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Fabrication of an electrochemical sensor based on carbon nanotubes modified with gold nanoparticles for determination of valrubicin as a chemotherapy drug: Valrubicin-DNA interaction

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ABSTRACT

In this study, an electrochemical sensor was fabricated based on gold nanoparticles/ ethylenediamine/ multi-wall carbon-nanotubes modified gold electrode (AuNPs/en/MWCNTs/AuE) for determination of valrubicin in biological samples. Valrubicin was effectively accumulated on the surface of AuNPs/en/MWCNTs/AuE and produced a pair of redox peaks at around 0.662 and 0.578 V (vs. Ag/AgCl) in citrate buffer (pH 4.0). The electrochemical parameters including pH, buffer, ionic strength, scan rate and size of AuNPs have been optimized. There was a good linear correlation between cathodic peak current and concentration of valrubicin in the range of 0.5 to 80.0 μmol L^{-1} with the detection limit of 0.018 μmol L^{-1} in citrate buffer (pH 4.0) and 0.1 mol L^{-1} KCl. Finally, the constructed sensor was successfully applied for determination of valrubicin in human urine and blood serum. In further studies, the different sequences of single stranded DNA probes have been immobilized on the surface of AuNPs decorated on MWCNTs to study the interaction of oligonucleotides with valrubicin.

1. Introduction

Over the last decade, gold nanoparticles (AuNPs) have attracted increasing research attention for applications in catalysis and sensors [1]. AuNPs generally possess excellent catalytic activity and offer a hospitable environment for biomolecules. AuNPs catalyze oxygen reduction [2] and enable direct determination of glucose oxidase [3,4], hemoglobin [5] and cytochrome *c* [6]. A vast body of original research publications in the current chemical literature indicates that AuNPs have been used to improve electroanalytical measurements with almost all commonly employed detection methods. Their function is most commonly to improve the detectability and sensitivity of detection, although often they allow modification of the selectivity in electrochemical detections. In contrast, carbon nanotubes are one of the most intensively investigated nanomaterials, since their discovery in 1991 [7]. The intrinsic properties of carbon nanotubes, including high surface area, high electrical conductivity, and hollow geometry, are making them attractive in many scientific and industrial fields [8,9].

Nanocomposites are combinations of nanomaterials with other molecules or nanoscaled materials, such as nanoparticles or nanotubes. In general, these novel nanocomposites have different physical and chemical properties from the constituent particles or wires, and thus allow new kinds of applications. Among these nanocomposites, AuNPs/CNTs composite is of particular interest, due to its easy fabrication protocols and broad potential applications. AuNPs/CNTs nanocomposite combines the excellent physical and chemical properties of both gold nanoparticles and carbon nanotubes. The easy modification surface of gold nanoparticle and the excellent conductivity of carbon nanotube as well as the high surface area, point towards a broad range of applications, such as biosensing, gas sensing, and electrochemistry [10].

Valrubicin (N-trifluoroacetyladriamycin-14-valerate) is a second-generation anthracycline: a cell cytostatic drug derived from the highly effective anthracycline doxorubicin (Adriamycin) [11,12]. Anthracyclines have been known since 1960s and are accepted as antitumoral drugs because of their cytostatic effect [13,14]. Chemotherapeutic drugs are effective molecules; however they are often associated with severe side effects including severe toxicity to skin and tissues [11,15,16]. Since its introduction in 1998, valrubicin has been used in bladder cancer therapy [17,18]. The antitumorigenic activity was achieved by contact with the bladder wall and subsequent absorption by cancer cells in which valrubicin expressed its cytostatic effect, resulting in a reduced proliferation of tumoral cells [18,19]. The absence of irritation and skin toxicity as ulceration and necrosis, which is unique among anthracyclines, in combination with the negligible systemic absorption on repeated

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dermal application, makes valrubicin relevant for the treatment of hyperproliferative debilitating but not life-threatening disease [18–21].

DNA plays a major role in the life process because it carries heritage information and instructors for the biological synthesis of proteins and enzyme through the process of replication and transcription of genetic information. DNA is quite often the main cellular target for studies with smaller molecules of biological importance like carcinogens, steroids and several classes of drugs. The investigation of drug–DNA interactions is of current general interest and importance [22–24], especially for the designing of new DNA-targeted drugs there is vitro screening. Generally, the interactions of small molecules with DNA involve three binding modes via, intercalation, groove binding and long-range assembly on the molecular surfaces of nucleic acids [25]. The intercalative binding is stronger than the other two binding modes because the surface of intercalative molecule is sandwiched between the aromatic, heterocyclic base pairs of DNA [26–32].

According to high cancer prevalence in modern societies, scrutiny and surveillance on chemotherapy medicines is an important job. Thus, the aim of this work was to fabricate an electrochemical sensor based on carbon nanotubes and gold nanoparticles to study the electrochemical behavior and quantitative measurement of valrubicin as a chemotherapy medicine in biological samples including human urine and blood serum. By cited properties for nanomaterials that have been employed to make this sensor, it has been expected to increase the sensitivity and efficiency of the method. To the best of our knowledge, this is the first report on the analytical determination of valrubicin since its discovery in 1998. In addition, some sequences of oligonucleotides bonded covalently on AuNPs/MWCNT modified electrode for study on the interaction with valrubicin. It was found that cytosine and guanine bases have more interaction with valrubicin and produce higher sensitivity towards reduction of valrubicin.

2. Experimental

2.1. Reagents and solutions

All chemicals used in the measurements were analytical grade and used without further purification. Multi-wall carbon nanotubes (diameter: 10-20 nm, length: 1-2 μm, purity >95%) were obtained from Sigma-Aldrich Chemicals. Gold nanoparticles with average diameters of ~13 and ~40 nm were prepared by the reduction of $HAuCl_4 \cdot 3H_2O$ (Merck, Germany) with sodium citrate. Tris (2-carboxyethyl) phosphine (TCEP) was purchased from Aldrich, USA as a reducing agent. Valrubicin was purchased from Rockville, USA and used as received. The stock solution of valrubicin (7.02×10^{-4} mol L^{-1}) was prepared by dissolving the required amount of valrubicin hydrochloride in ethanol and diluting to 25 mL with distilled water.

All oligonucleotides with various sequences were purchased and synthesized by First BASE Laboratories Sdn Bhd (Selangor, Malaysia). The base sequences were listed as, 15-mers probe thiolated DNA single sequence (ssDNA): 5′ SH-$(CH_2)_6$-CGC GGG CCG GGC CGG-3′; 15-mers probe thiolated DNA single sequence (ssDNA): 5′ SH-$(CH_2)_6$-ATA TTA TAA ATT ATA-3′; 15-mers probe thiolated DNA single sequence (ssDNA): 5′ SH-$(CH_2)_6$-CGA GTA TGC ATG ACC-3′; and 8-mers probe thiolated DNA single sequence (ssDNA): 5′ SH-$(CH_2)_6$-CGC GCG GG-3′.

2.2. Apparatus

All the voltammetric measurements were carried out using Autolab 302N electrochemical system (Metrohm Co., Ltd. Switzerland). A conventional three-electrode system was employed, including a bare Au electrode (AuE) modified with AuNPs/en/MWNTs film as working electrode, an Ag/AgCl (3.0 mol L^{-1} KCl) electrode as reference electrode and a graphite bare electrode as an auxiliary electrode. A pH meter (Metrohm, model 827) was used for all the pH measurements. The morphology of fabricated electrochemical sensor was studied by a field emission scanning electron microscope coupled with energy dispersive X-ray spectroscopy (FESEM-EDX), Jeol JSM 7600F.

2.3. Preparation of real samples

Serum samples were collected from five volunteers and mixed together before frozen in fridge. Into each of the 10 centrifugation tubes containing a certain concentration of valrubicin, 2.0 mL of human serum sample was transferred, and then mixed well with 20.0 mL of methanol to precipitate the serum proteins [33]. The precipitated proteins were separated using centrifuge for 20 min at 4000 rpm. The clear supernatant layer was filtered through a 0.2 μm polytetrafluroethylene (PTEF) filter to produce protein-free human serum. The pretreated human serum sample was diluted with citrate buffer (pH 4.0) before analysis.

Urine samples were filtered through a cellulose acetate filter (0.2 mm pore size, Supelco) and collected in dark glass bottles previously cleaned with hydrochloric acid and washed with deionized water. The samples were stored in darkness at 4 °C until the analysis was performed.

2.4. Modification of Au electrode with MWCNTs

The bare AuE was pretreated carefully with 0.05 μm alumina slurry on a polishing cloth, rinsed thoroughly with 1:1 HNO_3–H_2O (V/V), and then washed with pure ethanol and redistilled water, respectively. 10 mg of the untreated MWCNTs was added to a large amount of concentrated nitric acid (wt. 68%), and then sonicated for about 4 h. The mixture was filtered and washed with doubly distilled water until the filtrate was neutral. The treated MWCNTs were dried in an oven at 50 °C for 2 h. MWCNT suspension was accomplished as follows: 5.0 mg of treated MWCNTs was sonicated in 10.0 mL of N,N-dimethylformamide (DMF) for about 30 min after which a homogeneous suspension was obtained. The pretreated AuE was coated evenly with 15.0 μL of MWCNTs suspension, and then the solvent was evaporated under an ultraviolet lamp. Before using, the modified electrodes were washed repeatedly with double-distilled water to remove the loosely bound modifiers [3].

2.5. Fabrication of AuNPs/en/MWCNTs/AuE based electrochemical sensor

The decoration of MWCNTs with AuNPs on the surface of gold electrode was constructed after preparation of AuNPs suspension as follows. At first, AuNPs with average diameters of ~13 and ~40 nm were prepared by the reduction of $HAuCl_4$ with sodium citrate following the literature methods [9,34]. All glassware used for the preparation of AuNPs were thoroughly washed with freshly prepared aqua regia (HNO_3:HCl) (1:3), rinsed extensively with ultra-high purity water sequentially and then dried in an oven at 100 °C for 2 h. A 60 mL solution of 0.01% (w/v) $HAuCl_4$ was brought to a boil with vigorous stirring in a round-bottom flask fitted with a reflux condenser. Then, different amounts of 1.0% (w/v) sodium citrate were added to the $HAuCl_4$ solution (for 13 nm and 40 nm AuNPs, 4.5 and 0.6 mL of sodium citrate were used, respectively). The reaction mixture was maintained at the boiling point with continuous stirring for about 15 min. The suspension was stored at 4 °C until further use. Before modification of MWCNTs/AuE with AuNPs, electrografting of ethylene diamine (en) films on the surface of MWCNTs/AuE was completed electrochemically using cyclic voltammetry between 0.0 mV and 1400 mV (vs. Ag/AgCl) at 10 mV s^{-1} in a solution of 0.1 mol L^{-1} en-ethanol containing 0.01 mol L^{-1} of KCl as the electrolyte for six times. After modification, the surface was rinsed with ethanol, and then followed by double-distilled water and dried with nitrogen gas. To assemble the gold nanoparticles, en/ MWCNTs/AuE was immersed in citrate-capped AuNPs for 2 h at 4 °C in the dark. After treatment, the modified electrode was rinsed with double-distilled water, dried with a gentle stream of N_2 and used immediately [9]. Fig. 1 shows the