Chemical Engineering Journal 315 (2017) 573–582

Contents lists available at ScienceDirect

Chemical Engineering Journal

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

One-step synthesis of size-tunable gold nanoparticles immobilized on chitin nanofibrils via green pathway and their potential applications

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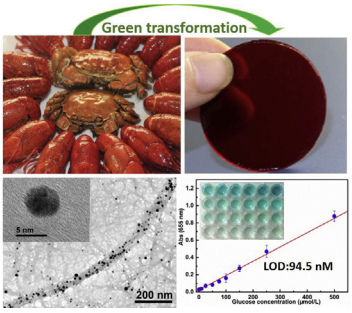
HIGHLIGHTS

- CNFs was used as reductant and stabilizer in synthesizing AuNPs for the first time.
- AuNPs were firmly immobilized along CNFs with high stability.
- Size and morphology of AuNPs were tunable by the reaction parameters.
- CNFs-AuNPs exhibited peroxide mimic catalytic performance.
- CNF-AuNPs were applied in glucose detection with LOD as low as 94.5 nM.

GRAPHICAL ABSTRACT

(a) Chitin nanofibrils (CNFs) were used as both reductant and stabilizer in the synthesis of gold nanoparticles (AuNPs) for the first time. (b) The size and morphology of the AuNPs were tunable by adjusting the reaction parameters. (c) Size and morphology of AuNPs were tunable by adjusting the reaction parameters. (d) The CNF-AuNPs were applied in glucose detection with a limit of detection as low as 94.5 nM.



ARTICLE INFO

Article history:
Received 27 November 2016
Received in revised form 16 January 2017
Accepted 17 January 2017
Available online 19 January 2017

Keywords:
Renewable resource
Chitin nanofibrils
Gold nanoparticle
Green catalysis
Glucose detection

ABSTRACT

Chitin is the second most abundant natural biopolymer on earth after cellulose with vast application potential. In the present work, the chitin nanofibrils (CNFs) fabricated through a simple physical method were validated, for the first time, to be capable of simultaneously generating and immobilizing gold nanoparticles (AuNPs) via a one-step synthesis. In our findings, CNFs acted as both reductant for the in-situ synthesis of AuNPs and stabilizer for the generated AuNPs, due to the reducibility and chelation capacity of the amino groups on chitin. When the CNFs concentration varied from 2.0 to 7.1 mg/mL, well dispersed AuNPs with controllable diameters (7–30 nm) were synthesized and immobilized along the chitin nanofibrils, showing high stability for at least three months. This new pathway was an environmentally friendly process, and even generated small AuNPs with diameters of less than 10 nm. Moreover, the CNF-AuNPs displayed peroxidase mimic behavior and catalyzed the oxidation reaction of 3,3′,5,5′-tetramethylbenzidine (TMB) by H_2O_2 to produce a blue solution. When combined with glucose oxidase, the CNF-AuNPs could sensitively detect glucose with a limit of 94.5 nM, which was highly specific, safe, cheap, and visible with low contamination. This work has put forward a facile "green" pathway for the heterogeneous synthesis of the size controllable and stable AuNPs immobilized on the biocompatible chitin nanofibrils and their potential in biosensing were evaluated.

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http://dx.doi.org/10.1016/j.cej.2017.01.065
1385-8947/

1. Introduction

It is worth noting that diabetes induced metabolic disturbance has become a social health problem all over the world, which raises the blood glucose level of 3–8 mM in normal human to 9–40 mM in diabetes patients [1]. Therefore, detecting of the blood glucose level with rapid, reliable, and low-cost methods becomes an important research goal. Biosensing approaches, which involve natural enzymes combined with electrochemical or colorimetric methods, have been widely applied [2]. However, natural enzymes are generally expensive, difficult to purify, and easily denatured, which restrict their widespread applications [3]. In recent years, nanoparticulate artificial enzymes, or "nanoenzymes", have attracted enormous interests by showing advantages in low-cost, easy preparation, high stability, and tunability [4]. It has been generally acknowledged that gold nanoparticle (AuNP) is one of the most important nanoenzymes [5], due to its excellent biocompatibility, high stability, unique optical/electronic properties, easy availability for versatile biofunctionalization and high catalytic activity [6–8]. Although bulk gold has long been known as catalytically inert, AuNPs have been utilized in various biochemical reactions under different conditions. For instance, cysteamine-capped AuNPs and bovine serum albumin (BSA)-stabilized Au clusters (AuNCs) have been reported to exhibit peroxidase-like activity [9,10], whereas citrate-capped AuNPs displayed glucose oxidase (GOx)-like activity [11,12].

However, the agglomeration tendency of AuNPs results in large aggregates with decreased catalytic activity and reduced life-time [13]. Thus, the design and preparation of AuNPs with long-term dispersion stability and high catalytic efficiency remains a primary challenge [14]. In the last decade, polymers such as polyvinyl alcohol [15], polyvinyl pyrrolidone [16] and polystyrene [17] have been commonly used for the size-controlled synthesis of AuNPs, but they have run into issues with non-biodegradability and non-biocompatibility, generally with combination of additional reducing or stabilizing agents [18,19]. As a result, natural polymers with excellent biocompatibility and biodegradability as well as abundant active groups for functionalization, have attracted growing interests in the bio-template synthesis of AuNPs [20]. Moreover, they have been demonstrated to be great potentials in optic apparatus [21], catalysis [22,23], enzyme immobilization [24,25], electrically conducting materials [18] and biosensing [26].

It is well known that chitin is the second most abundant biomass after cellulose, which widely exists in exoskeletons of crustaceans, insects, fungi and algae [27]. Despite its excellent biocompatibility, biodegradability and important bioactive functions [28], chitin has long been discarded as shellfish wastes in the fishing and seafood industries. In 2015, a comment published on *Nature* entitled 'Sustainability: Don't waste seafood waste' revealed the great but underestimated potential of chitin in biochemical and biomaterial production [29]. Chitin contains abundant hydroxyl and amino groups, and the latter endow chitin with various functions [30], such as reducing agent for AuNPs synthesis [31] and adsorbing metal ions via chelation or ion exchange mechanisms [32–35]. Partially deacetylation has been applied to fabricate chitin nanofibrils (CNFs) with high aspect ratio and an increased content of amino groups [36–38]. In the present work, deacetylated chitin nanofibrils were fabricated from chitin derived from the seafood waste via a physical method and then recruited as the sole reductant for the one-step synthesis of AuNPs as well as stabilizer to immobilize them. Furthermore, the positively charged AuNPs immobilized along the chitin nanofibrils possessed peroxidase-like activity and played important roles in the glucose detection via colorimetric method. This new pathway to construct the nanocomposite is an environmentally friendly process and puts forward new ideas for the application of chitin based nanomaterials in the field of biosensing, green catalysis and related fields.

2. Experimental

2.1. Materials

The raw chitin (RC) powder was purchased from Zhejiang Golden-Shell Biochemical Co. Ltd. (China) with a degree of acetylation (DA) of 90%. The weight-average molecular weight (M_w) was determined to be 5.96×10^5 in 5% (w/v) LiCl–DMAc by dynamic light scattering (DLS, ALV/GGS-8F, ALV, Germany). β-glucose and $HAuCl_4{\cdot}4H_2O$ were purchased from Aladdin Ltd. (Shanghai, China). Other chemical reagents (analytical grade) were purchased from Sinopharm Chemical Reagent Co., Ltd and used as received. Ultrapure water (Millipore) was used for all the experiments.

2.2. Preparation of chitin nanofibrils (CNFs)

A suspension of CNFs was prepared based on a previously reported method [37], with minor modification. First, partially deacetylated chitin were prepared from the commercial chitin powder and used as the starting materials for CNFs preparation. Briefly, 100 g raw chitin powder was suspended in 2500 ml 33% (w/w) NaOH aqueous solution, and 0.3 g $NaBH_4$ was added to prevent the alkali-induced depolymerization. The slurry was stirred at room temperature for 0.5 h and then at 90 °C for 3 h. The resultant powder was rinsed with distilled water and air-dried at 45 °C, to obtain the partially deacetylated chitin powder.

The degree of deacetylation (DD) value of the partially deacetylated chitin was determined by titration [39] as follows: 0.25 g vacuum-dried sample was dispersed in plenty (20 mL) HCl solution of 0.1 mol/L and then titrated with 0.1 mol/L NaOH. The pH value and corresponding volume of NaOH solution was recorded and a titration curve was plotted. This plot had two inflection points, where the corresponding volumes were recorded as V_1 and V_2. The DD value was calculated according to the following equation

$$DD = \frac{v2 - v1 \times c \times 0.016}{0.0994 \times m} \quad (1)$$

where c refers to the concentration of NaOH solution, and m refers to the weight of sample. The DD value for the partially deacetylated chitin used in this work was thus calculated to be 19.8%.

The partially deacetylated chitin powder was suspended in ultrapure water at a solid content of 0.5% (w/v). Several drops of 1 M acetic acid were added to control the pH value in range of 3–4 to facilitate the nanofibrillation. The resultant suspension (every 400 ml) was treated with household blender for 1 h, followed by sonication on an ultrasonic cell disruptor (JY98-IIIDN, Ningbo Scientz Biotechnology Co., Ltd., China) at a power of 800 W for 1 h in an ice bath. Subsequently, the suspension was centrifuged at 8000 rpm to remove any visible precipitate. The supernatant was concentrated by rotary evaporation and dialyzed against water until the pH no longer changed (about 6.6). The resultant suspension of CNFs was stored at 4 °C for use and the solid concentration was measured by weighting vacuum dried CNFs suspension with calculated volume.

2.3. Synthesis of CNF-AuNPs

CNF-AuNPs were synthesized by the reduction of $HAuCl_4$ with CNF suspension. Typically, 0.25 mL of 20 mM $HAuCl_4$ were added to 4.75 mL CNF suspensions with different concentrations and stirred at room temperature (25°°C) for 30 min to form a yellowish