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# An electrochemical sensor for warfarin determination based on covalent immobilization of quantum dots onto carboxylated multiwalled carbon nanotubes and chitosan composite film modified electrode

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ABSTRACT

A method is described for the construction of a novel electrochemical warfarin sensor based on covalent immobilization of CdS-quantum dots (CdS-QDs) onto carboxylated multiwalled carbon nanotubes/chitosan (CS) composite film on the surface of a glassy carbon electrode. The CdS-QDs/CS/MWCNTs were characterized by field-emission scanning electron microscopy (FE-SEM), transmission electron microscopy (TEM), Fourier transform infra-red (FTIR) spectroscopy, XRD analysis and electrochemical impedance spectroscopy (EIS). The sensor showed optimum anodic stripping response within 90 s at an accumulation potential of 0.75 V. The modified electrode was used to detect the concentration of warfarin with a wide linear range of 0.05–80 μM and a detection limit (S/N = 3) of 8.5 nM. The proposed sensor has good storage stability, repeatability and reproducibility and was successfully applied for the determination of warfarin in real samples such as urine, serum and milk.



## 1. Introduction

Warfarin [3-(α-acetonylbenzyl)-4-hydroxycoumarin, WAR] is a coumarin derivative, and widely used as an oral anticoagulant in various cardiovascular and cerebrovascular disorders such as venous thromboembolism, pulmonary embolism, atrial fibrillation, valvular heart disease and coronary heart diseases [1–3]. WAR is a drug with a narrow therapeutic index and warrants careful monitoring of the patient. In order to ensure the effectiveness and safety of oral anticoagulants, the dose must be adjusted accurately and frequently. Therefore, the therapeutic window of warfarin is very narrow. Exceeding the therapeutic window of this drug causes unwanted bleedings [4]. WAR is a weakly acidic drug ($pK_a = 5.19$) with an enolic group [5] and it was known to inhibit tumor spread, and to stimulate granulocytes, lymphocytes and macrophages [6]. Therapeutic concentration of WAR is about 2.0–5.0 μg $ml^{-1}$ and has a long half-life (20–60 h) [7].

A number of methods have been used for the determination of WAR including high performance liquid chromatography [8–15], liquid chromatography [16,17], capillary electrophoresis [18], phosphorescence [19], fluorescence [20], cloud point extraction [21], and spectrofluorimetric [22]. Electrochemical techniques are alternative methods for the WAR determination because they are simple, fast, sensitive and low cost. The electrochemical detection of WAR has been reported using multiwall carbon nanotubes/molecular imprinting polymer [23] and hanging mercury drop [24] as the working electrodes.

It is known that the modification of conventional electrodes has attracted much attention in the last 2 decades because it provides powerful means to bring new qualities to the electrode surface which was exploited for electrochemical purposes [25–31]. Chemically modified electrodes can be obtained by attaching a suitable electron mediator on electrode surface and can be applied in various fields including electroanalysis and electrocatalysis [32–43]. Among the wide range of electrode modifiers, carbon nanotubes have attracted the attention of electrochemists because of their advantageous features such as excellent long term stability, high conductivity, resistance to surface fouling, providing large surface area and their ability to promote electron-transfer process [44–46].

Chitosan (CS) has been gradually used for constructing sensors due to its attractive properties that include excellent film-forming ability, high permeability, good adhesion, nontoxicity, cheapness and a susceptibility to chemical modification. It also facilitates the electron transfer after its swelling in the reaction mixture due to its hydrophilic nature [47,48]. Besides, chitosan can be used as a dispersant to form a stable CNT–chitosan composite which can form a stable film on the electrode surface.

Quantum dots (QDs) are semiconductor nanocrystals that possess a size-tunable optical and electronic properties, which have been used in several areas, including catalysis, coatings, textiles, data storage, biotechnology, health care, biomedical, pharmaceutical industries and

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most recently, in bioanalytical chemistry [49]. The surface modification of QDs can change their optical, chemical, electrochemical and photocatalytic properties [50]. Therefore, QDs modified with different functional groups on the surface could provide a new chemistry for the application in electroanalytical chemistry [51]. One of these modifiers is cysteine, which its capped QD has been used as a fluorescent probe for copper ions [52]. Thus, it seems that cysteine can also be used as a proper surface modifier in the electrochemical field. To a better platform for nanoscale sensing, the capped QDs can be attached to a suitable nanoparticle such as MWCNTs [53]. The development of practical strategies for assembling QDs onto MWCNT surface is an area of considerable interests which its combination exhibits synergistic effects towards target analysis.

In the present work, the advantages of using CdS-QD/CS/MWCNT/GC electrode combined with differential pulse stripping voltammetry (DPSV) are presented for the analytical determination of WAR. In order to enhance the electrode sensitivity and electronic transmission, multiwall carbon nanotubes (MWCNTs) immobilized on the surface of GCE. The L-cysteine capped CdS quantum dots (CdS-QDs) for creating a nanostructured platform and chitosan as the stabilizing agent to prevent the MWCNT aggregation are used as further modifiers. However, due to the synergistic effects of the modifiers, the combination of MWCNTs with chitosan and CdS-QDs improved the electron transport activity of the composite film as catalyst for analyte analysis. On the other hand the composite film facilitated the electron transfer more than those of MWCNTs, chitosan or CdS QDs alone [48,54–56]. The behavior of the modified electrode and its application for WAR electroanalysis were investigated by cyclic voltammetry. The prepared modified electrode was successfully applied for voltammetric determination of low level of WAR in real samples.

## 2. Experimental

### 2.1. Chemicals

Carboxylated multiwall carbon nanotubes with purity 95% (10 nm diameters) and 1–2 μm length were obtained from DropSens (Llanera, Spain). Warfarin sodium, N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), chitosan (95% deacetylation) and cysteine were purchased from Sigma-Aldrich (St. Louis, USA). All reagents were of analytical-reagent grade and used without further purification. Double distilled water was used thoroughly.

### 2.2. Apparatus

Electrochemical experiments were performed via using a μAutolab III (Eco Chemie B.V.) potentiostat/galvanostat by NOVA 1.8 software. A conventional three-electrode cell was used with a saturated Ag/AgCl as reference electrode, a Pt wire as counter electrode and a modified glassy carbon (1.8 mm diameter) as working electrode. The cell was a one-compartment cell with an internal volume of 10 ml. All experiments were typically conducted at room temperature. JENWAY pH-meter (model 3345) was also applied for pH measurements. To obtain information about the morphology of the electrode surface, transmission electron microscopy (TEM) (Zeiss EM 900), scanning electron microscopy (SEM) (Tscan Company, Czech Republic), X-ray diffractometer (XRD) (X'Pert Pro MPD, PANalytical, Netherlands) and Fourier transform infra-red (FTIR) spectroscopy (ALPHA model of Bruker, Germany) were used.

### 2.3. Synthesis of CdS-quantum dots by seed assistant technique

CdS-quantum dots (QDs) have been synthesized in accordance with the literature published before [57]. The functionalized cysteine capped CdS quantum dots were facilely prepared and described as follows: a 1.0 mM portion of cysteine dissolved in 100 ml of deionized water and then purged with pure nitrogen for 60 min under magnetic stirring. The pH value of the solution was adjusted between 8.5 and 9.0 using 0.5 M Tris solution. Subsequently, 0.50 mM portion of $Cd(NO_3)_2$ was dropped slowly into the above solution and reacted for 30 min resulting in a molar ratio cysteine:Cd of 2:1. Finally, 0.50 mM portion of $S^{2-}$ (from a $Na_2S$ solution) dissolved in 10 ml water was dropped slowly into the vortex of the solution to reach a molar ratio of S:Cd of 1:1. At this point, the mixture solution is colorless. The seed solution was injected into this mixture solution under strong magnetic stirring, which was obtained by directly mixing 2 ml of $10^{-4}$ M $Cd^{2+}$ solution and the same amount of $S^{2-}$ solution. All steps were performed under magnetic stirring. The bright yellow-green colloid obtained after the reaction solution was sealed, incubated for 2 h at 47 °C bath water, and then flushed with $N_2$ for 30 min to remove most of the unreacted sulfide after stirring for 30 min. The colloid solution was stored at room temperature without any precipitation during several months.

### 2.4. Preparation of the modified electrode

The procedure of the fabrication of the sensor is illustrated in Scheme 1. To prepare a modified electrode, glassy carbon electrode was polished with emery paper followed by alumina (0.05 μm) and then thoroughly washed with double distilled water. To remove the adsorbed particles the electrode was further cleaned in an ultrasound bath. 5 μl of functionalized MWCNT suspension in DMF (1 mg $ml^{-1}$) was cast on the surface of GCE and dried in air to form a MWCNT film on the electrode surface. Then, chitosan (0.5% in acetic acid, 200 μl) was added to 10 ml of 1 M KCl and electrodeposited onto the MWCNT/GC electrode through cyclic voltammetry by applying 20 successive deposition cycles at −0.15 to 0.20 V at a scan rate of 20 mV $s^{-1}$. Then the resulting electrode was immersed into the solution containing CdS-QDs (pH 7.0) in the presence of 0.1 mM EDC and NHS for 12 h to form CdS-QDs/CS/MWCNTs/GCE. During this process the cysteine capped, CdS-QDs will covalently bond to the carboxylic groups of the functionalized MWCNTs and amine groups of CS. Finally, the modified electrode was washed thoroughly with double distilled water to remove the unbound materials and then after washing it was dried in air and kept at room temperature for further use.

### 2.5. Characterization of modified electrode by FTIR

To record FTIR spectra of the modified electrode at different stages of its construction, the deposited material was scrapped off the GC electrodes, grinded with dry potassium bromide (KBr) and this powder mixture was then pressed in a mechanical press to form a translucent pellet through which the beam of the spectrometer can pass. Then this pellet was kept in the socket of FTIR spectrophotometer and its spectrum was recorded. Band intensities in IR spectrum were expressed as transmittance (T).

### 2.6. Preparation of real samples

Serum, urine and breast milk samples were collected from a 36 year old patient volunteer after using a tablet containing 5 mg warfarin (from APOTEX Co.). 0.5 ml methanol, as serum protein denaturation and precipitating agent, was added to 1 ml of the serum sample. After vortexing for 40 s, the precipitated protein was separated out by centrifugation for 4 min at 10,000 rpm. The clear supernatant layer was filtered through a 0.45 μm Milli-pore filter to produce a protein-free human serum. A 200 μl of this solution was transferred into a 10.0 ml volumetric flasks containing phosphate buffer (pH = 2) and different amounts of standard solution of warfarin. After adjusting the volume of solutions, their warfarin contents were determined using an optimized proposed procedure.