

Eco-Friendly Synthesis of Fucoidan-Stabilized Gold Nanoparticles

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Abstract: Problem statement: Metallic gold nanoparticles (AuNPs) are widely used in many applications including medical, pharmaceutical, diagnostics and sensors. The chemical synthesis of AuNPs normally requires synthetic materials which might cause the toxicological concerns. The use of naturally occurring materials like fucoidans for successful synthesis of AuNPs is of interests. **Approach:** Fucoidans as sulfated polysaccharides from marine algae *Cladosiphon okamuranus* (o-fucoidan) and *Kjellmaniella crassifolia* (t-fucoidan) were used for synthesis of AuNPs. The suitable condition for the synthesis was investigated to obtain the nanometric size of AuNPs. The synthesized AuNPs were characterized for the size, morphology and Surface Plasmon Resonance (SPR). **Results:** The fucoidan-stabilized AuNPs containing optimum weight ratio of gold atom to fucoidan yielded the spherical sizes with an average of 8-10 nm and the absorption maxima of SPR band around 527-530 nm. The AuNPs stabilized by o-fucoidan structured as linear polymer were more monodisperse than those stabilized by t-fucoidan structured as branched polymer. **Conclusion:** Fucoidan can be employed solely for AuNP synthesis and the sulfate constituent in fucoidan is important for gold reduction and stabilization. The simple yet eco-friendly synthesis of AuNPs stabilized by fucoidans would be attractive for application use of metallic nanoparticles.

Key words: Gold nanoparticles, fucoidan, polysaccharides, synthesis

INTRODUCTION

Gold nanoparticles (AuNPs) are attractive for many applications including pharmaceuticals, diagnostics and biosensors (Ghosha *et al.*, 2008; Zhao *et al.*, 2008; Peng *et al.*, 2009). The advantages of AuNPs come from their biocompatibility and ability to target delivery of therapeutic agent (Patra *et al.*, 2008). The synthesis of AuNPs normally involves a reduction of positively charged gold atom and an addition of stabilizing agents. The chemical used for gold synthesis is normally citrate, sodium borohydride and organic compounds (Sun *et al.*, 2003; Kimling *et al.*, 2006). In order to reduce the use of chemical and toxic waste to the environment, the synthesis of AuNPs using natural compounds is of interests. Among them, polysaccharide or polysaccharide-based biopolymer has been employed

to synthesize AuNPs. Polysaccharides that have been recently reported for synthesis of AuNPs include all those containing amine groups in particular chitosan, heparin and hyaluronan (Huang and Yang, 2004a; 2004b; Kemp *et al.*, 2009). In addition, the ability of dried seaweed *Sargassum wightii* to stabilize AuNPs was thought to arise from extracellular polysaccharides present in seaweed (Singaravelu *et al.*, 2007).

Fucoidan is a polysaccharide extract from cell wall of marine brown algae such as Laminariales, Fucales, Chordariales, Desmarestiales and Dictyotales. Fucoidan shows some pharmacological activities including anti-inflammation, anti-coagulant, anti-viral, anti-cancer and anti-peptic ulcer activities (Shibata *et al.*, 2003; Yang *et al.*, 2006; Cumashi *et al.*, 2007; Teruya *et al.*, 2007; Li *et al.*, 2008). Moreover, fucoidans are also applied in cosmetic application as anti-aging compound and whitening agent (Fitton *et al.*, 2007). The fucoidan

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structure is a linear backbone of α -1 \rightarrow 3-linked fucopyranose with additional sulfate group and may have a branched structure (Li *et al.*, 2008). Nonetheless, there is no evidence whether fucoidan can facilitate the formation of AuNPs. The green synthesis of AuNPs using natural fucoidans would provide an alternative to chemical method. The aim of study is to synthesize AuNPs using fucoidan extract from marine algae and the characteristics of synthesized AuNPs are investigated.

MATERIALS AND METHODS

The two different species of marine algae, Okinawa mozuku fucoidan from *Cladosiphon okamuranus* (o-fucoidan; $M_w = 380,000 \text{ g mol}^{-1}$) and Takara kombu fucoidan from *Kjellmaniella crassifolia* (t-fucoidan; $M_w = 200,000 \text{ g mol}^{-1}$) were obtained from Toyo Science and Takara Bio, Japan. The structures of o-fucoidan and t-fucoidan are represented in Fig. 1. Hydrogen tetrachloroaurate (III) trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) solution (30 % w/w) was

purchased from Sigma (St. Louis, USA). All chemicals were used as received.

Synthesis and characterization of gold nanoparticles: Fucoidan was dissolved in 50 mL deionized water to obtain various concentrations (0.01, 0.02, 0.04, 0.06, 0.08, 0.10, 0.12, 0.14, 0.16, 0.20 and 0.40% w/v). Then, 3.45 μL of 30% w/w hydrogen tetrachloroaurate (III) trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) solution was added to obtain 20 ppm of gold atom. The mixture was heated to 80°C on a water bath with magnetic stirring for 45 min. The mixture was visually observed for appearance and determined for Surface Plasmon Resonance (SPR) spectrophotometrically using Shimadzu UV-Vis 160A. The morphology and size of AuNPs were visualized using Transmission Electron Spectroscopy (TEM) on a 200 KV Joel JEM-2100 equipped with Semafore 4.01 program for size analysis and the zeta potential was determined using Zetasizer NanoZS (ZEN3600).

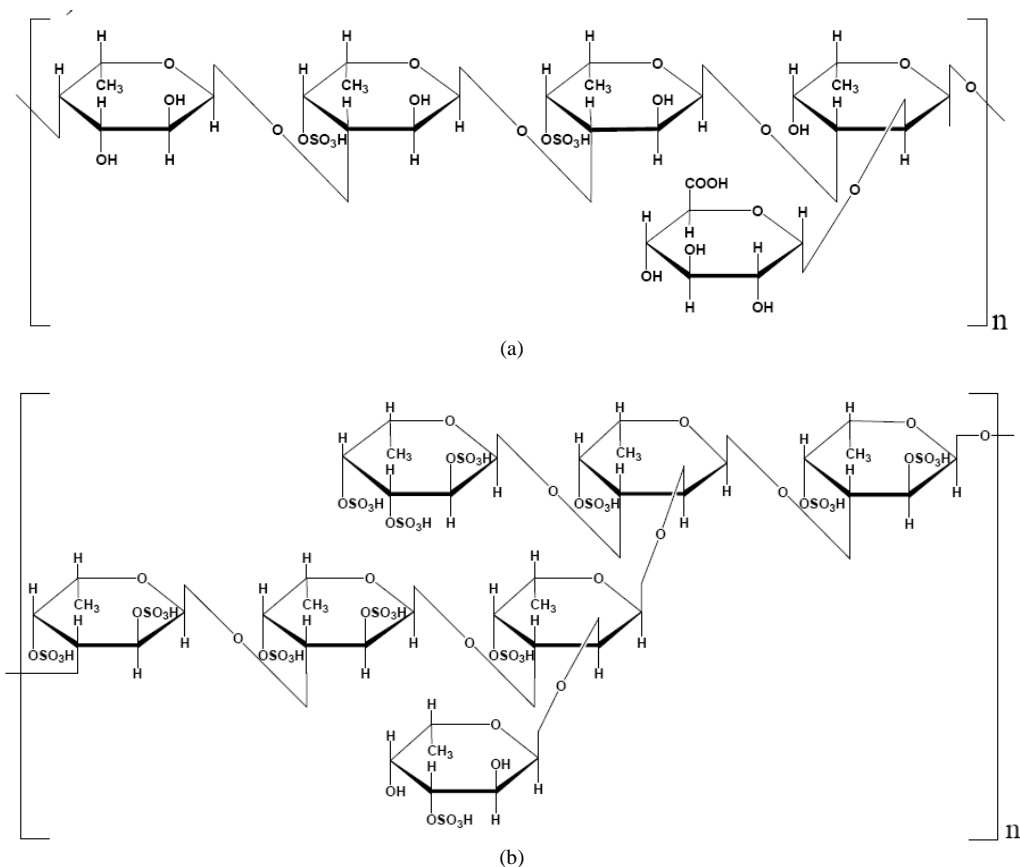


Fig. 1: Structures of o-fucoidan (a) and t-fucoidan (b)

For the study on the effect of duration time on the synthesis of fucoidan-stabilized AuNPs at ambient temperature, the fucoidan was weighed and dissolved in water to obtain 0.04% w/v for o-fucoidan and 0.10% w/v for t-fucoidan before the gold solution was added. The mixture was stirred until the ruby-red color of AuNP solution appeared. The synthesized AuNPs were determined for the change in SPR band at 1, 8, 24, 48 and 72 h.

RESULTS

From the result, red solution of AuNPs was observed when fucoidan concentrations were in the range of 0.01-0.12% w/v (Fig. 2) which corresponded to the weight ratios of gold atom to fucoidan ranging from 1:50 to 1:60. The SPR bands of AuNPs containing gold to fucoidan at different weight ratios, 1:5, 1:10, 1:20, 1:30, 1:40, 1:50 and 1:60, were further characterized. The overall peak absorbance of the nanoparticles was downward to blue shift for both fucoidans upon decreasing weight ratio of gold to fucoidan, from 530 nm (1:5 ratio) to 519 nm (1:60 ratio) for o-fucoidan (Fig. 3a), and from 547 nm (1:5 ratio) to 522 nm (1:60 ratio) for t-fucoidan (Fig. 3b). The weight ratios of gold atom to fucoidan which yielded the absorption maxima, A_{max} , were 1:20 ($A_{max} = 0.39$, 527 nm) for o-fucoidan and 1:50 ($A_{max} = 0.38$, 529.5 nm) for t-fucoidan. Upon decreasing the ratio beyond their optimum points, lower absorbance of o-fucoidan stabilized AuNPs (o-AuNPs) and AuNPs stabilized by t-fucoidan (t-AuNPs) was found. Although the SPR bands of AuNPs stabilized by either o-fucoidan or t-fucoidan were similar, we observed that the appearance of o-AuNPs was ruby red while those stabilized by t-AuNPs was red purple. The TEM images showed the morphology of spherical o-AuNPs and t-AuNPs at 1:20 and 1:50 weight ratios of gold to o-fucoidan and t-fucoidan, respectively (Fig. 4). The TEM results indicated that the average size of o-AuNPs (8.54 ± 2.96 nm, $n \geq 400$) became smaller and narrower in size distribution than t-AuNPs (10.74 ± 4.78 nm, $n \geq 400$).



Fig. 2: Appearance of AuNPs stabilized by o-fucoidan (a) and t-fucoidan (b) upon decreasing weight ratios of gold atom to fucoidan (from left to right), 1:5, 1:10, 1:20, 1:30, 1:40, 1:50 and 1:60

Since the fucoidan contains chemical constituents with negative charges such as sulfate, the zeta potential of AuNPs were expectedly in negative values, -34.70 ± 4.99 mV ($n = 4$) for o-AuNPs and -28.50 ± 3.66 mV ($n = 4$) for t-AuNPs. In another experiment, we demonstrated if fucoidan-stabilized AuNPs can be simply synthesized without the need of heat. The weight ratios of gold to fucoidan for synthesis were 1:20 and 1:50 for o-AuNPs and t-AuNPs, respectively. The sample was rigorously stirred at room temperature for different time periods and the SPR band of AuNPs was observed. The results showed the effect of time period on the peak absorbance of AuNPs. The highest absorbance of o-AuNPs and t-AuNPs was obtained within 48 h (Fig. 5).

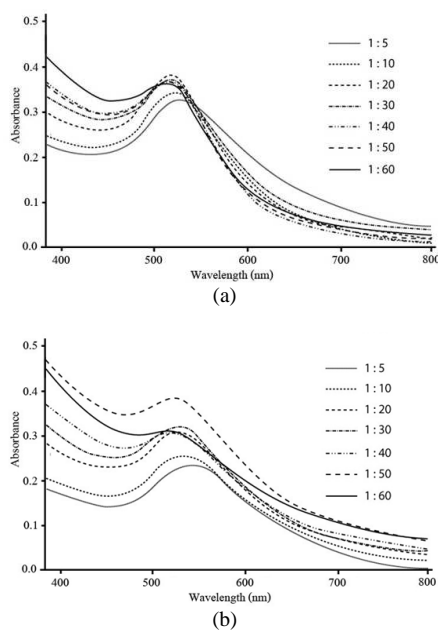


Fig. 3: UV-Vis spectrum of fucoidan-stabilized AuNPs prepared at 80°C using various weight ratios of gold to o-fucoidan (a) and t-fucoidan (b)

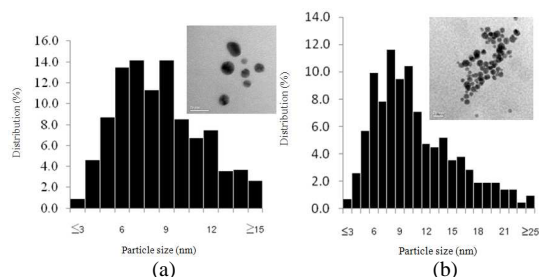


Fig. 4: TEM images (inset, scale bar of 20 nm) and histogram of size distribution of o-fucoidan-stabilized AuNPs (a) and t-fucoidan-stabilized AuNPs (b)

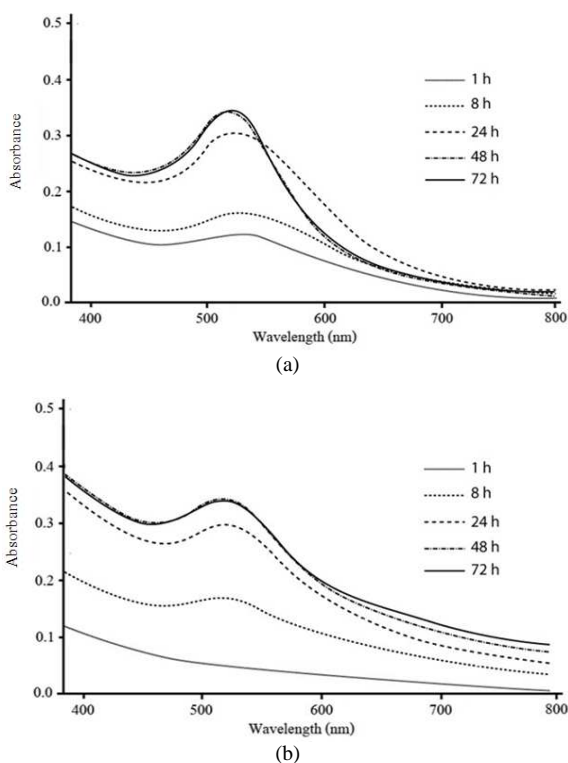


Fig. 5: UV-Vis spectrum of fucoidan-stabilized AuNPs prepared at ambient temperature and different time, using weight ratios of 1:20 gold to o-fucoidan (a) and 1:50 gold to t-fucoidan (b)

DISCUSSION

The ruby-red color of the gold solution usually indicates the presence of AuNPs in the size range below 25 nm, which corresponds to the SPR band around 520-530 nm (Link and El-Sayed, 1999). The red-purple color of t-AuNPs seemed to correlate with the red shift of the absorbance peak to longer wavelengths than those of o-AuNPs, compared at the same ratio. The red shift at absorption maxima may also indicate the aggregation behavior of nanoparticles (Lee and Perez-Luna, 2005; Schofield *et al.*, 2007). Normally, the AuNPs having size of less than 25 nm show the SPR band of lower than 530 nm or so-called the intrinsic size region where the wavelength shift in the absorption maxima is not significant and the measured absorbance directly determines the concentration of nanoparticles present (Link and El-Sayed, 1999). AuNPs at the absorption maxima were consequently used for further studies on size distribution and homogeneity of nanoparticles by using TEM analysis. Although the fucoidans from two different species of marine algae can facilitate the formation of AuNPs within the 10-nm size range, we observed some differences in that t-

AuNPs were less monodispersed and more aggregated in nature. The findings from TEM seemed to support our SPR results earlier on the longer wavelength at absorption maxima of t-AuNPs.

Although o-fucoidan and t-fucoidan initiated the formation of nanometer-sized AuNPs, the difference in SPR spectrum and size distribution noticed might be arisen from fucoidan structure. While polymer chain of o-fucoidan composes of mainly linear chain of α -(1 \rightarrow 3)-linked sulfated fucopyranose with substituting residues of glucuronic acid at the C-2 position of L-fucopyranose for every 4 molecules of the main chain. In contrary, a branched structure of sulfated fucopyranose was found in t-fucoidan at C-2 position of the main chain. Since sulfate group is the site for gold reduction and stabilization, amount and location of the chemical group are likely to be responsible for nanoparticle dispersity. For t-fucoidan, the sulfate esters present in a main chain together with additional ones at side chains can possibly interact with several nanoparticles, hence raising them to stay in contact with each other and cause the occurrence of gold nanocluster. Whether the other factors namely molecular weight and concentration of o-fucoidan and t-fucoidan may affect the AuNP dispersity are questionable. However, from the study of Huang and Yang (2004a) on chitosan-stabilized AuNPs, the five-fold lower in concentration and eight-fold less in molecular weight of chitosan did not obviously cause the nanoparticles more dispersity. In addition, o-AuNPs and t-AuNPs were able to be synthesized at room temperature and the peak absorbance was in the time-dependent manner up to 48 h. The findings were in agreement with other report on the time duration required for complete formation of AuNPs synthesized using dried seaweed at an ambient temperature (Singaravelu *et al.*, 2007).

CONCLUSION

Conclusively, the present study provides an alternative for eco-friendly synthesis of spherical and monodisperse AuNPs using sulfated polysaccharides from algae fucoidans. The different structure of fucoidan is less likely to significantly impact on nanoparticle formation but does affect on the SPR, size distribution and dispersity. Fucoidan-stabilized metallic AuNPs can be further investigated for their potential in various nanotechnology-based applications.

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