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Synthesis and antioxidant properties of gum arabic-stabilized selenium nanoparticles

Huiling Kong[a], Jixin Yang[b], Yifeng Zhang[a], Yapeng Fang[a,b,*], Katsuyoshi Nishinari[a], Glyn O. Phillips[a,c]

[a] Glyn O. Phillips Hydrocolloid Research Centre at HUT, School of Food and Pharmaceutical Engineering, Faculty of Light Industry, Hubei University of Technology, Wuhan 430068, China
[b] Department of Chemistry, Glyndwr University, Plas Coch, Mold Road, Wrexham LL11 2AW, UK
[c] Glyn O. Phillips Hydrocolloid Research Center, Glyndwr University, Plas Coch, Mold Road, Wrexham LL11 2AW, UK

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ABSTRACT

Selenium nanoparticles (SeNPs) were prepared by using gum arabic (GA) as the stabilizer in a facile synthetic approach. The size, morphology, stability and antioxidant activity *in vitro* of the gum arabic-selenium nanocomposites (GA-SeNPs) were characterized by transmission electron microscopy (TEM), dynamic light scattering (DLS), Fourier-transform infrared spectroscopy (FTIR), atomic force microscopy (AFM) and ultraviolet–visible spectrophotometry (UV–vis). SeNPs (particle size of ~34.9 nm) can be stabilized in gum arabic aqueous solutions for approximately 30 days. FTIR results show that SeNPs were combined to the hydroxyl groups of GA. In the present work, the alkali-hydrolyzed GA (AHGA) was also prepared and its efficiency in stabilizing SeNPs was compared with GA. It was concluded that the branched structure of GA was a significant factor for the functionality. The hydroxyl radical scavenging ability and DPPH scavenging ability of GA-SeNPs were higher than those of AHGA-SeNPs and could reach 85.3 ± 2.6%, 85.3 ± 1.9% at a concentration of 4 mg/ml, respectively.

1. Introduction

Selenium (Se) is a nutritional trace element with remarkable antioxidant characteristics that are of fundamental importance to human health [1–4]. It can inhibit many inflammatory cell mechanisms through antioxidant selenoenzymes as one selenium atom is absolutely required at the active site of all selenoenzymes in the form of the 21st amino-acid selenocystein [5,6]. However, Se has a very narrow margin between the thresholds of functionality and toxicity. It was shown to suppress the growth of tumor cells *in vivo* and *in vitro* [7–10]. In particular, selenium nanoparticles (SeNPs) have excellent bioavailability, high biological activity and low toxicity [10]. For instance, consumption of 200 μg Se per day by cancer patients reduces mortality and depresses the incidence of many diseases including lung, colorectal and prostate cancers [11–13]. Nano-Se has a 7-fold lower acute toxicity than sodium selenite in mice (LD50 113 and 15 mg Se/kg body weight, respectively) [14]. Some studies demonstrated the antioxidant properties of hollow spherical selenium nanoparticles, which may have potential use as special anti-oxidative drugs [11,15]. Moreover, aging cells accumulate oxidative damage [16–18]. It has been reported that SeNPs in the size range from 5 to 200 nm were efficient for free radical scavenging both *in vivo* and *in vitro* [19,20]. Biologically synthesized selenium nanoparticles with diameter less than 100 nm have potential application as food additives with antioxidant properties [12,21].

Gum arabic (GA) is one of the widely accepted ingredients in the food and pharmaceutical industry. It is a branched, neutral or slightly acidic complex polysaccharide existing as mixed calcium, magnesium, and potassium salts. The GA (Acacia Senegal species) has demonstrated high heterogeneity, which is made up of approximately 44% galactose, 13% rhamnose, 27% arabinose, and 16% glucuronic acid and 4-*O*-methyl glucuronic acid [22–24]. It also contains 2–3% peptide moieties as an integral part of the structure. Three major fractions in GA were identified, including arabinogalactan protein complex (AGP), arabinogalactan (AG) and glycoprotein (GP) [25–27].

Abbreviations: AFM, atomic force microscopy; AG, arabinogalactan; AGP, arabinogalactan protein; AHGA, alkali-hydrolyzed GA; DLS, dynamic light scattering; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DR, deoxyribose; EDTA, ethylenediaminetetraacetic acid; FTIR, Fourier-transform infrared spectroscopy; GA, gum arabic; GP, glycoprotein; GPC, gel permeation chromatography; HBP, hyperbranched polysaccharide; HRTEM, high-resolution transmission electron microscopy; $NaBH_4$, sodium borohydride; SeNPs, selenium nanoparticles; TBA, 2-thiobarbituric acid; TCA, trichloroacetic acid; TEM, transmission electron microscopy; UV–vis, ultraviolet–visible spectrophotometer.

* Corresponding author at: Glyn O. Phillips Hydrocolloid Research Centre at HUT, School of Food and Pharmaceutical Engineering, Faculty of Light Industry, Hubei University of Technology, Wuhan 430068, China. Tel.: +86 27 88015996.
E-mail addresses: y.fang@glyndwr.ac.uk, fangyp@mail.hbut.edu.cn (Y. Fang).

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Pure SeNPs do not represent a stable system in aqueous solutions; therefore, stabilization and functionalization of them by suitable chemical reagents is essential towards their specific interaction with biological targets [28]. As is well known, biomacromolecules have been applied as templates for controlling inorganic crystal nucleation and growth [29–34]. Zhang et al. showed that a water-soluble hyperbranched polysaccharide (HBP) extracted from sclerotia of *Pleurotus tuber-regium* functioned as a stabilizer and capping agent of SeNPs [31]. However, the extraction process of HBP was tedious and the yield was low. In recent years, the ability of GA to act as a biocompatible shell for nanostructures has triggered enormous interest in medical research [35]. GA, which is easily accessible and cheap, has many functional properties making it an ideal candidate as stabilizer and emulsifier in the food industry and beyond. In this work, a facile and green method to synthesize and stabilize selenium nanoparticles was developed by using gum arabic as a stabilizing agent. The SeNPs were prepared using GA and alkali-hydrolyzed GA (AHGA) to demonstrate their usefulness in stabilizing the nanostructures and clarify the effect of structural characteristics on the antioxidant ability of SeNPs *in vitro*. The size, morphology, bonding mechanism, stability and antioxidant action of GA-SeNPs were characterized by transmission electron microscope (TEM), atomic force microscope (AFM), Fourier transform infrared spectroscopy (FTIR), dynamic light scattering (DLS) and ultraviolet–visible spectroscopy (UV–vis) *etc*. The stabilizing mechanism was found to be related to the branched structure of gum arabic and the interaction of hydroxyl groups with selenium nanoparticles. The anti-oxidant properties of the gum arabic-stabilized SeNPs were also studied and found to be linked to the stability of the nanoparticles.

2. Materials and methods

2.1. Materials

Gum arabic (GA) was provided by San-Ei Gen F.F.I. Inc. (Osaka, Japan) in spray dried form. The powder contains 5.56% moisture, as well as 8.76 ppm Fe and 1.43 ppm Cu. Selenium dioxide and ascorbic acid were purchased from Tianjin Chemical Reagent Institute (Tianjin, China) and Xilong Chemical Co. Ltd. (Puning, China), respectively. Iron (III) chloride hexahydrate, potassium ferricyanide, trichloroacetic acid (TCA), hydrogen peroxide, methanol and sodium borohydride ($NaBH_4$) were purchased from Chinese Medicine Group Chemical Reagent Co. Ltd. (Shanghai, China). Deoxyribose (DR) was purchased from Amresco and 2,2-diphenyl-1-picrylhydrazyl (DPPH) was from Aladdin Industrial Corporation. 2-Thiobarbituric acid (TBA), ethylenediaminetetraacetic acid (EDTA) and protease (Type XIV from *Streptomyces griseus*) were purchased from Sigma–Aldrich (USA). All the chemicals were of analytical grade and used without further purification. Milli-Q water was used in all the experiments.

2.2. Modification and characterization of GA

Alkali-hydrolyzed GA (AHGA) was prepared according to the procedures reported in the literature [36]. Briefly, GA (2 g) was treated with 200 ml of 4 M $NaBH_4$ and 2 M NaOH for 6 h at 100 °C. Excessive $NaBH_4$ was neutralized using 12.5 ml of 1 M acetic acid in 1062.5 ml of methanol in an ice bath. The resulting precipitate was washed three times with methanol, deproteinized, and dialyzed against water (Mw cutoff: 14 kDa) for 72 h to remove any free salts, followed by freeze drying.

The GA samples before and after alkaline hydrolysis were characterized by gel permeation chromatography coupled with multi-angle laser light scattering (GPC-MALLS). The GPC-MALLS system consists of a Superose 6 10/300GL column (GE Healthcare, USA), a DAWN HELEOS multiangle light scattering detector (Wyatt Technology Corporation, USA) operated at 658 nm, an Optilab rEX refractometer (Wyatt Technology Corporation, USA), and a SPD-10Avp series UV detector (Shimadzu Technologies, Japan) carried out at 214 nm. 0.2 M aqueous NaCl solution filtered through a 0.2 μm Millipore filter was used as an eluent, delivered by a Waters 515 HPLC pump (Waters Corporation, USA) at a constant rate of 0.4 ml/min. A refractive index increment d*n*/d*c* of 0.141 ml/g was used for molecular parameter analysis of GA.

2.3. Preparation of GA-SeNPs and AHGA-SeNPs

The same concentration of aqueous GA and AHGA solution (1 mg/ml, 11.25 ml) was added into a 20 ml sealed bottle, respectively, and they were mixed with 150 μl of 0.6 M selenious acid (selenium dioxide dissolved in the water) and 2.85 ml of water under magnetic stirring for 6 h. 4.5 ml of 0.1 M aqueous ascorbic acid solution was added dropwise into the resulting mixture, which was then stirred for 0.5 h at room temperature.

2.4. Size and morphology measurements of SeNPs

Transmission electron microscopy (TEM) of the diluted solutions of GA-SeNPs was measured on a JEOL JEM-2010 (HT) electron microscope at an accelerating voltage of 200 kV. The high-resolution transmission electron microscopy (HRTEM) image was acquired on a JEOL JEM 2010 FEF (UHR) microscope at 200 kV [34]. One drop of each sample solution was put onto copper grid and dried in air for 5 min for TEM observation. The average particle size of SeNPs was obtained from TEM measurements of three replications.

The morphology and size distribution of the samples were examined using AFM (Agilent Technologies, USA) in a tapping mode. A 10 μl drop of either 10 μg/ml GA or GA-SeNPs aqueous solution was deposited onto freshly cleaved mica and dried by nitrogen at room temperature and 45–60% humidity. A scanning probe made of SiN_4 with a cantilever length of 235 μm and a spring constant of 98 N/m was employed.

Dynamic light scattering (DLS, Zetasizer Nano ZS, Malvern) was used to monitor the change of particle size during storage. 1.0–1.5 ml of each sample was measured in a polystyrene cuvette at a fixed angle of 173° at 25 °C. Laser Doppler velocimety (LDV, Zetasizer Nano ZS, Malvern) was applied to obtain zeta potential values to check the bonding mechanism. Effect of pH was measured by pH-titration device (Zetasizer Nano ZS, Malvern). Both particle size, zeta potential and pHs of GA and GA-SeNPs were reported as the average values of triplicate measurements.

2.5. Analysis of bonding between GA/AHGA and SeNPs

FTIR spectra of the samples were recorded on a Nicolet 170SX FTIR spectrometer in the range of 4000–400 cm^{-1} using the KBr-disk preparation method.

2.6. Stability of gum arabic (GA/AHGA)-SeNPs

The GA/AHGA-SeNPs aqueous solutions were recorded by photographs to compare the stability of SeNPs during storage of 60 days at 25 ± 1 °C.