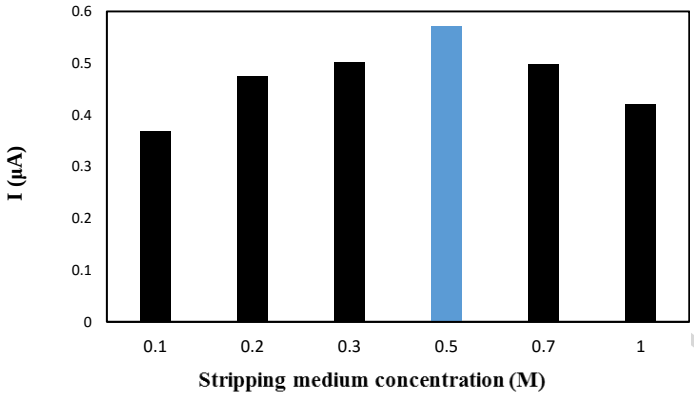

Fig. 10

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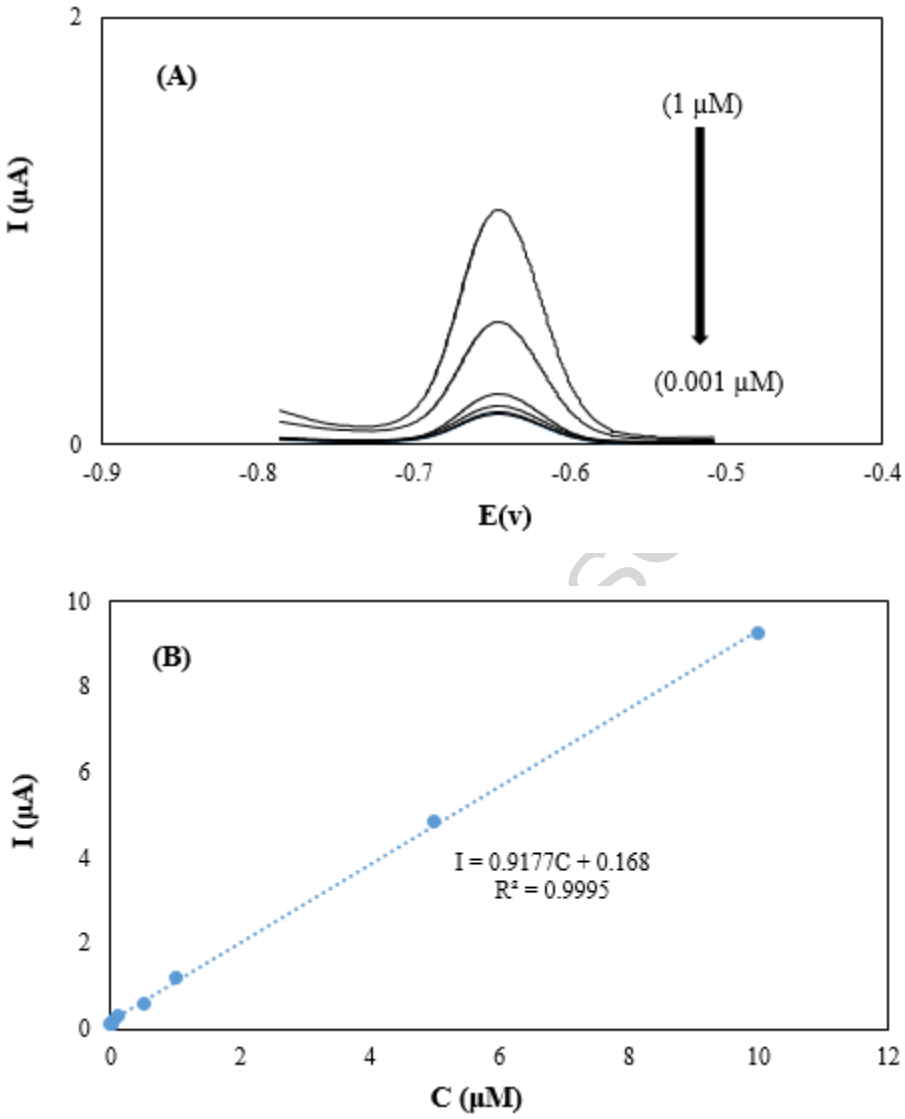

Fig. 11

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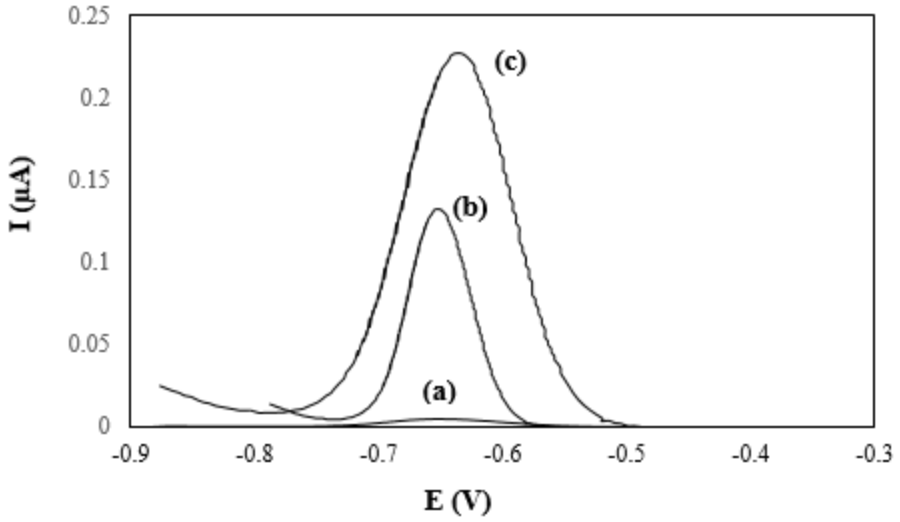

Fig. 12

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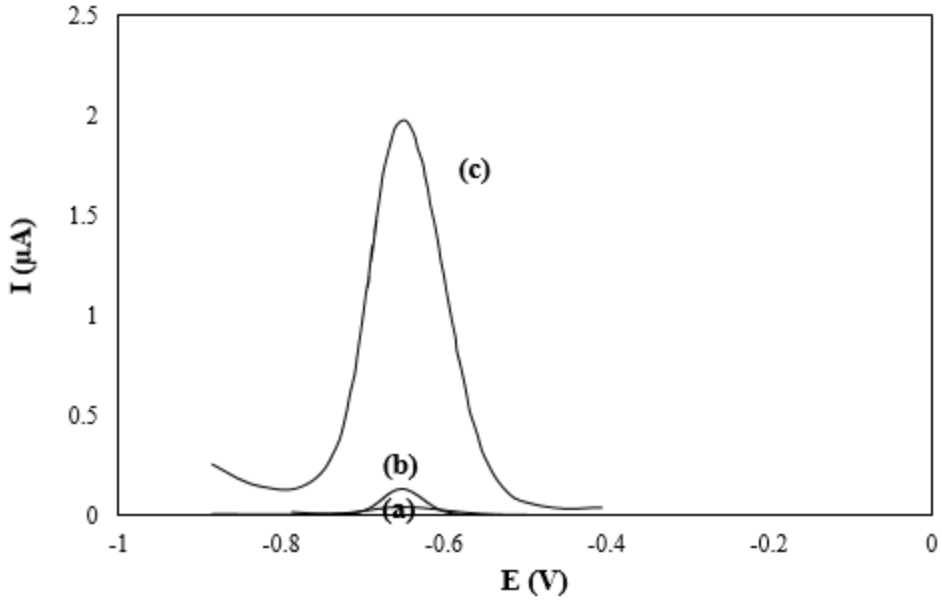

Fig. 13

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Table 1

Interfering compounds	Tolerance limit (C_{int}[a]/C_{DNR})
folic acid	100
glucose	150
urea	200
ascorbic acid	200

[a] C_{int} refers to interfering species concentration.

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Table 2

Sample	Added (μM)	Found[a] (μM)	Recovery (%)
	-	Not Detected	-
Urine	2.00×10^{-3}	$1.95\times10^{-3}\pm1.20\times10^{-4}$	97.5
	0.10	0.097±0.0015	97.0
	-	Not Detected	-
Serum	5.00×10^{-3}	$5.02\times10^{-3}\pm2.10\times10^{-4}$	100.4
	2.0	1.97±0.067	98.5

[a] Mean ± standard deviation (n=3).

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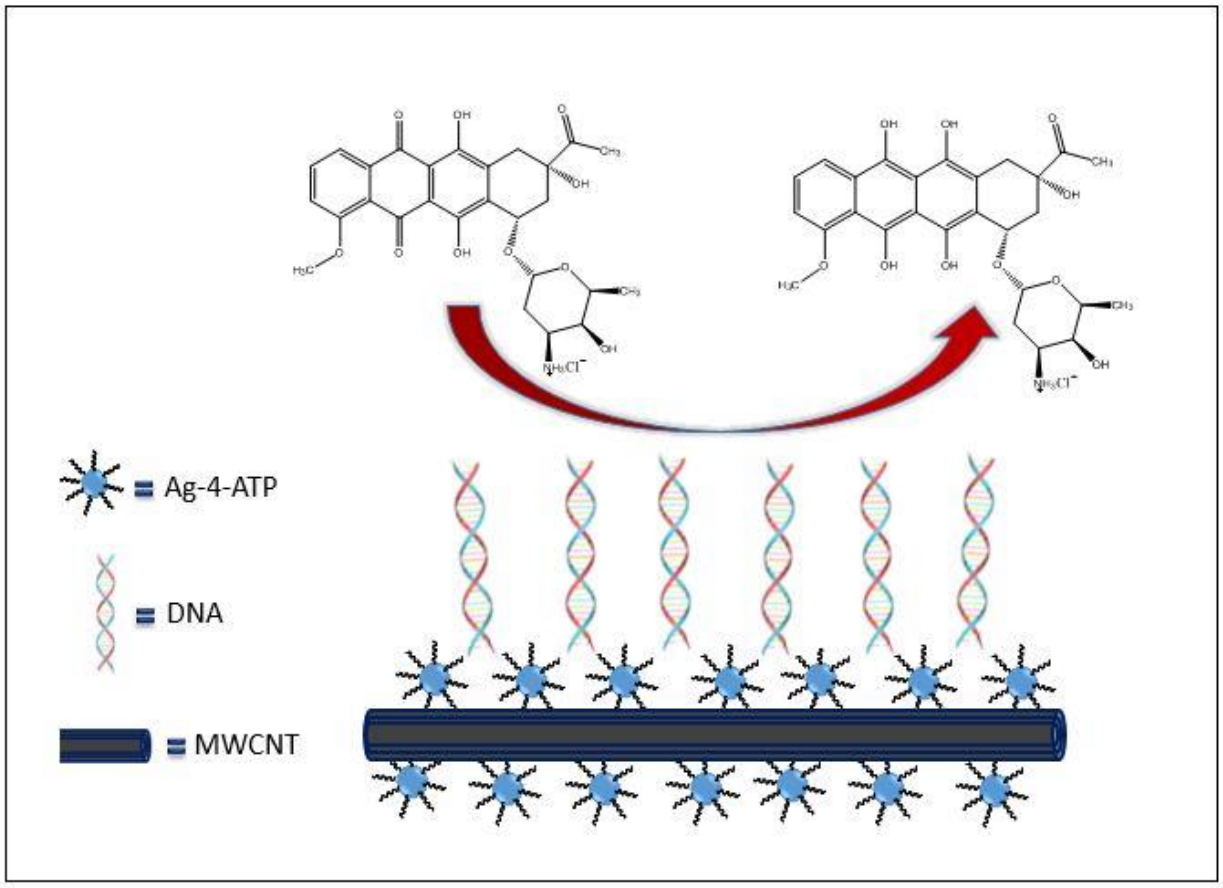

Graphical abstract

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Highlights

1. A DNA biosensor was developed for daunorubicin anticancer drug determination.
2. Ag-4-ATP-MWCNT nanocomposite coated with dsDNA biosensor exhibits excellent sensitivity and selectivity.
3. The as-prepared biosensor could be used for assay of serum and urine samples.

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