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Full Length Article

Porphyran-capped gold nanoparticles modified carbon paste electrode: a simple and efficient electrochemical sensor for the sensitive determination of 5-fluorouracil

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ABSTRACT

The application of carbon paste electrodes modified with porphyran-capped gold nanoparticles (CPE/AuNps-PFR) to detect an important anticancer drug, 5-fluorouracil (5-FU), is described. Gold nanoparticles (AuNps) were synthesized through a green one-pot route, by using porphyran (PFR) (a sulfated polysaccharide extracted from red seaweed) as reducing and stabilizing agent. The reaction temperature and the concentrations of $AuCl_4^-$ and PFR for AuNps-PFR synthesis were optimized by using a 2^3 full factorial design with central point assayed in triplicate. The smallest particle size (128.7 nm, obtained by DLS) was achieved by employing a temperature of 70 °C and $AuCl_4^-$ and PFR concentrations equal to 2.5 mmol L^{-1} and 0.25 mg mL^{-1}, respectively. The AuNps-PFR nanocomposite was characterized by UV–vis spectroscopy, FTIR, DLS, TEM, XRD and zeta potential, which proved that PFR was effective at reducing and capping the AuNps. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) experiments showed that the nanocomposite could enhance the electrochemical performance of the electrodes, as a consequence of the high conductivity and large surface area presented by the AuNps. The CPE/AuNps-PFR was able to electrocatalyze the oxidation of 5-FU by CV and differential pulse voltammetry (DPV). A linear relationship between the DPV peak currents and 5-FU concentration was verified in the range from 29.9 to 234 μmol L^{-1} in 0.04 mol L^{-1} BR buffer solution pH 8.0. Detection and quantification limits were found to be 0.66 and 2.22 μmol L^{-1}, respectively. Besides the good sensitivity, CPE/AuNps-PFR showed reproducibility and did not suffer significant interference from potentially electroative biological compounds. The good analytical performance of the modified electrode was confirmed for determining 5-FU in pharmaceutical formulations, with good percent recoveries (ranging from 96.6 to 101.4%) and an acceptable relative standard deviation (RSD = 2.80%).

1. Introduction

5-Fluorouracil (5-FU; Fig. 1A) is an antineoplastic agent that has been widely employed in cancer chemotherapy, mainly in the treatment of colorectal, pancreatic, stomachic and breast tumors [1–3]. 5-FU acts on cancer cells both by direct incorporation into nucleic acids and by inhibiting thymidylate synthase enzyme, which is involved in nucleotide synthesis [4]. Nevertheless, the possibility of occurrence of severe side-effects as a result of the non-specificity presented by 5-FU makes its determination in pharmaceuticals and biological fluids a topic of considerable relevance to the oncologic medicine [5].

Several analytical techniques have been proposed for 5-FU detection, such as HPLC [6], capillary electrophoresis [7], LC–MS/MS [8], GC–MS [9] and fluorescence spectrometry [10]. In spite of their good performance, these methods are generally expensive, time-consuming and require relatively complex procedures for sample preparation [11]. In view of this situation, development of more practical, fast and inexpensive methods is necessary for 5-FU determination [2]. In this context, electroanalytical techniques emerge as efficient alternatives to the aforementioned methods, due to advantages such as low-cost, good sensitivity, selectivity, fast response, possibility of miniaturization and short analysis time [2,11]. The use of electrochemical sensors based on the so-called chemically mod-

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ified electrodes (CME) makes the electroanalytical methods even more attractive, since the chemical modifier provides a greater sensitivity and selectivity to the electrodic material, and improves its electrocatalytic properties, which enhances the analytical performance of the method [12].

Metal nanoparticles have been extensively exploited for developing CME. Among them, gold nanoparticles (AuNps) have received special attention as chemical modifiers due to the unique and interesting properties that emerge when gold presents in the nanoscale. The high electrical conductivity along with the high surface area displayed by this kind of nanomaterial may increase the sensitivity of the modified electrodes, while their catalytic properties can reduce the overpotential of many electrochemical reactions, improving the detection process. Thus, AuNps-based CME can be considered as suitable devices for application in electroanalysis [13,14].

The main methods for producing AuNps are based on the chemical reduction of gold complexes in the presence of reducing and capping agents such as sodium citrate, sodium dodecyl sulfate and sodium borohydride. However, most of these reagents are toxic and might be harmful to the human health and to the environment [15]. In this sense, green methods that use materials from natural sources (i.e. plant extracts, biodegradable polymers, microorganisms, vitamins and polysaccharides) for AuNps synthesis have been constantly developed [15–19].

Porphyran (PFR; Fig. 1B) is a well-known sulfated polysaccharide that is extracted from red seaweed. The structure of PFR is composed by a linear backbone of alternating 3-linked-β-D-galactopyranose units and 4-linked-α-L-galactopyranose-6-sulfate or 3,6-anhydro-α-L-galactopyranose units [20]. The application of PFR and other sulfated polysaccharides for the green synthesis of metal nanoparticles has gained prominence as a result of its biocompatibility, biodegradability, nontoxicity, low cost and natural abundance [21]. Moreover, PFR can act simultaneously as a reducing and capping agent, which eliminates the need to add other reagents during the synthetic process [22]. Therefore, PFR can be considered as a convenient green material for nanoparticle production, and the nanocomposite based on these two materials may find a promising and attractive applicability in the chemical modification of electrodes.

In this sense, the present work reports the development of an efficient electrochemical sensor for 5-FU determination based on a carbon paste electrode modified with porphyran-capped AuNps (CPE/AuNps-PFR). The AuNps-PFR nanocomposite was obtained by a simple one-pot route, based on the principles of green chemistry [23]. The synthesis process was optimized through an experimental design in order to achieve a smaller particle size, and the final product was characterized by spectroscopic and morphological techniques. The CPE/AuNps-PFR showed sensitivity towards 5-FU and it was successfully applied for its detection in a pharmaceutical injection sample, by using differential pulse voltammetry.

2. Material and methods

2.1. Chemicals

All reagents were of analytical grade and were used without previous purification. Glassy carbon powder, 5-FU, potassium hexacianoferrate(II) trihydrate ($K_4[Fe(CN)_6]\cdot 3H_2O$), potassium hexacianoferrate(III) ($K_3[Fe(CN)_6]$), tetrachloroauric acid trihydrate ($HAuCl_4\cdot 3H_2O$), bovine serum albumin (BSA), ascorbic acid and D-glucose were purchased from Sigma-Aldrich, and D-galactose were obtained from Merck. All solutions were made with distilled water. Phosphate-buffered saline solution (PBS) was prepared at pH 7.4 by using 0.15 mol L^{-1} NaCl, 6.0 mmol L^{-1} Na_2HPO_4 and 1.0 mmol L^{-1} NaH_2PO_4. Britton-Robinson (BR) buffer solution was made by mixing 0.04 mol L^{-1} CH_3COOH, 0.04 mol L^{-1} H_3PO_4 and 0.04 mol L^{-1} H_3BO_3, and pH was adjusted with 2.2 mol L^{-1} NaOH. 5-FU stock solution (1.0×10^{-2} mol L^{-1}) was prepared daily, and stored under refrigeration (4.0 °C) when not used.

2.2. PFR extraction and characterization

PFR was isolated from *Porphyra* genus red seaweed (commercial form – *nori*), by aqueous extraction at 60 °C for 5 h by using a previously reported method [24]. The polysaccharide fraction was characterized by Fourier transform infrared spectroscopy (FTIR) and ^{13}C nuclear magnetic resonance spectroscopy (^{13}C NMR). FTIR spectra were recorded in a PerkinElmer Frontier spectrometer, in the range of 4000–500 cm^{-1} (transmission mode), by employing KBr pellet method. For ^{13}C NMR analysis, the polysaccharide sample was dissolved in deuterium oxide and the spectra were recorded at 80 °C in a Bruker Avance III spectrometer (100.61 MHz; acquisition time 2.0 s; internal standard: tetramethylsilane). Total sugar content was determined by phenol-sulfuric acid method using D-galactose as standard [25], while the sulfate content was turbidimetrically analyzed by gelatin-barium sulfate method, using sodium sulfate as standard [26].

2.3. Synthesis and characterization of porphyran-capped gold nanoparticles (AuNps-PFR)

The AuNps-PFR nanocomposite was synthesized by adding a given amount of PFR stock solution (10.0 mg mL^{-1}) to a known volume of $HAuCl_4\cdot 3H_2O$ solution, under magnetic stirring and heating in water bath, followed by the addition of some drops of 2.0 mol L^{-1} NaOH to adjust the pH to 11.0. The reaction mixture was kept in absence of light and under stirring for 2 h. After this time, the prepared AuNps-PFR suspension was naturally allowed to cool down to room temperature. The AuNps-PFR nanocomposite was stored under refrigeration (4 °C) in light-protected vessels.

The experimental variables $HAuCl_4\cdot 3H_2O$ concentration ([$AuCl_4^-$]), reaction temperature (T) and PFR concentration ([PFR]) were optimized through a 2^3 full factorial design (including a cen-

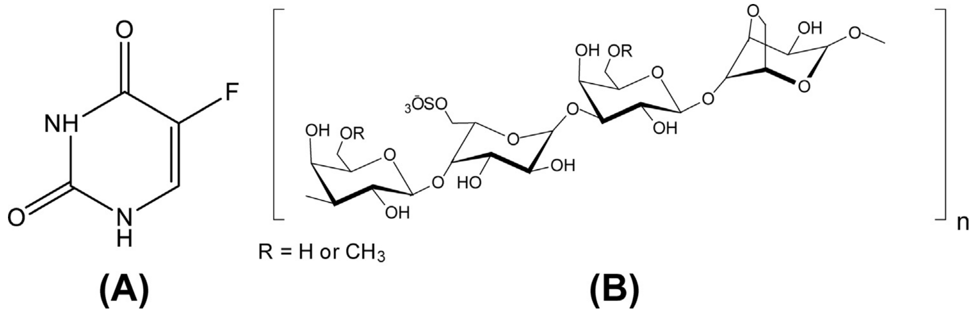

Fig. 1. Chemical structures of (A) 5-FU and (B) PFR [20].