



# One-step synthesis of potassium ferricyanide-doped polyaniline nanoparticles for label-free immunosensor

Sijing He<sup>a</sup>, Qiyu Wang<sup>b,\*</sup>, Yanyan Yu<sup>a</sup>, Qiuja Shi<sup>a</sup>, Lin Zhang<sup>a</sup>, Zuanguang Chen<sup>a,\*</sup>

<sup>a</sup> School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510006, China

<sup>b</sup> The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou 510630, China

## ARTICLE INFO

### Article history:

Received 27 September 2014

Received in revised form

3 January 2015

Accepted 7 January 2015

Available online 8 January 2015

### Keywords:

One-step synthesis

Potassium ferricyanide-doped polyaniline nanoparticles

Gold nanoparticles

Label-free immunosensor

Carcinoembryonic antigen

## ABSTRACT

A novel, label-free and inherent electroactive redox immunosensor for ultrasensitive detection of carcinoembryonic antigen (CEA) was proposed based on gold nanoparticles (AuNPs) and potassium ferricyanide-doped polyaniline (FC-PANI) nanoparticles. FC-PANI composite was synthesized via oxidative polymerization of aniline, using potassium ferricyanide ( $K_3[Fe(CN)_6]$ ) as both oxidant and dopant. FC-PANI acting as the signal indicator was first fixed on a gold electrode (GE) to be the signal layer. Subsequently, the negatively charged AuNPs could be adsorbed on the positively charged FC-PANI modified GE surface by electrostatic adsorption, and then to immobilize CEA antibody (anti-CEA) for the assay of CEA. The CEA concentration was measured through the decrease of amperometric signals in the corresponding specific binding of antigen and antibody. The wide linear range of the immunosensor was from  $1.0 \text{ pg mL}^{-1}$  to  $500.0 \text{ ng mL}^{-1}$  with a low detection limit of  $0.1 \text{ pg mL}^{-1}$  ( $S/N=3$ ). The proposed method would have a potential application in clinical immunoassays with the properties of facile procedure, stability, high sensitivity and selectivity.

© 2015 Elsevier B.V. All rights reserved.

## 1. Introduction

The levels of tumor markers in serum or tissue provide essential information for clinical cancer screening and disease diagnosis (Sidransky, 2002; Wulfkühle et al., 2003). Carcinoembryonic antigen (CEA), an acidic glycoprotein found in colorectal carcinomas, is a common tumor marker for clinical diagnosis of colorectal, pancreatic, gastric and cervical carcinomas (Goldenberg et al., 1976; Benchimol et al., 1989; Oikawa et al., 1989). The dominant quantitative determination of tumor markers in the clinic is attributed to the immunoassay techniques (Fowler et al., 1989; Brochot and Siddiqi, 1989).

Compared with conventional immunoassay techniques, electrochemical immunosensors based on the specificity of antigen-antibody interactions with electrochemical transduction have attracted considerable interest due to their intrinsic advantages, such as low cost, simple preparation, good portability, rapid analysis and sensitive measurements (Lai et al., 2012; Wang et al., 2006; Yin et al., 2011). Generally, the majority of electrochemical immunosensors are performed depending on label of antibodies or antigens, or utilize reversible redox species in the electrolyte

solution. However, the labeling process is time-consuming, expensive, complex, and easy to be denatured for the susceptibility of biomolecules (Lin et al., 2012; Qiu et al., 2009). The redox species in solution take a risk of being polluted and affecting the bioactivity of antibodies or antigens. Efforts are continuing towards the development of a novel label-free electrochemical immunosensor attached the redox species on the electrode surface, which eliminates direct contact with antibodies. Various redox compounds have been adopted to fabricate electroactive nanocomposites. Ferrocene was covalently bonded to the chitosan long chain as a novel redox active hybrid material for detection of hepatitis B surface antigen (Qiu et al., 2009). The graphene oxide-thionine-Au nanocomposites was synthesized with efficient redox-activity and conductivity (Han et al., 2013). Lu et al. (2014) developed a label-free immunosensor based on redox ultrathin AuPt nanowire decorated thionine/reduced graphene oxide matrix. A novel redox membrane was prepared using chitosan and  $K_3[Fe(CN)_6]$ , and Nafion was used to adhere the redox species to the membrane (Shi and Ma, 2011). A  $K_3[Fe(CN)_6]$ -chitosan-glutaraldehyde system capable of high signal indication was described (Weng et al., 2013). Among many redox mediators as signal molecule,  $K_3[Fe(CN)_6]$  has been widely employed as an electron transfer mediator due to good reversible redox properties and low cost. However, there are several challenges concerning immobilization of electron-shuttling mediators on the interfaces of electrode, especially for soluble mediators

\* Corresponding author. Fax +86 20 3994 3071.

\*\* Corresponding author

E-mail addresses: [1732259720@qq.com](mailto:1732259720@qq.com) (Q. Wang), [chenzg@mail.sysu.edu.cn](mailto:chenzg@mail.sysu.edu.cn) (Z. Chen).

with low molecular weight which can easily diffuse away from the electrode surface into the bulk solution, resulting in poor response performance. Increasing the molecular weights of the mediators via attaching to polymers could address this problem well (Okawa et al., 1999; Das et al., 2007).

Conducting polymers, especially polyaniline (PANI), have been extensively applied in biosensor field. The conjugated  $\pi$ -electron backbones in their chemical composition induce their unusual electrochemical characteristics including low ionization potential, high electrical affinity and high electronic conductivity (Teles and Fonseca, 2008). Biological and electronic properties of PANI could improve by doping different types of inorganic materials (Schultz and Karabulut, 2005). To the best of our knowledge, there are no reports on FC-doped PANI nanoparticles. In this one-step reaction in aqueous medium, protons from hydrochloric acid were attracted by quinonoid nitrogen atoms of the PANI long chains, which made quinone reduce to benzene, and then the PANI chains took positive charges. In order to keep the electric neutrality of the PANI chains, anions of dopant ( $[\text{Fe}(\text{CN})_6]^{3-}$ ) were incorporated as pendant groups along the conducting polyaniline backbone accompanied by protons (Scheme 1). The unique properties of AuNPs to provide a suitable microenvironment for biomolecules immobilization, and to facilitate electron transfer between the immobilized biomolecules and electrode substrates, have led to an intensive use of this nanomaterial for the construction of considerable immunosensors with enhanced analytical performance (Wang et al., 2013; Chen et al., 2012; Lu et al., 2012). Therefore, AuNPs were introduced to FC-PANI composite modified GE for anchoring anti-CEA.

Here, a simple and ultrasensitive electrochemical immunosensor was fabricated for the first time based on immobilization of antibody molecules on biocompatible redox-active AuNPs/FC-PANI composite. The formation of FC-PANI nanoparticles could avoid the leakage of  $[\text{Fe}(\text{CN})_6]^{3-}$  from the matrix,

retain its electrochemical activity and improve the efficiency of heterogeneous mediation. An electrode immobilized FC-PANI composite without any fixing materials (eg. chitosan or Nafion) possessed prominent biocompatibility, good conductivity, large specific surface area and excellent redox electro-activity, and thus could serve as an excellent platform for biomolecules immobilization and electrocatalysis. The detection of CEA was realized by monitoring the change in the electrode response of  $[\text{Fe}(\text{CN})_6]^{3-}$  as the redox mediator.

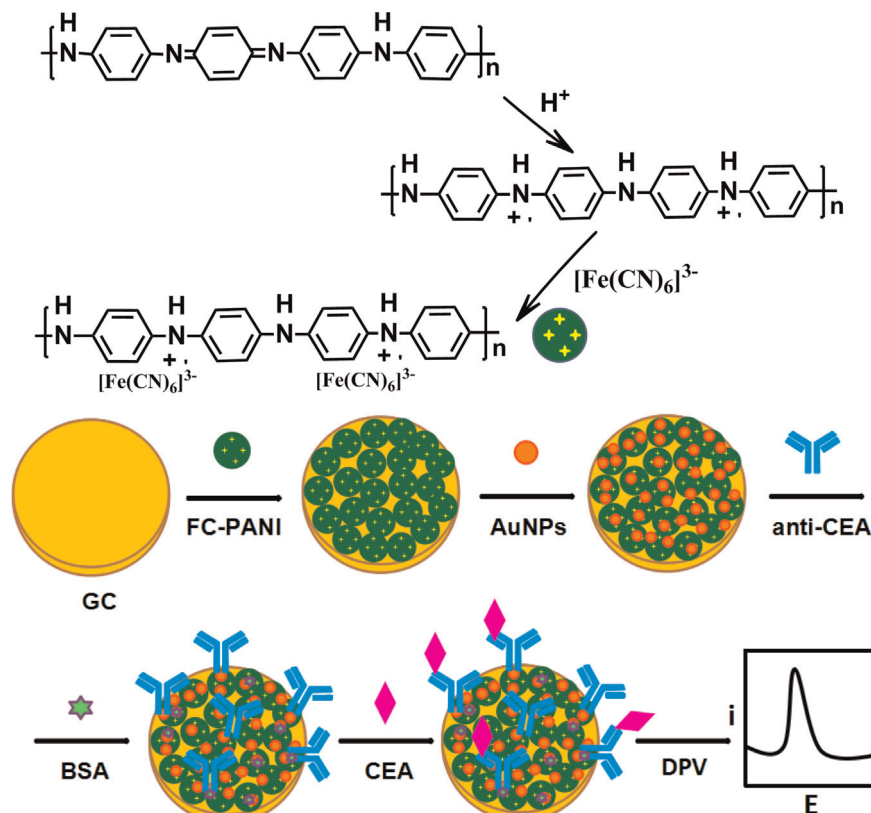
## 2. Experimental

### 2.1. Materials and reagents

Carcinoembryonic antigen (CEA) and anti-carcinoembryonic antigen (anti-CEA) were purchased from Biosynthesis Biotechnology Co. Ltd. (Beijing, China). Human immunoglobulin G (IgG) and albumin from bovine serum (BSA) were obtained from Chengwen Biological Company (Beijing, China). Gold chloride ( $\text{HAuCl}_4$ ), polyvinyl alcohol (PVA) and sodium borohydride ( $\text{NaBH}_4$ ) were provided by Sigma Chemical Co. (USA). Aniline, potassium ferricyanide ( $\text{K}_3[\text{Fe}(\text{CN})_6]$ ), sodium citrate, D-(+)-glucose, uric acid (UA) and ascorbic acid (AA) were from Aladdin chemistry Co. Ltd. (Shanghai, China). Acetic acid–sodium acetate (HAC–NaAc) buffer solution was employed as the supporting electrolyte. All chemicals were of analytical grade and used without further purification, and redistilled water was used throughout.

### 2.2. Apparatus

All electrochemical measurements were performed with a RST5100F electrochemical workstation (Suzhou Risetest



Scheme 1. Schematic illustration of the stepwise immunosensor fabrication process.

Download English Version:

<https://daneshyari.com/en/article/7232595>

Download Persian Version:

<https://daneshyari.com/article/7232595>

[Daneshyari.com](https://daneshyari.com)