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Magnetic nanoparticles embedded with graphene quantum dots and multiwalled carbon nanotubes as a sensing platform for electrochemical detection of progesterone

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ABSTRACT

Results of this paper related to fabrication of a simple, reproducible, stable and sensitive electrochemical sensing platform of progesterone (P4) based on graphene quantum dots (GQDs) nanocomposite. GQDs, Fe_3O_4 nanoparticles and functionalized multi–walled carbon nanotubes (f–MWCNTs) modified glassy carbon electrode (Fe_3O_4@GQD/f–MWCNTs/GCE) were constructed and the electrocatalytic properties of the modified electrode toward the oxidation of P4 were analyzed. GQDs with the size of about 15 nm were prepared by a facile and low expense bottom–up method by carbonization of citric acid and dispersing the carbonized products into alkaline solution. The characterization of the sensor was studied by transmission electron microscopy (TEM), X–ray diffraction (XRD), UV–vis spectroscopy, Fourier transform infrared spectroscopy (FT–IR) and voltammetry techniques. Electrochemical studies suggested that as–prepared sensor revealed some advantages in terms of high effective surface area, more reactive sites and excellent electrochemical catalytic activity toward the oxidation of P4. After optimization of analytical conditions, the peak currents for P4 were found to vary linearly with its concentrations in the wide range of 0.01–0.5 and 0.5–3.0 μM. The estimated detection limit and sensitivity of the electrode were 2.18 nM and 16.84 μA μM^{-1}, respectively. This sensor exhibited excellent stability, selectivity, sensitivity and reproducibility and could be successfully applied for determination of P4 in human serum samples and pharmaceutical products with excellent recoveries and without the interferences of coexisting substances.

1. Introduction

Sensitive, effective and rapid detection of specific biomolecules is critical in medical diagnosis, biological engineering and environmental protection [1]. In the last two decades with the highly developed nanotechniques, various types of nanomaterials including nanoparticles [2,3], carbon nanotubes [4,5], quantum dots [6,7], and electroactive component encapsulated nanoparticles [8,9] have been grabbing scientists' attention for applications in sensors, biocatalysis, electronics and medicines. The unique chemical and physical properties of nanomaterials and their enormous advantages, such as large surface to volume ratio, electrocatalysis and active surface sites, make them extremely suitable for designing new sensing devices, especially electrochemical sensors and biosensors [10]. Among all nanostructures, quantum dots are one of the best candidates which are superior in terms of their excellent properties [11,12].

Graphene quantum dots (GQDs) are a kind of 0D material with characteristics derived from both graphene and carbon dots (CDs), which can be regarded as incredibly small pieces of graphene [13–17] which are one, two or few–layered graphene sheets with lateral dimensions smaller than 100 nm [11,14,18–20]. By converting 2D graphene sheets into 0D GQDs, the GQDs exhibit new phenomena due to quantum confinement and edge effects, which are similar to CDs [15,20]. Their novel properties, such as remarkable conductivity, high surface–to–volume ratio, stable luminescence, good biocompatibility, and low toxicity, result in high performance sensors based on GQDs [21–24]. As compared to other carbon nanomaterials, such as graphene and carbon nanotube, GQDs possess larger number of carboxyl groups, much smaller volumes, and have good electrical conductivity. Therefore, it is considered as an excellent candidate for construction of nanoscaled, sensitive, stable and cost–effective electrochemical sensor [13,14,20,25,26].

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As an important magnetic material, magnetite (Fe_3O_4) nanoparticles (MNPs) have attracted an increasing interest for application in sensors, which arising from their high surface area, high biocompatibility, low toxicity, easy preparation, and high adsorption ability. Furthermore, Fe_3O_4 nanoparticles have a unique ability to promote fast electron transfer between electrode and the active site of the redox reaction [27,28]. However, pure magnetite nanoparticles may not be very stable over time because they are very likely to aggregate to reduce the energy associated with ratio of surface area to volume and the strong dipole–dipole attraction between particles [29–31]. To overcome these limitations, various approaches are used to change the surface of Fe_3O_4 NPs via the loading of other materials during or after the synthesis process [32,33].

Nowadays, carbon nanotubes mainly multi–walled carbon nanotubes (MWCNTs) have enjoyed widespread attention in the task of sensors and biosensors, owing to their high surface area, high electrical conductivity, good stability, unique electronic and nano-structural properties. It is also known that the surface of the electrode became more porous after adding MWCNTs [34–37]. In addition, Van der Waals and chemical interactions between MWCNTs and graphene materials and electrostatic interactions between MWCNTs and magnetic nanoparticles, resulting in more stable and conductive platform at the surface of modified electrode [38,39].

One of steroid hormones derived from cholesterol with a vital role in human and animal health is progesterone. Progesterone (4–pregnone–3,20 dione, P4) is a non–saturated α,β ketone that is important in the stabilization and maintenance of pregnancy in mammals, acting in the synthetic route of various biologically active steroids [40,42] and playing a role in regulating the sequence of the ovulatory cycle by controlling other hormones [43–45]. Also in the pharmaceutical industry, P4 is the active principle of many drugs used in hormone replacement therapies [46]. An imbalance of P4 in the human organism can cause malformations of the reproductive system and also infertility problems. Therefore, P4 is an ideal candidate as a marker for early diagnosis of pregnancy [41,44,47].

Various techniques have been employed for P4 determination, consist of high performance liquid chromatography (HPLC) and liquid chromatography (LC) coupled with different detectors (DAD [48], UV [40,49], MS [50], and DCE [51]), time–resolved fluoroimmunoassay [52], fluorescent immunoassay [53], and chemiluminescence enzyme immunoassay (CLEIA) [54]. These approaches possess high sensitivity. Nevertheless, most of these methods suffer from poor selectivity, use of unstable and corrosive reagents, sample preparation steps, considerable time delays between sampling and obtaining results, and require expensive equipments which can adversely affect the analytical results [43,55]. Thus, there has been continuous effort to develop reliable and sensitive electrochemical methods, which can permit cost–effective, convenient, rapid and selective determination of P4 in clinical laboratories.

Taking into account the properties of Fe_3O_4, GQDs and MWCNTs mentioned above, assembling of these nanomaterials generates a versatile 3D hybrid network with synergic and electrocatalytic characteristics of them. Accordingly, the focus of the present paper is the use of Fe_3O_4@GQD/f–MWCNTs nanocomposites based modified electrodes for sensitive detection of P4. The developed sensor has some advantages such as simplicity of electrode preparation, wide linear range, low detection limit, durability, sensitivity and selectivity, and low cost for the determination of P4 in various real samples. Furthermore, the effects of supporting electrolyte, pH, scan rate, accumulation time and potential and interference effect were studied in details. To the best of our knowledge, up to now, no other works in the literatures have been reported on applying the proposed electrode for determination of P4 in human serum samples and pharmaceutical products.

2. Materials and methods

2.1. Reagents and solutions

All reagents and solvents were of the highest purity available from Merck (Darmstadt, Germany) and were used without further purification. Progesterone and other hormones were from Aburaihan Pharmaceutical Co. (Tehran, Iran). Citric acid (CA), iron(III) chloride ($FeCl_3{\cdot}6H_2O$), iron(II) chloride ($FeCl_2{\cdot}4H_2O$) were obtained from Sigma–Aldrich (St. Louis, Missouri, USA). The MWCNTs (>95%) was obtained from Plasma Chem. GmbH (Berlin, Germany). Aqueous solutions were prepared with phosphate buffer solution (PBS) 0.1 M in the pH range 3.0–9.0 by mixing solutions of 0.1 M $Na_2HPO_4.12H_2O$ and 0.1 M $NaH_2PO_4.H_2O$. The stock solution of P4 was prepared fresh at the time of experiments. All solutions were prepared and diluted using high quality deionized water.

2.2. Apparatus

Electrochemical measurements were conducted using the Autolab PGSTAT 30 electrochemical analyzer (Ecochemie BV, Utrecht, the Netherlands) controlled by Nova 1.7 software. GCE was purchased from Azar Electrode Company (Urmia, Iran). All electrochemical studies were performed at 25 ± 1 °C. A conventional three–electrode cell was employed, incorporating a modified working electrode, a saturated Ag/AgCl reference electrode, and a platinum wire counter electrode. The pulse amplitude of 0.025 V, pulse interval time of 0.5 s and scan rate of $0.01\ V\,s^{-1}$ over the potential range of 0.5–1.2 V *vs.* Ag/AgCl were selected as optimum instrumental parameters for differential pulse voltammetry (DPV) measurements. All the pH values were measured with a Metrohm pH meter (model 827, Swiss made). A model EM10C–100 KV (Zeiss) transmission electron microscope, Philips PW1840 X–ray powder diffractometer and Shimadzu 8900 Fourier transform infrared spectrometer (Kyoto, Japan) were used to characterize the morphology, size and structure of the nanomaterials. The amperometry analysis was carried out by PalmSens analyzer at the potential of 0.9 V under continuous stirring. Electro–chemiluminescence (ECL) method (Roche, Cobas E411) was used as a reference method for P4 determination.

2.3. Synthesis of graphene quantum dots (GQDs)

The GQDs were prepared by directly pyrolyzing of CA (Scheme 1) [56]. In a typical procedure of GQDs preparation, 2.0 g CA was put into a 5.0 mL beaker and heated to 200 °C using a heating mantle. About 5 min later, the CA was liquated. Subsequently, the color of the liquid was changed from colorless to pale yellow, and then orange in 30 min, implying the formation of GQDs. The obtained orange liquid for preparing GQDs was added drop by drop into 50 mL of $20\ mg\,mL^{-1}$ NaOH solution, under vigorous stirring. After neutralized to pH 7.0 with NaOH, the aqueous solution of GQDs was obtained.

2.4. Synthesis of the Fe_3O_4@GQDs nanocomposite

Fe_3O_4 nanoparticles were prepared according to the modified Massart method [57] via the co-precipitation of a mixture of $FeCl_3{\cdot}6H_2O$ and $FeCl_2.4H_2O$. First, 0.809 g $FeCl_3{\cdot}6H_2O$ was added to 50 mL GQDs solution at room temperature under a nitrogen atmosphere with vigorous stirring. Then, temperature was increased to 80 °C, and 0.323 g of the $FeCl_2{\cdot}4H_2O$ was added slowly to the solution containing Fe^{3+}/GQDs which was vigorously stirred. Then,