Sensors and Actuators B 245 (2017) 753–764

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Analysis of the heterogeneous structure of iron oxide/gold nanoparticles and their application in a nanosensor

Y. Bustami[a,b,*], M. Moo-Young[a], W.A. Anderson[a]

[a] Department of Chemical Engineering, University of Waterloo, Ontario, N2L3G1, Canada
[b] School of Biological Sciences, Universiti Sains Malaysia, Penang, 11800, Malaysia

ARTICLE INFO

Article history:
Received 19 April 2016
Received in revised form 16 January 2017
Accepted 23 January 2017
Available online 27 January 2017

Keywords:
IONPs/AuNPs
Electrostatic self-assembly
Glucose oxidase immobilization
Glucose detection

ABSTRACT

Magnetic iron oxide-gold nanoparticles (IONPs-AuNPs) could provide a useful platform for biosensing purposes. The aim of this study was to investigate the formation of such particles using a simple electrostatic self-assembly technique, and then to demonstrate their application using a glucose detection assay. AuNPs were attached to the IONPs surface by ionic interaction, as verified using UV–vis spectrophotometry. Then, a carbodiimide-coupling technique was used to covalently attach glucose oxidase (GOx) onto the nanoparticles' surface and the bioactivity was measured using an ABTS colorimetric assay. For characterization, UV–vis spectrophotometer, DLS, zeta potential, TEM, EDX, XPS and FTIR techniques were used. The particle diameter obtained from TEM was 16.1 ± 11.1 nm and EDX confirmed the presence of Au and Fe elements. In addition, FTIR results exhibited strong vibrational modes around 1654, 1546 and 3369 cm^{-1} that appeared to be primarily due to immobilization of GOx onto Fe/Au. The colorimetric assay also showed a significant increase in green color intensity, due to oxidation of ABTS, with increasing glucose concentrations ranging from 20 μM to 100 μM. IONPs/Au nanoparticles showed a good potential for application in this colorimetric assay, thus suggesting an excellent basis for a nanosensor system using these particles.

1. Introduction

In recent years, a large number of studies have focused on the construction of magnetic nanoparticles coated with gold nanoparticles, since this provides an excellent platform for multifunctionality. At the nanosized scale, both nanoparticles develop into a new hybrid material where the surface effects dominate and contribute to a superparamagnetism phenomenon for the magnetite phase [1], and a high plasmon field and potential for surface functionalization for the gold nanoparticle portion [2]. Based on these special characteristics, the selective separation or removal of magnetic nano- and micro- particles and composites from the complex samples is easily performed. This process can be very important for bio-applications and environmental technology because most of the biological materials and contaminants of interest have no magnetic properties. When this biological material or contaminant is coupled to a magnetic material, an efficient selective separation is enabled [3,4].

Gold nanoparticles (AuNPs), a nanosized metal particle that has a high plasmon field upon receiving optical energy [5], are used widely for analytical signals. The AuNPs capacity for surface functionalization receives a great deal of attention as it exhibits a strong interaction with thiolated linkers or biomolecules. Amine groups and cysteine residues in proteins are known to bind strongly with gold colloids [6–8] due to chemisorption.

It is known that the coating of iron oxide nanoparticles (IONPs) with AuNPs is a potentially versatile approach, particularly for biodiagnostic applications. Since IONPs are not stable, are easily oxidized and have limited options for bio-functionalization, hence, further chemical functionalization needs to be performed to form biocompatible nanoparticles. AuNPs have been shown to be a useful shell coating for magnetite as it adds functionality as well as improves its stability in aqueous dispersions [9]. The composition of the core, shell and interface structure exhibits a unique physiochemical property and makes these composites suitable for many nano-biotechnology research applications.

However, the formation of core-shell nanoparticles is quite challenging as the Au shell forms a poor diffusion barrier against the core layer and there are significant difficulties in controlling the uniformity and thickness of the metal coating [10]. Therefore, some researchers have suggested the formation of alloy nanoparticles or

* Corresponding author at: School of Biological Sciences, Universiti Sains Malaysia, Penang, 11800, Malaysia.
E-mail address: ybustami@usm.my (Y. Bustami).

http://dx.doi.org/10.1016/j.snb.2017.01.142
0925-4005/

nanoalloys. A few studies have reported the preparation of nanoalloys using pulsed laser deposition [11], carbon film deposition at very high temperature (1600 K) in the presence of helium flow [12] and electrodeposition [13]. One study used high-temperature colloidal synthesis to decompose iron pentacarbonyl in the presence of gold nanocrystals [14]. In addition, dumbbell-like Au–Fe_3O_4 particles exhibited bifunctional properties with high magnetization and excellent catalytic activity toward nitrophenol reduction [15], when fabricated using thermal decomposition of the iron-oleate complex in the presence of Au seeds.

Some studies have provided information on the interfacial reactivity, the structural and electronic properties of various morphologies of Au–Fe_3O_4 heterostructures, and an understanding of the interaction between the magnetite and the gold nanoparticle surfaces [15–17]. However, information on IONPs-AuNPs fine heterostructure, charge transfer and their interfacial relationships are still limited and ongoing investigation needs to be done. In addition, it is also of interest to further investigate these heterogeneous nanoparticles for applications as a supporting material for biomolecules such as enzymes. Several enzymes and biomolecules have been successfully immobilized onto IONPs-AuNPs nanoparticles, for examples glucose oxidase [18], sulfite oxidase [19] and tyrosinase.

In our work, the heterogeneous structure of hybrid iron oxide nanoparticles-gold nanoparticles (IONPs-AuNPs) was investigated, as well as their potential application in nanosensors. The IONPs were complexed with AuNPs using a simple and easy electrostatic self-assembly technique, adapted from a layer-by-layer deposition technique which exploits surface layer electrostatic attractions for a quick and easy deposition of heterogeneous nanoparticles. Instead of using polyelectrolyte, we manipulated the interfacial charge between IONPs and AuNPs since IONPs surface charges can easily be tuned to positive or negative values by a simple pH adjustment. Subsequently, these nanoparticles were used to immobilize glucose oxidase on the surface, and these were then used for the colorimetric detection of glucose in aqueous solution using an ABTS assay.

2. Materials and methods

2.1. Chemicals

The chemical reagents used in this work were ferrous sulfate heptahydrate ($FeSO_4.7H_2$, ReagentPlus®, >99%), hydrogen tetrachloro-aurate (III) ($HAuCl_4.3H_2O$, ≥99.9%), sodium hydroxide (NaOH, ACS Reagent, ≥97%), trisodium citrate ($Na_3C_6H_5O_7$, anhydrous, ≥98%, GC), nitric acid (HNO_3, ACS Reagent 70%) diluted to 65%, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS, 10 mg/tablet), *N*-ethyl-N′- (3-dimethylaminoprophyl) carbodiimide hydrochloride linker (EDC-linker, commercial grade, powder), *N*-hydroxysuccinimide (NHS, 98%), D-glucose (≥99.5%, GC), glucose oxidase Type VII from *Aspergillus Niger* (GOx), horseradish peroxidase lyophilized powder (HRP), 11-Mercaptoundecanoic acid (11-MUDA, M_w: 218.36 g/mol), PBS buffer (tablet), Tween 20 (viscous liquid), 2-(*N*-morpholino)ethanesulfonic acid buffer (MES buffer, ≥99.5%) and sodium acetate anhydrous buffer (NaAc). All reagents were purchased from Sigma Aldrich Canada Co. (Oakville ON) and were used as received without further purification.

2.2. Synthesis of IONPs

IONPs were synthesized using a reverse co-precipitation method. This method was adapted from Mahmed et al. [20] with slight modification. First, 50 mL NaOH (1 M) and 1 mL $Na_3C_6H_5O_7$ (1 mM) were mixed in 50 mL deionized water. Then, 55.6 mg of $FeSO_4.7H_2O$ was added into the mixture with vigorous stirring for 10 min at room temperature. After adding the salts into the alkaline solution, black precipitates were observed, suggesting the formation of IONPs. The resulting precipitate was then immediately microwave irradiated for up to 30 s and then was readily collected by a permanent magnet after the solution cooled down to room temperature. The black precipitates were then air-dried overnight. This black precipitate (12 mg) was treated with 10 mL HNO_3 (6 M) by stirring vigorously for 10 min and the color immediately changed to brown-reddish. The IONPs were resuspended in deionized water, were centrifuged for 10 min at 6500 rpm, and separated using a permanent magnet. The separated IONPs were then added to 1 mL deionized water with the IONPs final concentration of 12 mg/mL, and this was stored at room temperature until use.

2.3. Synthesis of AuNPs

Citrate-capped AuNPs were synthesized using the Turkevish method by mixing 500 μL $HAuCl_4{\cdot}3H_2O$ (10 mM) into a solution containing 300 μL trisodium citrate ($Na_3C_6H_5O_7$) (100 mM) and 10 mL deionized water. The solution was microwave irradiated for 45 s with 10 s interval mixing and the formation of AuNPs was observed as the solution color changed to dark red.

2.4. Synthesis of IONPs-AuNPs

AuNPs were coated onto the IONPs surface using a variation of the layer-by-layer deposition technique. For this electrostatic self-assembly methodology, 12 mg/mL of IONPs treated with HNO_3 were mixed with 2.5 mL of the citrate-capped AuNPs for 1 h. The nanoparticles were then separated by a permanent magnet overnight. The separated IONPs-AuNPs were stabilized with 3.5 mL of PBS-T (10 mM, pH 4) by vigorous mixing for 1 h. Subsequently, the solution was centrifuged for 10 min at 6500 rpm and the IONPs-AuNPs were separated using a permanent magnet, washed several times with PBS-T, and were stored at 4 °C until use.

2.5. Detection of glucose

2.5.1. Carbodiimide-coupling strategy

IONPs-AuNPs were weighed out to approximately 29 mg and added to 950 μL MES buffer, (10 mM, pH 4). In a separate tube, 28.85 mg of 11-MUDA was dissolved in 10 mL methanol, and 50 μL of this freshly prepared solution was then added to the IONPs-AuNPs solution followed by mixing at room temperature for 1 h. In order to remove the unbound 11-MUDA, the solution was washed several times and IONPs-AuNPs were separated using a permanent magnet. Finally, 1 mL of MES buffer (10 mM, pH 4) was added to the nanoparticles. In a separate tube, 0.312 mg of EDC-linker and 1.24 mg of NHS were mixed in 1 mL MES buffer (10 mM, pH 4). Subsequently, this solution was added to the carboxylate-modified nanoparticles (IONPs-AuNP-COOH) and this was incubated at 4 °C for 30 min without mixing. To remove excess EDC-linker and NHS, the solution was centrifuged for 10 min at 6500 rpm and washed several times with PBS-T (10 mM, pH 4). The nanoparticles were separated using a permanent magnet and 1 mL MES buffer (10 mM, pH 4) was added to the nanoparticles, which were then stored at 4 °C until use.

2.5.2. Immobilization of glucose oxidase (GOx)

Carboxylate-modified nanoparticles (180 μL) was mixed with 20 μL of GOx (1 mg/mL) and was incubated at 4 °C for 1 h. Subsequently, the nanoparticles-bioconjugate (IONPs-AuNP-GOx) was centrifuged for 10 min at 6500 rpm and separated using a permanent magnet. The unbound GOx was carefully pipetted from