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RESEARCH ARTICLE

Production of Electrochemiluminescence (ECL) Biosensor Using Os-Pd/HfC Nanocomposites for Detecting and Tracking of Human Gastroenterological Cancer Cells, Tissues and Tumors

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Abstract

In the current paper, the first step for using Os-Pd/HfC nanocomposites as luminophore in producing Electrochemiluminescence (ECL) biosensor-namely increasing the sensitivity of biosensor through creating Os-Pd/HfC nanocomposites and using it instead of other nanocomposites for detecting and tracking of human gastroenterological cancer cells, tissues and tumors, is evaluated. Further, optimization of tri-n-propylamine (TPrA), 2-(dibutylamino) ethanol (DBAE) and benzyl peroxide (BPO) concentrations and Os-Pd/HfC nanocomposites as four main and effective materials in the intensity of luminescence for detecting and tracking of human gastroenterological cancer cells, tissues and tumors are considered so that the highest sensitivity obtains. In this regard, various concentrations of four materials were prepared and photon emission was investigated in the absence of human gastroenterological cancer cells, tissues and tumors.

Keywords

Biosensor, Electrochemiluminescence (ECL), Os-Pd/HfC Nanocomposites, Photomultiplier, Cadmium Oxide (CdO) nanoparticles, Human gastroenterological cancer cells, Tissues and tumors

Introduction

Biosensors are systems for measuring the concentration of biomolecules such as proteins, enzymes, nuclides and etc. which produce by various methods and materials depending on the type of biosensor and biomolecule. In optical method of Electrochemiluminescence (ECL), a luminophore excites at the presence of activator agent due to applying electrical potential and hence, emits photon. In optical ECL biosensor, the concentration of biomolecules can be measured using this method and stabilizing the luminophore on the biomolecules. In other words, biomolecules play the role of electrical potential carrier to luminophore. Hence, the applied potential to luminophore varies with concentration of biomolecules and therefore, the intensity of emitted photons varies [1-27]. The advantages of ECL method compared to other optical methods are 1) It does not necessary to have an excitation source which cause to reduction of optical interferences; 2) Having strong time and position separation power; 3) Simplicity, low cost, high speed and low time of measurement [28-63].

In the produced optical biosensor, as the first sample in the country, Os-Pd/HfC nanocomposites was used which is one of the most used luminophores, applied in production of ECL biosensors due to its high quantum efficiency and small size. Small size of Os-Pd/HfC nanocomposites leads to its easy conjugation with biomolecules which minimizes the interference in immune system of biomolecules [64-85]. In the produced optical sensor, tri-n-propylamine (TPrA), 2-(dibutylamino) ethanol (DBAE) and benzyl peroxide (BPO) are used as activator agents for Os-Pd/HfC nanocomposites. It should be noted that the purity of Os-Pd/HfC was already and experimentally confirmed in detail [35].

One of the basic characteristics of biosensor is its high sensitivity. Sensitivity of a biosensor is the minimum amount of concentration detection of biomolecules. According to this definition, sensitivity of the pro-

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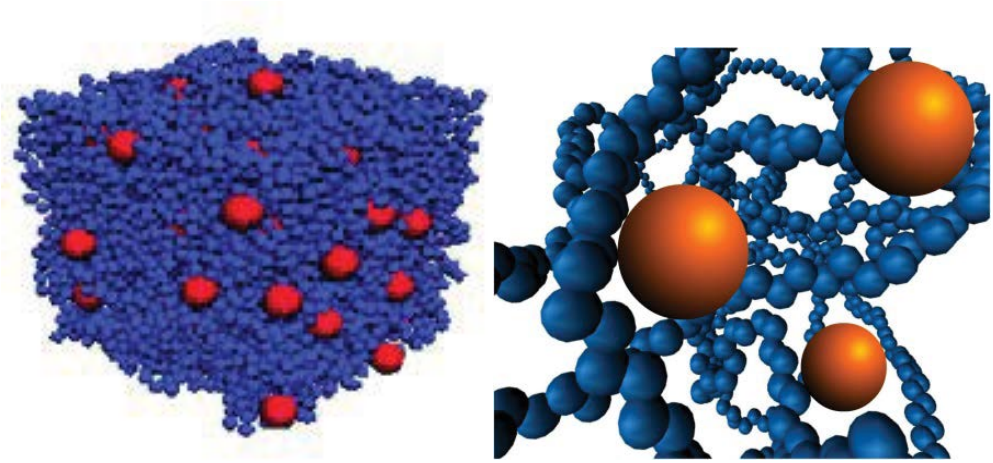

Figure 1: Schematic views of static (left) and rheological (right) properties of Os-Pd/HfC nanocomposites were studied by computer modeling and simulation.

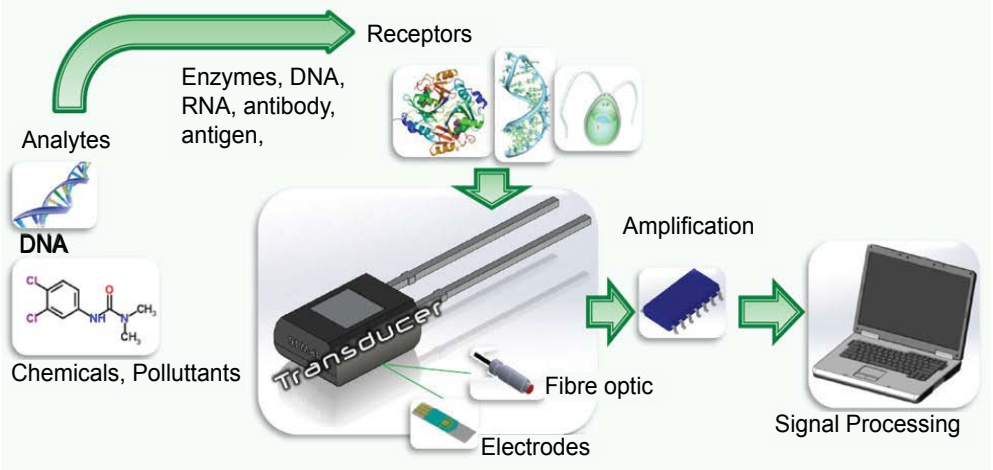

Figure 2: Biosensors are devices which use a biological recognition element retained in direct spatial contact with transduction system (IUPAC definition). Biosensors can be straightforwardly depicted as devices that convert a physical or biological event into a measurable signal; they are composed of a biosensing element (i.e. enzyme, tissue, living cell) that provides selectivity and a transducer that converts the chemical responses into a processable signals. Specifically, biosensor consists of three parts: The first element is the biomediator (a biomimic or biologically derived material e.g. tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids, and biological sensitive elements created with genetic engineering), the second element is the transducer (physicochemical, optical, piezoelectric, electrochemical, etc.) that transforms the signal resulting from the analyte's interaction with the biological element into a signal that can be measured and quantified; the third element is the associated electronics or signal processor, responsible for a user-friendly way of the results visualization. Some biosensors require a process of biomediator immobilization to the sensor surface (metal, polymer or glass and other materials) using physical or chemical techniques [191-194].

duced biosensor increases proportional to increase in intensity of emitted photons from luminophore. Hence, Os-Pd/HfC nanocomposites were used for this reason.

SiO_2 and CdO nanoparticles enhance the intensity of photons due to some advantages. Two time-ionized SiO_2 and CdO nanoparticles easily coop Os-Pd/HfC nanocomposites ions due to having negative charge and enhance the optical stability of Os-Pd/HfC nanocomposites because of their optical property. At the other hand, as these molecules are of large active surface, they are able to charge (coop) a large amount of Os-Pd/HfC nanocomposites. However, Os-Si or Os-Cd nanoparticles cannot individually stabilize on biomolecules such as antibodies and hence, Cadmium Oxide nanoparticles are used to solve this problem [86-110].

The produced CdO nanoparticles have negative charge on their surfaces due to the production type and therefore, they can easily absorb functional groups with positive charge (such as amino groups). Many biomolecules are of functional groups with positive charge. To settle CdO nanoparticles with negative charge on Os-Si or Os-Cd, layers with positive charge such as amino groups can be used. Due to small size of nanoparticles, a large number of them settle on Os-Si or Os-Cd (Figure 1). In addition, regarding the fact that CdO nanoparticles are strong electric conductors, they enhance electron transferring process (electrical potential) to Os-Pd/HfC nanocomposites coop into SiO_2 or CdO [111-171].

In the current experimental work, in addition to sample preparation and producing sensor device, the effect

of nanocomposite concentration also is investigated. As it is necessary to prevent any interference on the structure of Os-Pd/HfC nanocomposites, this issue is investigated in sample preparation and using them in electrochemical system.

On the other hand, Electrochemiluminescence (ECL) is the process where species generated at electrodes undergo electron-transfer reactions to form excited states that emit light. This chapter outlines the principles, history, applications, advantages, limitations, and possibilities for improving the performance of this technology. Electrochemiluminescence is a means of converting electrical energy into light (radiative energy). It involves the production of reactive intermediates from stable precursors at the surface of an electrode. These intermediates then react under a variety of conditions to form excited states that emit light. Traditionally, ECL was generated via annihilation, where the electron transfer reaction is between an oxidized and a reduced species, both of which are generated at an electrode by alternate pulsing of the electrode potential. Electrochemiluminescence can also be generated in a single potential step utilizing a coreactant. Coreactant ECL has been used in a wide range of analytical applications including clinical diagnostics, chromatography, food and water testing, and biowarfare agent detection. ECL can also be used in the analysis of various species. Furthermore, electrochemiluminescence has also been used to monitor enzymatic reactions. ECL labels have distinct advantages over detection methods as they are sensitive, nonhazardous, inexpensive, diagnostic of the presence of a particular label, linear over a wide range, and incorporate simple and relatively inexpensive equipment [172-190]. It should be noted that a schematic of biosensing device was presented in Figure 2 [191-194].

Materials, Research Method and Experimental Techniques

Tri-n-propylamine (TPrA), 2-(dibutylamino) ethanol (DBAE) and benzyl peroxide (BPO) were supplied from

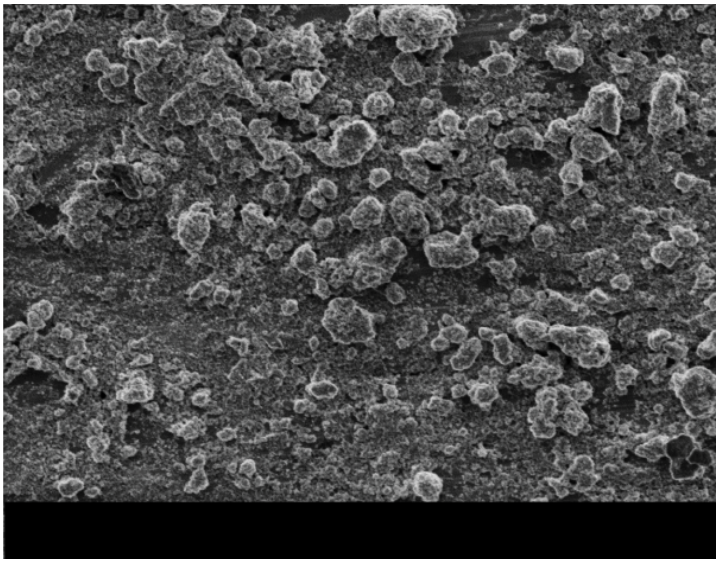

Figure 3: SEM image for the produced CdO nanoparticles with scale 500 (nm).

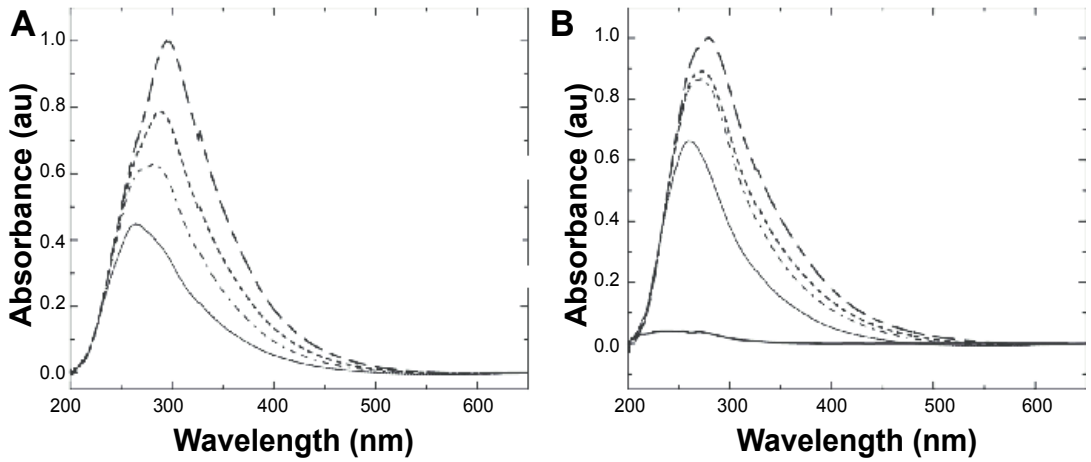

Figure 4: Comparative graphs of UV-Visible absorptive and emitted spectrum for Os-Pd/HfC nanocomposites (Photo shows SEM image for a) Os-Si; b) Os-Cd). It should be noted that each graph shows UV-Visible absorptive and emitted spectrum with the passage of time.

Sigma-Aldrich Co. Os-Pd/HfC nanocomposites and other chemicals used in this work were supplied from American International Standards Institute (AISI) and also Bio Spectroscopy Core Research Laboratory at Faculty of Chemistry, California South University (CSU), Irvine, California, USA.

For preparing Cadmium Oxide nanoparticles with negative charge on the surface, 5 (mg/mL) $HCdCl_4$ was added to boiling water and trisodium citrate was added to the boiling solution as reducing agent.

In the next step, the produced samples were deposited on Glassy Carbon Electrode (GCE) electrodes with various concentrations using Graphene Oxide (GO). GO was created in order to stabilize sample on the electrode through making π-π bonding between the created elements and electrode as well as speeding up the electron transferring between them. Then, the produced collection was placed into a solution containing tri-n-propylamine (TPrA), 2-(dibutylamino) ethanol (DBAE) and benzyl peroxide (BPO) and by applying the required electric potential difference between this electrode and reference electrode and counter used in electrochemical system, optical emission from material was produced and measured. Detection of emitted photons was performed by photon proliferator of Hamamatsu ip21.

Results and Discussion

In order to recognize the size of produced CdO nanoparticles, SEM imaging was used. Figure 3 shows a sample of SEM image produced from CdO nanoparticles. In this regard, the average size of these particles is between 15-20 (nm). Figures 4a and Figures 4b show SEM image for Os-Si and Os-Cd, respectively. Size of these nanoparticles is about 50 (nm). By comparing the obtained sizes, it was indicated that Os-Si or Os-Cd nanoparticles are able to load a large number of SiO_2 or CdO nanoparticles.

Figure 4 shows absorptive and emitted graphs of Os-Pd/HfC nanocomposites. Comparison of absorptive curves indicates 5 (nm) shift of wavelength in the spectrum of Os-Si and Os-Cd at 450 (nm) which confirms cooping of Os-Pd/HfC nanocomposites into CdO or SiO_2. In addition, according to emission curve, nanocomposite production does not change the emitted spectrum of Os and its luminescence nature and increasing the emission intensity of nanocomposite indicates copping a large number of Os-Pd/HfC nanocomposites.

As ray emitted from samples is used to affect biomolecules, its amount was measured through selecting optimum concentration of nanocomposite on the produced samples and concentration of required tri-n-propylamine (TPrA), 2-(dibutylamino) ethanol (DBAE) and benzyl peroxide (BPO) solvent before applying luminescence on biomolecules. In this regard, the intensity of luminescence of samples in solvents with various concentrations was measured to find optimum concentration of nanocomposite in the produced samples for a constant concentration of solvent, as shown in Figure 5. From this test, optimum amount of Os-Pd/HfC nanocomposites was determined as 8 (mg/mL) and then, samples with optimum concentration of nanocomposite were tested at different concentrations of tri-n-propylamine (TPrA), 2-(dibutylamino) ethanol (DBAE) and benzyl peroxide (BPO) solvent and again, optimum luminescence was obtained as 20 (mM). Figure 6, Figure 7 and Figure 8 indicates this optimum amount.

Conclusions, Perspectives, Useful Suggestions and Future Studies

As the production of Electrochemiluminescence (ECL) biosensor is performed for the first time in the

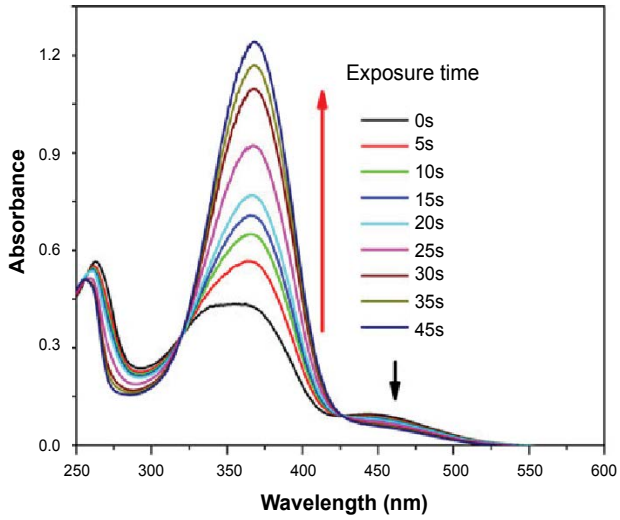

Figure 5: Photon emission for various concentrations of nanocomposite (from 1 (mg/mL) to 10 (mg/mL)). (Figure: Optimization graph of concentration of Os-Pd/HfC nanocomposites).

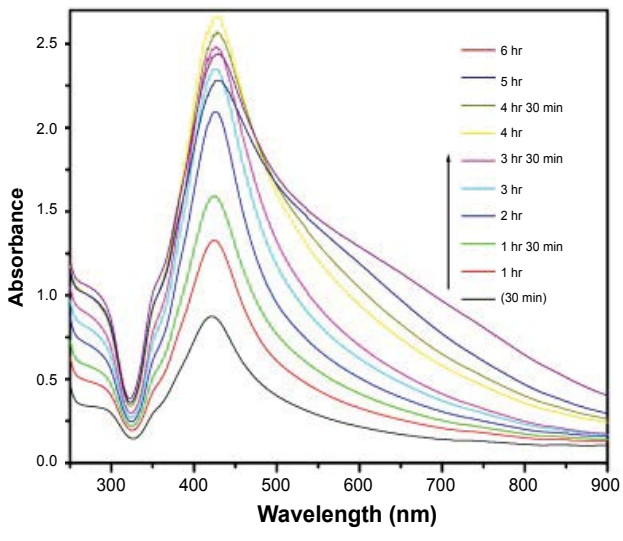

Figure 6: Photon emission for various concentrations of tri-n-propylamine (TPrA) (from 10 (mM) to 100 (mM)). (Figure: Optimization graph of concentration of tri-n-propylamine (TPrA)).

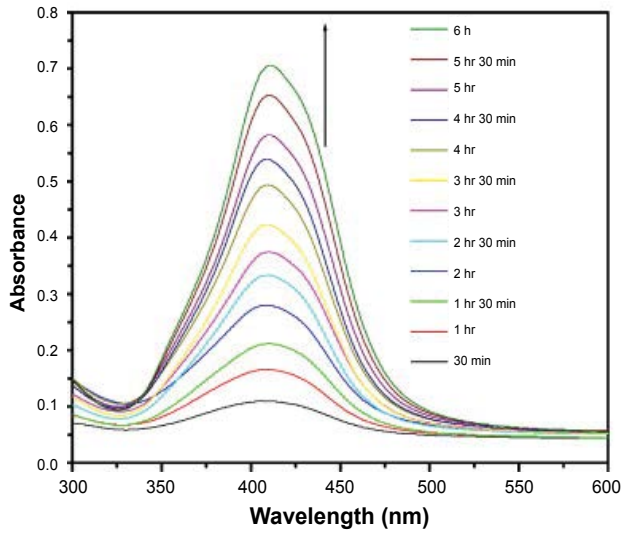

Figure 7: Photon emission for various concentrations of 2-(dibutylamino) ethanol (DBAE) (from 10 (mM) to 100 (mM)). (Figure: Optimization graph of concentration of 2-(dibutylamino) ethanol (DBAE)).

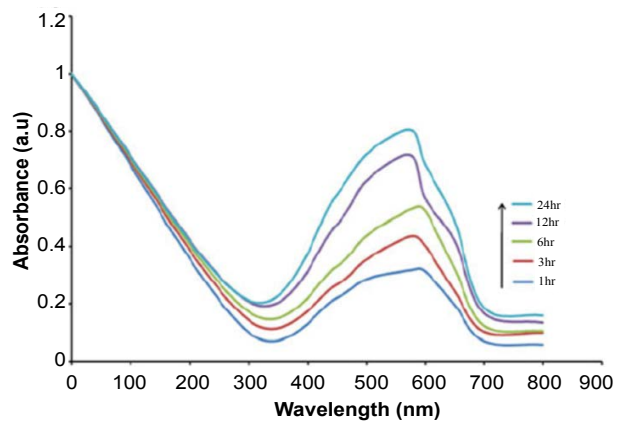

Figure 8: Photon emission for various concentrations of benzyl peroxide (BPO) (from 10 (mM) to 100 (mM)). (Figure: Optimization graph of concentration of benzyl peroxide (BPO)).

world, it was necessary to provide appropriate conditions such as high sensitivity and optimizing the effective factors for detecting and tracking of human gastroenterological cancer cells, tissues and tumors before any measurement. Lack of these conditions will lead to loss of detecting and tracking of human gastroenterological cancer cells, tissues and tumors.

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