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Characteristics Study on Biosynthesized Au Nanoparticles Supported onto Cross-Linked Chitosan Beads

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Abstract: In this study chitosan beads were synthesized and cross-linked using glutaraldehyde in order to enhance the chemical resistance and mechanical stability of chitosan beads. Herein the biopolymer chitosan-GLA beads were used as a support for Au nanoparticles. This process contains two steps; using chitosan-GLA beads to adsorb Au(III) ion from aqueous solution of HAuCl₄, followed by reduction of adsorbed Au(III) ion to Au(0) using the aqueous *Psidium guajava* leaf extract as reducing and stabilizing agent. The chitosan-GLA/Au(0) characterized using Fourier Transform Infrared (FTIR) spectroscopy, field emission scanning electron microscope/energy dispersive scanning electron microscopic (FESEM), (EDX), X-Ray Diffraction (XRD) and BET surface area analyses which proves the successful immobilization and reduction of Au(III) ion to Au(0) form.

Key words: Chitosan beads, glutaraldehyde, Au nanoparticles, adsorption, *Psidium guajava*

INTRODUCTION

Nanometrials are receiving a considerable amount of attention from different fields of science because of their unique properties in which they differ from those bulk materials. Additionally, nanoparticles have a wide range of application such as electronics, cosmetics, optical display and biomedical application (Thakkar *et al.*, 2010). Among various metal nanoparticles, gold exhibits a significant potential of application in different areas like medical science (Elghanian *et al.*, 1997), catalysis and drug delivery (Niemeyer, 2001). The most common methods used for the synthesis of Au(0) is the chemical reduction of gold(III) ion solution by using chemical reducing agent such as NaBH₄ and citrate but such reducing agent produce toxic chemicals and hazardous. Due to these environmental problems, there is a need to develop environmental and friendly process for NPs production that does not involve toxic chemicals in the synthesis process. Different biological systems such as bacteria (He *et al.*, 2007), fungi (Kumar *et al.*, 2007) and plants (Sathishkumar *et al.*, 2009; Shankar *et al.*, 2003)

have been used for Au(0) synthesis, however plant extract have several advantageous as compared to other biological methods as it is easy, available, non-toxic and eliminate the complicated techniques for cell culture (Gardea-Torresdey *et al.*, 2003).

The adsorption of metal ions using low-cost natural materials as potential adsorbent including biopolymers, zeolites, clay, agricultural waste such as rice hull, banana pith, saw dust and pumpkin waste (Kamari and Ngah, 2009; Ngah *et al.*, 2005) is recognized as an emerging, economical and effective technique that widely used for the removal of toxic metals from aqueous environments. The advantage of this method as compared to the other methods is the low generation of residue, easy recovery of metal and the possibility for reuse of the adsorbent (Ngah *et al.*, 2002).

Chitosan, a derivative biopolymer obtained from the deacetylation of chitin occurs naturally and abundantly from the exoskeletons of insects, crustaceans' shells and fungi cellular walls (Muzzarelli *et al.*, 2012). Chitosan is one of the bio absorbable and non toxic polymers which are processed in different biofunctionalities. Recently

chitosan, which is used as an adsorbent is attracting a lot of attention due to its hydroxyl and amino functional groups that show high potential for adsorption of metal ions (Hasan *et al.*, 2008). Therefore, many studies focused on supporting of metal nanoparticles such as using zeolites (Riahi *et al.*, 2002), metal oxides and biopolymer like chitosan or chitosan (Cumbal *et al.*, 2003; Wu and Ritchie, 2006). While chitosan showed excellent properties and it is found that it rarely to be used as support which is due its solubility in most dilute mineral and organic acids solutions. Hence to overcome this problem, chitosan is chemically modified by using cross-linking agent such as glutaraldehyde (GLA), Ethylene Glycol Diglycidyl Ether (EGDE), epichlorohydrin (ECH), genipin and triphosphate (TPP) (Ngah *et al.*, 2005; Ngah and Fatinathan, 2008).

In the present study, we attempted to use chitosan due to its strong adsorption properties towards metal ions and applied environmental green method to synthesize well dispersed Au nanoparticles in a biopolymer support which will be useful for further biological or catalytic applications.

METHODOLOGY

Preparation of chitosan beads: Chitosan solution was prepared by dissolving 2 g of chitosan flakes in 60 mL of 5% (v/v) acetic acid solution. The chitosan solution was stirred and kept over night to ensure the completely dissolved chitosan flakes. Then this viscous solution put drop wise into a precipitation bath containing 250 mL of 0.5 M NaOH by using a peristaltic pump to neutralize the acetic acid within the chitosan gel and to form spherical uniform beads. The obtained wet chitosan gel beads were washed thoroughly with distilled water to remove any excess NaOH until neutrality (pH = 7).

Preparation of cross-linked chitosan beads: The prepared wet chitosan beads were suspended in 2.5% glutaraldehyde solution to obtain a ratio of 1:1 with chitosan. The chitosan beads in resulting glutaraldehyde solution were left to stand for 24 h at room temperature. Then the cross-linked chitosan beads were washed for several times with distilled water subsequently, the beads were filtered and dried in an oven at 60°C. Here, the newly formed beads called chitosan-GLA beads.

Preparation of *Psidium guajava* leaf extract: *Psidium guajava* leaves used in these experiments were obtained locally from Pontian, Malaysia. The leaves were

washed with distilled water to remove any dust or impurities and allowed to dry for 1-2 weeks at room temperature. The dried leaves were then ground and sieved through a 20 mesh sieve. One gram of the powdered *Psidium guajava* leaves was soaked in 100 mL of deionized water for 24 h in a 500 mL Erlenmeyer flask. This was then followed by boiling the solution for 5 min after which it was filtered. The filtrate was collected and kept in refrigerator at 4°C for further experiments.

Immobilization of Au(0) onto cross-linked chitosan-GLA beads: The chitosan-GLA/Au(0) beads prepared by the wetness impregnation method. The 0.5 g of chitosan-GLA beads were immersed in to 30 mL of an aqueous solution of HAuCl₄ (0.3 mM) and kept under stirring for 4 h. The chitosan-GLA beads (appearing dark red) that had adsorbed Au(III) ions were filtered, cleansed by using deionised water for several times and dried in an oven at 60°C overnight in order to proceed to Au(III) ion reduction. The experiments conducted at room temperature. The obtained Au-chitosan complex was reduced using 10 mL of *Psidium guajava* leaf extract and stirred for overnight. The chitosan-GLA/Au(0) was washed with deionized water in an oven at 60°C.

Swelling test for chitosan beads, chitosan-GLA beads and chitosan-GLA/AuNPs beads: An appropriate amount of chitosan samples (0.05 g) were added into beaker containing dilute acid, deionized water and dilute alkaline solutions, respectively for a period of 24 h at room temperature. The swollen chitosan were filtered and dried. Swollen chitosan samples were weight out and the percentage of swelling was calculated based on the following equation:

$$S (\%) = \frac{W_f - W_i}{W_i} \times 100 \quad (1)$$

where, W_f is the weight of swollen beads (g) and W_i is the weight of dry beads (g).

RESULTS AND DISCUSSION

Characterization of chitosan beads: The surface area with the help of BET data of chitosan flakes, chitosan-GLA beads and chitosan-GLA/Au (0) beads were illustrated in Table 1.

From the results presented in Table 1, it was observed that the surface area of chitosan-GLA beads is higher than chitosan flakes which is attributed to the to

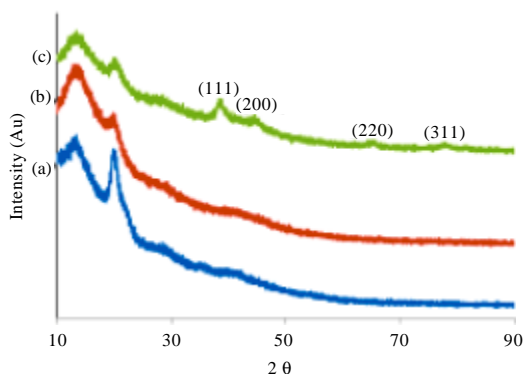


Fig. 1: X-Ray Diffusion (XRD) of (a) Chitosan flakes, (b) Chitosan-GLA beads and (c) Chitosan-GLA Au(0) beads

Table 1: Physical characteristics of chitosan flakes, chitosan-GLA beads and chitosan-GLA Au(0) beads

Sample	BET surface area (m ² g ⁻¹)
Chitosan flake	3.10
Chitosan-GLA beads	4.63
Chitosan-GLA/Au(0) beads	4.74

Table 2: The CHN analyses of chitosan flakes, chitosan-GLA beads and chitosan-GLA/Au(0) beads

Sample	C (%)	H (%)	N (%)
Chitosan flakes	40.91	7.54	7.80
Chitosan-GLA beads	43.58	7.81	6.95
Chitosan-GLA/AuNPs beads	51.63	8.83	5.50

Table 3: Swelling behavior of chitosan beads, chitosan-GLA beads and chitosan-GLA/Au (0) beads in different medium

Sample	Swelling (%)		
	5% (v/v) acetic acid	Deionized water	NaOH (0.01 M)
Chitosan beads	Soluble	43.8	40.9
Chitosan-GLA beads	20.6	6.2	16.5
Chitosan-GLA/Au(0) beads	26.7	11.9	14.9

higher molecular mass of cross linking agent. Same observation was noticed after adsorption and reduction of Au(III) ion on cross-linked chitosan due to the presence of Au(0) in chitosan-GLA matrix.

From Table 2, it was found that modification with cross-linking agents decreased the mass percentage of N atom on the chitosan chain. This is due to the chemical reaction between the cross-linking agents and chitosan. While the mass percentage of C atom and H is increased. This can be related to the reaction between chitosan and the cross-linking agent (glutaraldehyde).

Swelling test of chitosan beads, chitosan-GLA beads and chitosan-GLA/Au(0) beads: One of the important physical tests to provide information about crystalline nature

of adsorbent is the percentage of swelling. Table 3 shows the swelling percentage of chitosan flakes, chitosan-GLA and chitosan-GLA/Au(0). The percentage of swelling has been calculated by using the Eq. 1. Based on this result, we can conclude that chitosan flakes is soluble in acid media but it show higher percentage of swelling in deionized water and alkaline medium. However, after the chitosan has been cross-linked with GLA, the swelling capacity is reduced but it shows some degree of swelling in acid medium due to the amino groups protonation in chitosan (Jeon and Holl, 2003). Similar to chitosan-GLA beads, the chitosan-GLA/Au(0) beads gives the highest swelling percentage which can explained by the organic surfactant (capping agent) from *Psidium guajava* leaf extract which is soluble in acidic medium.

Swelling test of chitosan flakes, chitosan-GLA beads and chitosan-GLA/Au(0) beads

X-ray diffraction analysis: The XRD study was done for chitosan flakes, chitosan-GLA beads and chitosan-GLA/Au(0) beads. Figure 1a illustrates the presence of two broad peaks 12 and 26°, respectively which are the natural crystal peaks of chitosan flakes. After the chitosan were cross-linked by glutaraldehyde (curve b in Fig. 1), the intensity of the natural crystal peaks became weak. Due to the lowering in the crystallinity of chitosan flakes, probably large number of H bonds in chitosan flakes destroyed after cross-linking process.

Furthermore, when the Au(III) ion adsorbed onto the cross-linked chitosan beads and reduced the presence of peaks at 38, 44.45, 65.14 and 77.6° that can be clearly seen and corresponding to (111), (200), (220) and (311), respectively Brags reflection regarding face centered cubic lattice (fcc) gold crystal, respectively “curve c” in Fig. 1 (Jin and Bai, 2002).

Fourier-transform infrared spectra analysis: The FTIR studies expressed in Fig. 2 were used to observe the transmutation of the functional groups in chitosan molecule after the modification, adsorption and reduction process. Figure 2a shows a broad and strong bands ranging from 3200-3600 cm⁻¹ which indicates the presence of OH and NH₂ groups which is consistent with the peaks at 1082 and 1152 cm⁻¹ assigned to the alcoholic C-O and C-N stretching vibrations, respectively.

In Fig. 2b after chitosan has been cross-linked with GLA appearing of a significant new peak at around 1660 cm⁻¹ can be attributed to imine group (C=N). More

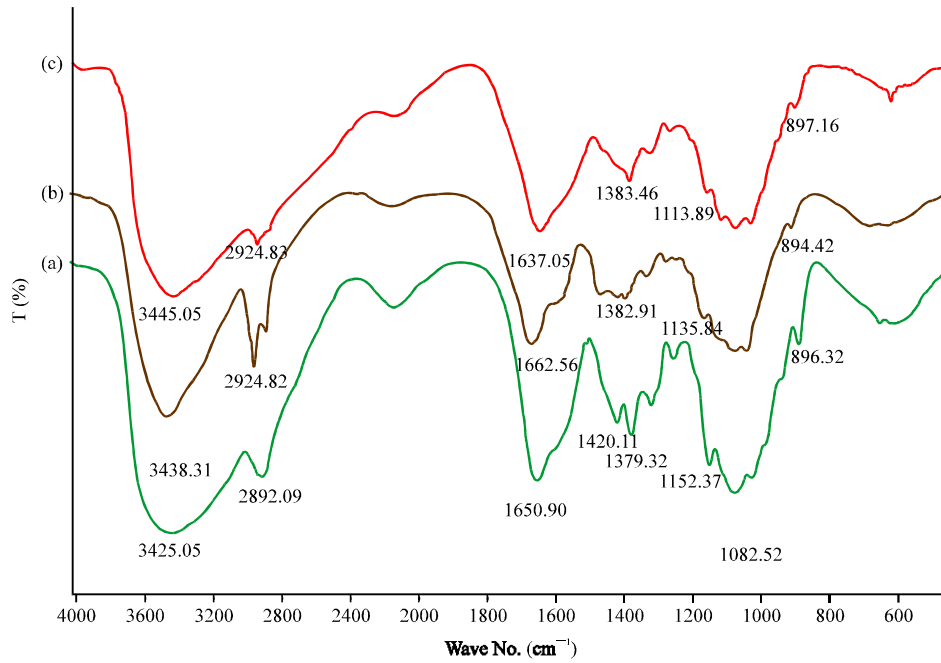


Fig. 2: Fourier Transform Infrared (FTIR) spectra of (a) Chitosan flakes, (b) Chitosan-GLA beads and (c) Chitosan-GLA/Au(0) beads

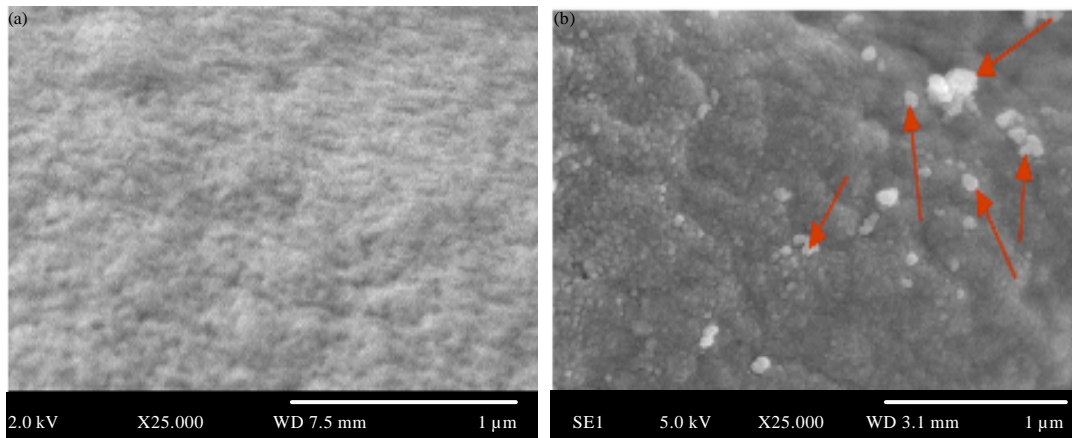


Fig. 3(a-b): (a) Chitosan-GLA beads and (b) Chitosan-GLA/Au(0) beads

ever, the intensities around 1420 cm^{-1} peak (primary amino group, -NH_2) is reduced which indicates that most of amino groups are involved in the cross-linking process.

The absence of peak at around 1720 cm^{-1} confirms that there is no unreacted aldehyde group (Monteiro and Airoldi, 1999). A comparison of the spectrum for chitosan-GLA beads with that of Au(0) loaded beads (Fig. 2c) showed the red shifting in the $\text{C}=\text{N}$ stretching which suggesting the interaction between metal and N atom. The intensity of -NH group was reduced after

attached with Au(III) ion due to the molecular weight of Au^{+3} atom (Jin and Bai, 2002).

FESEM analysis: Field Emission Scanning Electron Microscope/energy (FESEM) was employed to analyze the surface morphology of chitosan-GLA beads and chitosan-GLA/Au(0) (Fig. 3a, b). This showed that the surface morphology of chitosan-GLA beads has changed as it is smoother and less cracking. In contrast observed in Fig. 3b after adsorption and reduction of Au(III) ion,

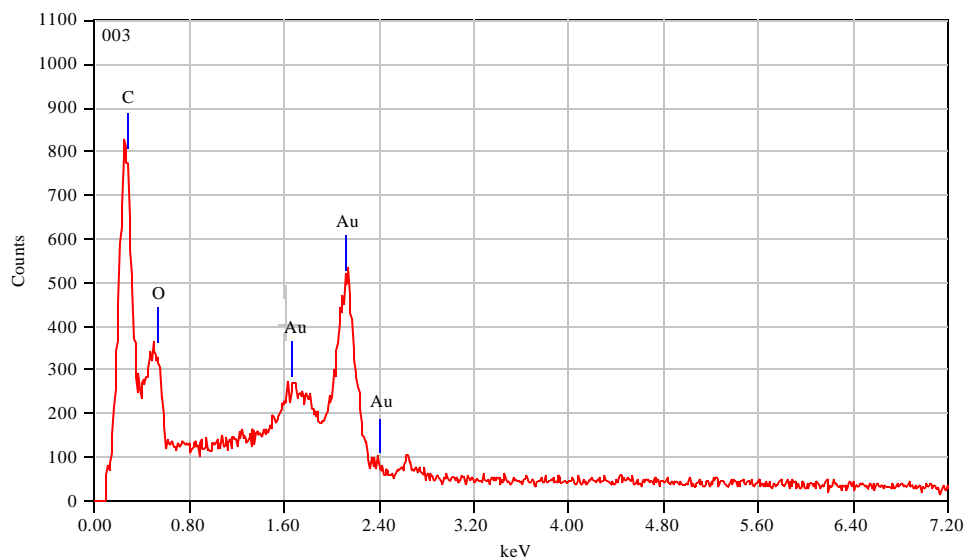


Fig. 4: Energy Dispersive X-ray (EDX) spectrum of chitosan-GLA/Au(0) beads

the surface was totally changed to more irregular and rough beside the presence of AuNPs on the chitosan matrix (represented with red arrows).

The EDX spectrum of chitosan-GLA/Au(0)beads is shown in Fig. 4.

CONCLUSION

In this study, the adsorption and bioreduction of Au(III) ion from aqueous solution on cross-linked chitosan beads from aqueous solution were investigated. Techniques such as FESEM, EDX, XRD, CHN and FTIR were used for the characterization of chitosan flakes, chitosan-GLA beads and chitosan-GLA/Au(0) beads. It was confirmed that the adsorption and reduction process has occurred successfully. The FESEM results showed the formation of irregular shape of gold nanoparticles on the surface of chitosan beads by using simple environmental reduction process. The FTIR showed that the adsorption and reduction of Au(III) ion on chitosan is chemical adsorption because of relevant changes observed on the functional groups. It can be concluded that the cross-linked chitosan beads are effective absorbent for metal ion collection and this type of bioreduction show green and environmental advantageous in order to increase the chitosan beads application.

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