



Contents lists available at ScienceDirect

# Advanced Powder Technology

journal homepage: [www.elsevier.com/locate/apt](http://www.elsevier.com/locate/apt)



## Original Research Paper

# Alkaline phosphatase mediated synthesis of carbon nanotube–hydroxyapatite nanocomposite and its application for electrochemical determination of luteolin

Feng Gao <sup>a,b,\*</sup>, Xiaoqian Chen <sup>a</sup>, Hidekazu Tanaka <sup>b,\*</sup>, Ayaka Nishitani <sup>b</sup>, Qingxiang Wang <sup>a</sup>

<sup>a</sup> College of Chemistry and Environment, Fujian Province Key Laboratory of Morden Analytical Science and Separation Technology, Minnan Normal University, Zhangzhou 363000, PR China

<sup>b</sup> Department of Chemistry, Graduate School of Science and Engineering, Shimane University, 1060 Nishikawatsu, Matsue, Shimane 690-8504, Japan

### ARTICLE INFO

#### Article history:

Received 8 June 2015  
Received in revised form 27 December 2015  
Accepted 12 February 2016  
Available online xxx

#### Keywords:

Luteolin  
Alkaline phosphatase  
Carbon nanotubes  
Hydroxyapatite  
Electrochemical sensor

### ABSTRACT

A nanocomposite of hydroxyapatite (HAP)–carbon nanotube (CNT) was synthesized by *in-situ* transformation of CNT–calcium phenyl phosphate (CaPP) with the assist of alkaline phosphatase. The nanocomposite was characterized by powder X-ray diffraction (XRD), Fourier transform infrared (FTIR) and transmission electron microscope (TEM). Then the HAP–CNT was cast onto a glassy carbon electrode (GCE) and used as a sensing platform for the electrochemical determination of luteolin. The electrochemical experiments indicated that the HAP–CNT modified GCE (HAP–CNT/GCE) could greatly enhance the electrochemical response of luteolin. A series of experimental parameters including the type and pH value of supporting electrolyte, accumulation time and accumulation potential were optimized. The electrochemical parameters such as electron transfer rate constant and electron transfer coefficient of luteolin at the HAP–CNT/GCE were also investigated. Under the optimal conditions, the reduction peak currents presented a good linear relationship with the concentration of luteolin in the range of  $4.0 \times 10^{-7}$ – $1.2 \times 10^{-5}$  M. The detection limit was estimated to be  $8.0 \times 10^{-8}$  M.

© 2016 Published by Elsevier B.V. on behalf of The Society of Powder Technology Japan. All rights reserved.

## 1. Introduction

Flavonoids, a type of natural polyphenolic compounds that are abundant in fruits and vegetables, received great interest in the biochemical and pharmacological fields due to their antioxidant free-radical scavenging, anti-inflammatory, anti-carcinogenic and other beneficial properties [1]. The luteolin (3',4',5',7-tetrahydroxy flavone) is one of the most important flavonoids that can be obtained from in celery, green pepper, parsley, perilla leaf and chamomile tea. Recent studies have shown that this compound has significant biological effects including anti-inflammatory, anti-bacterial and anti-oxidant, as well as anti-proliferative activity against cancer cells [2–4]. The accurate and sensitive determination of luteolin is a critical step for the evaluation of its biological and medical effects. Up to date, various methods have been

reported for the determination of luteolin, including high-performance liquid chromatography [5,6], capillary electrophoresis [7,8], spectrophotometry [9], et al. However, these methods have their inherent shortcomings of time-consuming, low sensitivity, expensive instrument and/or complicate operation process. Recently, based on the excellent electrochemical activity of luteolin from the catechol group on B ring (3',4'-dihydroxyl), the electrochemical method receives considerable interest for luteolin determination due to its fascinating advantages of high sensitivity, rapid response, simplicity of operation and cost-effectiveness [10].

Calcium hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ , HAP) is a major component of biological hard tissues such as teeth and bones in animal organism [11,12]. It has been established that the surface of HAP possesses abundant of P–OH groups acting as adsorption sites for various molecules [13]. Therefore, it had been extensively applied in many areas owing to its good biocompatibility and rich multi-adsorbing sites. For example, it can be used as adsorbents for chromatography to separate protein and enzyme, catalysts for alcohols dehydration, and basic material for artificial teeth and bones preparation [14]. Nanostructured HAP particles with a higher surface area would be more desirable for their use in many

\* Corresponding authors at: College of Chemistry and Environment, Fujian Province Key Laboratory of Morden Analytical Science and Separation Technology, Minnan Normal University, Zhangzhou 363000, PR China. Tel.: +86 596 2210114; fax: +86 596 2520035 (F. Gao). Tel./fax: +81 852 32 6823 (H. Tanaka).

E-mail addresses: [fgao1981@mnnu.edu.cn](mailto:fgao1981@mnnu.edu.cn) (F. Gao), [hidekazu@riko.shimane-u.ac.jp](mailto:hidekazu@riko.shimane-u.ac.jp) (H. Tanaka).

fields. However, the conductivity of HAP crystals is too low for the material to be considered a candidate for its use in electrochemical devices [15,16]. One approach to improve the conductivity of HAP is to dope the material with metal ions or electro-conductive nanoparticles [17,18].

Carbon nanotubes (CNTs) with one-dimensional and hollow structure are one of the most fascinating nanometer materials. Since the discovery by Iijima [19], CNTs have attracted worldwide interests in diverse areas including materials science [20], sensors [21,22], transistors [23,24], field-emission display [25], and energy storage [26,27] due to their high mechanical strength, excellent thermal conductivity, unique electronic properties and thermal stability. These outstanding characters make CNTs ideal candidates to enhance stiffness, strength and electronic conductivity of the composite materials. As a consequence, the nanocomposite of CNT and HAP (CNT–HAP) received great interests, especially in electrochemical field. For example, Safari et al. [28] have synthesized the CNT–HAP through a microwave-assisted solid state metathesis reaction, and the electrochemical impedance spectroscopy (EIS) result revealed that the composite had better charge transfer capacity than the pristine CNTs due to formation of organized electrical functions between the two components. Li et al. prepared a nanocomposite film of HAP–TiO<sub>2</sub>–CNT via the ultrasonication mixing method, and used the nanocomposite as a platform to immobilize glucose oxidase for glucose sensing analysis [29]. However, it is difficult to precisely control the chemical and structural uniformity of CNT–HAP prepared by physical mixing or microwave assisted solid state metathesis in these work. The direct deposition of HAP on CNTs via liquid-phase reaction is a proper route to resolve this problem [30]. But because the CNTs are hydrophobic and easy to aggregate in aqueous solution, the functionalization of CNTs via strong acid oxidation to improve the dispersibility is necessary before the controllable deposition of HAP [31].

In this work, the nanocomposite of HAP–CNT was synthesized through a novel alkaline phosphatase mediated transformation process using CNT-calcium phenyl phosphate (C<sub>6</sub>H<sub>5</sub>PO<sub>4</sub>Ca·nH<sub>2</sub>O: CaPP) as the source (Scheme 1). Firstly, the phenyl phosphoric acid was interacted with CNTs via  $\pi$ – $\pi$  stacking and hydrophobic force to obtain a well dispersed phenyl phosphoric acid-introduced CNT solution. Secondly, the CaPP was prepared on CNT surface by reacting phenyl phosphoric acid on CNT with Ca<sup>2+</sup> added in the solution. Followed by, the phenyl phosphoric acids in CaPP on CNT surface were hydrolyzed by adding alkaline phosphatase at 36.5 °C to recrystallize as HAP. Thus, the HAP particles were generated and *in-situ* deposited on the surface of the CNT. The transformation process was characterized by powder X-ray diffraction (XRD), Fourier transform infrared (FTIR) and transmission electron microscope

(TEM). Moreover, based on its multiple active sites, high surface area and excellent conductivity, the nanocomposite was used as an electrochemical sensing material for the determination of luteolin. Under the optimum conditions, differential pulse voltammetry (DPV) showed a good correlation with the concentration of luteolin over the range from  $4.0 \times 10^{-7}$  to  $1.2 \times 10^{-5}$  M, and detection limit was estimated to be  $8.0 \times 10^{-8}$  M. This work opens a new way for the synthesis of HAP–CNT composite, and broadens its application in electrochemical field.

## 2. Experimental

### 2.1. Materials and apparatus

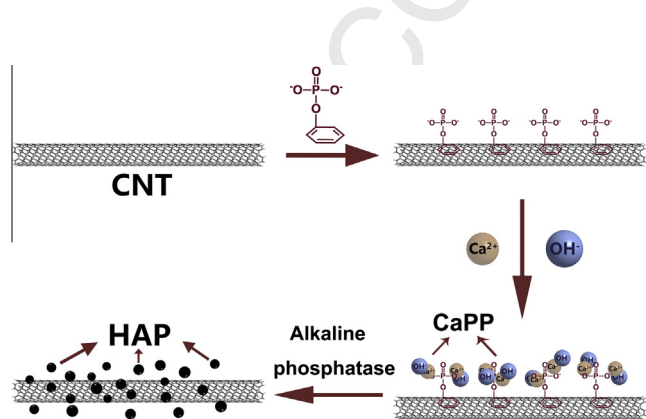
Luteolin was purchased from Sigma–Aldrich Co., Ltd. (China). Multi-walled carbon nanotubes (diameter 40–60 nm) and Ca(OH)<sub>2</sub> were purchased from Wako Pure Chemical Industries Co., Ltd. (Japan). Phenyl phosphoric acid was purchased from Tokyo Chemical industry Co., Ltd. (Japan). Alkaline phosphatase from bovine intestinal mucosa with specific enzyme activity of 1.5 units mg<sup>-1</sup> was purchased from Biozyme Laboratories (Japan). Tris (hydroxymethyl) aminomethane (Tris) was provided by Xilong Chemical Company (China) and Tris–HCl buffer solution was prepared by adding appropriate HCl in 10 mM Tris. Phosphate buffer saline (PBS) with various pH was prepared through mixing 0.02 M NaCl and 0.05 M NaH<sub>2</sub>PO<sub>4</sub>–Na<sub>2</sub>HPO<sub>4</sub> with different ratios. Britton–Robinson (B–R) buffer solution was prepared by adding 0.4 M acetic acid and 0.4 M boric acid into a solution of 0.4 M phosphoric acid. Chitosan (CS) was purchased from Aladdin Reagent Co., Ltd. (China). All the other chemicals were of analytical reagent grade and obtained commercially. Doubly distilled water was used throughout the experiments.

Powder X-ray diffraction (XRD) patterns of the materials were obtained from a Rigaku diffractometer with a Ni-filtered Cu  $\alpha$  radiation (30 kV and 16 mA, Japan). Particle morphology was observed by a TOPCON transmission electron microscope (TEM, Japan). Transmission IR spectra were recorded by a KBr method using a JASCO Fourier transform infrared (FTIR) spectrometer with a resolution of 4 cm<sup>-1</sup> (Japan). Electrochemical measurements were carried out on a CHI 650C electrochemical analyzer (China) in connection with a conventional three-electrode system: a glassy carbon electrode (GCE diameter = 2 mm) modified with different materials was used as the working electrode, Ag/AgCl electrode as the reference electrode, and Pt wire as the counter electrode.

### 2.2. Preparation of HAP–CNT

First, the CaPP–CNT nanocomposite was prepared by a simple sonication method [32]. In brief, 10.0 mg CNTs were dispersed in 100 mL water free from CO<sub>2</sub> in a sealed polypropylene vessel and ultrasonicated for 2 h. Then 50.0 mL of 0.106 mM aqueous phenyl phosphoric acid solution was added. After the mixture was sonicated for 1 h, 100 mL of 8.85 mM Ca(OH)<sub>2</sub> was added, and then aged at 37 °C for 24 h. Afterwards, the product were filtered, thoroughly washed with water and finally dried at 50 °C in an air oven for 1 day, thus the sample of CaPP–CNT was obtained.

Transformation of HAP–CNT from CaPP–CNT was carried out as follows: 235 mg of the synthesized CaPP–CNT was dispersed into 210 mL of water free from CO<sub>2</sub> under stirring at room temperature. The aqueous solution including 15 mg alkaline phosphatase was then added into the solution suspending the CaPP–CNT particles. The pH of the suspension was adjusted to 9.6 by adding 1.0 M aqueous NH<sub>3</sub> solutions and the suspension was treated at 36.5 °C for 48 h in an incubator. After aging, the product was filtered, thoroughly washed with water and finally dried at 50 °C in an air oven



**Scheme 1.** Diagram for the transformation process from CaPP–CNT to HAP–CNT with the assist of alkaline phosphatase.

Download English Version:

<https://daneshyari.com/en/article/10260355>

Download Persian Version:

<https://daneshyari.com/article/10260355>

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