

Biosynthesized gold nanoparticles supported on magnetic chitosan matrix as catalyst for reduction of 4-nitrophenol

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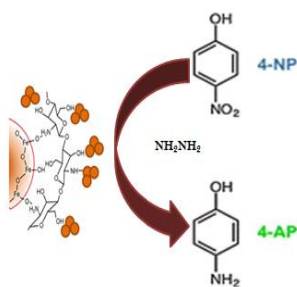
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Article history

Received 25 February 2019
 Revised 23 April 2019
 Accepted 30 May 2019
 Published Online 25 June 2019

Graphical abstract



Abstract

The design and environmentally-safe synthesis of magnetically recoverable solid-supported metal nanoparticles with remarkable stability and catalytic performance have significant industrial importance. In the present study, we have developed an inexpensive bioinspired approach for assembling gold nanoparticles (AuNPs) in magnetic chitosan network under green, mild and scalable condition. AuNPs were well loaded on the surface of the magnetic support due to the presence of hydroxyl (-OH) and amino (-NH₂) groups in chitosan molecules that provided the driving force for the complexation reaction with the Au(III) ions. Reduction of the Au(III) to Au(0) was achieved by using *Melicope ptelefolia* aqueous leaf extract. The synthesized magnetic chitosan supported biosynthesized Au nanocatalyst was characterized using Fourier Transform Infrared (FT-IR), Carbon, Hydrogen and Nitrogen (CHN), Transmission Electron Microscopy (TEM), X-Ray Diffraction (XRD) and Atomic Absorption Spectroscopy (AAS) analyses. FTIR spectrum of magnetic chitosan showed peaks at 1570 cm⁻¹, which indicate for N-H bending vibration and at 577 cm⁻¹ which designates the Fe-O bond. CHN analytical data further supported the coating of chitosan onto the magnetite. TEM analysis showed an amorphous layer around the magnetite core, proving the coating of chitosan on the magnetite surface and the average particle size of AuNPs calculated was 7.34 ± 2.19 nm. XRD analysis showed six characteristic peaks for magnetite, corresponding to lattice planes (220), (311), (400), (422), (511) and (440) in both the magnetite and magnetic chitosan samples (JCPDS file, PDF No. 65-3107). Meanwhile, XRD analysis of catalyst showed characteristic peaks of AuNPs at 2θ (38.21°, 44.38°, 62.2°, 77.32° and 80.76°), which correspond to (111), (200), (220), (311) and (222) lattice planes (JCPDS file, PDF No.04-0784). AAS analysis showed the loading of AuNPs as 5.4%. The rate constant achieved for the reduction of 4-nitrophenol to 4-aminophenol in the presence of hydrazine hydrate using 10 mg of catalyst was 0.0046 s⁻¹. The magnetic chitosan supported AuNPs is effective as catalyst for the reduction of 4-nitrophenol.

Keywords: [Magnetic chitosan, Gold nanoparticles, Biosynthesis, *Melicope ptelefolia*, 4-nitrophenol]

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INTRODUCTION

Nanomaterials are receiving a considerable amount of attention from different fields of science because of their unique properties in which they differ from those of bulk materials. Among various metal nanoparticles, gold exhibits a significant potential of application in different areas like medical science, catalysis and drug delivery [1].

The most common method used for the synthesis of Au is the chemical reduction of Au(III) ions solution by using chemical reducing agent such as sodium borohydride (NaBH₄) and citrate but such reducing agents are toxic and hazardous [2]. Due to these problems, there is a need to develop environmental friendly process for gold nanoparticles (AuNPs) production that does not involve toxic chemicals in the synthesis process. Among different biological systems employed for AuNPs synthesis, plants extract has several advantages as compared to fungi and bacteria, as it is easily available, non-toxic and eliminate the use of complicated techniques for cell cultures [3].

Melicope ptelefolia is a plant species belonging to the family Rutaceae. It is a medicinal and widely grown plant in Malaysia and is locally known as 'Tenggek Burung'. It is a popular salad and consumed raw by the Malay community. Leaves of this plant have its own benefits such as to revitalize the body and to release tension. In addition, its leaves have been reported to help in controlling and reducing high blood pressure and *diabetes mellitus* if consumed regularly [4]. This plant can be used as a reducing agent in this research due to the presence of biomolecules in its leaf extract. These biomolecules are responsible to reduce metal ions to form metal atom [5]. As in [6], *Hygrophila spinosa* aqueous leaf extract has been used as reducing agent for HAuCl₄ to obtain gold nanoparticles in order to apply in pharmaceutical industry.

Chitosan (CS), a derivative biopolymer obtained from the deacetylation of chitin, occurs naturally and abundantly from the exoskeletons of insects, crustaceans' shells and fungi cellular walls. Chitosan is one of the bioabsorbable and non-toxic polymers, which is 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 [7].

Magnetic separation technique has some advantages, such as high efficiency and cost-effectiveness. In this regard, magnetite (Fe_3O_4) has been widely used as magnetic material due to its excellent magnetic properties, chemical stability and biocompatibility. Thus, by using Fe_3O_4 as the main support, the catalyst can be easily separated from the reaction media with the aid of an external magnet, leading to fast and efficient recovery route [8].

4-nitrophenol is a common organic waste water pollutant which is widely used in the preparation of pesticides, explosives and pharmaceutical industries. This compound poses significant health risk due to its carcinogenic activities. On the other hand, 4-aminophenol is an important intermediate for the production of pharmaceuticals substances, photographic materials and rubber materials [9].

In the present study, we have developed an inexpensive bioinspired approach for decorating small gold nanoparticles (AuNPs) in a magnetic chitosan network using *Melicope ptelefolia* leaf extract under green, mild and scalable condition. Preparation of magnetic chitosan matrix is significant due to prevent agglomeration between metal-metal atoms after being reduced. Structural features as well as catalytic performance of the prepared hybrid nanocomposite have been investigated in the reduction of 4-nitrophenol to 4-aminophenol.

EXPERIMENTAL

Materials

All glasswares were washed thoroughly with deionised water and dried overnight in the oven before being used for experiment. Deionised water was used throughout this study. Commercial grade solvents were distilled before being used. All chemicals were obtained commercially from Sigma-Aldrich and were used as received without further purifications. *Melicope ptelefolia* (Tenggek Burung) leaves were purchased from a local market in Johor Bahru.

Synthesis of magnetite (Fe_3O_4) nanoparticles

Fe_3O_4 was prepared according to literature method with some modifications [8]. A mixture of iron(III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) (2.7 g, 10 mmol) and iron(II) chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$) (1.0 g, 5 mmol) was dissolved in deionised water (130 mL) under N_2 flow. Ammonium hydroxide (NH_4OH) (11 mL, 28% v/v) was added under rapid mechanical stirring (700 rpm). The resultant solution was heated to 60 °C and stirred for 1 h under N_2 flow. The reaction mixture was let to cool at room temperature before washing it using deionised water twice. The product formed was collected by using external magnet and redispersed in trisodium citrate (200 mL, 0.3 M). Then, the solution was further heated at 80 °C for 1 hr. The precipitate formed (Fe_3O_4) was collected by using external magnet and washed with acetone. The product was kept in a desiccator for further reaction.

Synthesis of magnetic chitosan (Fe_3O_4 -CS) nanoparticles

Magnetic chitosan (Fe_3O_4 -CS) was prepared following the method as reported with some modifications [9]. Fe_3O_4 (0.5 g) was dispersed in deionised water (150 mL) while chitosan (75%-85% deacetylated) flakes (0.25 g) were dissolved separately in acetic acid (70 mL, 2 wt%) with continuous stirring. Both solutions were mixed under vigorous stirring for 1 h. Then, the resultant solution was transferred quickly into a beaker. The product obtained was collected by using external magnet, washed with acetone and kept in a desiccator for further use.

Preparation of *Melicope ptelefolia* leaf extracts solution

Fresh *Melicope ptelefolia* leaves were washed thoroughly with deionised water to remove any dust or impurities and dried at room temperature for 1-2 weeks. The dried leaves were ground into powder using an electrical blender. *Melicope ptelefolia* leaf powder (4 g) was extracted with deionised water (200 mL) in a Soxhlet apparatus for 8 h. The resultant leaf extract was transferred into a porcelain crucible and slowly heated to remove the solvent. The dried, powdered solid obtained was kept in a vacuum desiccator for further use. A 10 % w/v

leaf extract solution was prepared by dissolving 10 mg of dried powder in 100 mL deionised water.

Immobilization of Au(III) ions onto Fe_3O_4 -CS nanoparticles

Tetrachloroauric(III) acid (HAuCl_4) (0.3 mM, 25 mL) was mixed with Fe_3O_4 -CS (30 mg) in a centrifuge tube. The mixture was shaken mechanically (480 rpm) for about 24 h at room temperature. The product (Fe_3O_4 -CS-Au(III)) was collected by an external magnet, washed with deionised water and allowed to dry. The separated solution was analyzed by Atomic Absorption Spectroscopy (AAS).

Bioreduction of immobilized Au(III) to Au(0) nanoparticles using *Melicope ptelefolia* leaf extract

Fe_3O_4 -CS-Au(III) obtained from the previous reaction was directly treated with *Melicope ptelefolia* leaf extract solution (12 mL, 10% w/v). The mixture was shaken for 2 h at room temperature. The final product (Fe_3O_4 -CS-AuNPs) was collected using an external magnet, washed with deionised water and dried in the desiccator for further use.

Catalytic reduction of 4-nitrophenol to 4-aminophenol

Catalytic reduction of 4-nitrophenol was performed following literature method [10]. Hydrazine hydrate (0.375 mM, 1.6 mL) was added into 4-nitrophenol (0.125 mM, 1.6 mL). Then, the Fe_3O_4 -CS-AuNPs (10 mg) catalyst was added into the reaction mixture with stirring. The UV-vis absorption spectrum of the reaction mixture was recorded with time to monitor the change in absorption intensity of the reaction mixture in the scanning range of 200-500 nm.

RESULTS AND DISCUSSION

Fe_3O_4 -CS-AuNPs was synthesised via a simple and inexpensive method by the immobilisation of AuNPs on the magnetic chitosan support using *Melicope ptelefolia* leaf extract in this work. The leaf extract acted as a reducing and stabilizing agent in the preparation of AuNPs on the surface of the magnetic chitosan. The presence of biomolecules such as *p*-*O*-geranylcoumaric acid and 2,4,6-trihydroxy-3-farnesylgeranylacetophenone in the *Melicope ptelefolia* leaf extract are believed to be responsible in the reduction of Au(III) to Au(0) [11].

FTIR spectroscopic analysis was carried out to identify functional groups presented in CS, Fe_3O_4 , Fe_3O_4 -CS and Fe_3O_4 -CS-AuNPs. The FTIR spectra of the samples were as presented in Figure 1.

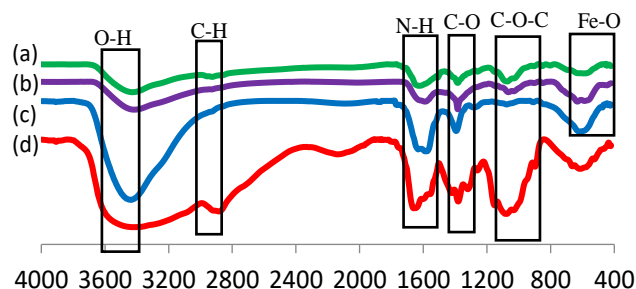


Figure 1 FTIR absorption spectra of (a) Fe_3O_4 -CS, (b) Fe_3O_4 -CS-AuNPs, (c) Fe_3O_4 and (d) CS.

Absorption bands at 3401 cm^{-1} and 3370 cm^{-1} observed in the IR spectrum of magnetite and magnetic chitosan are indicative of OH group. The presence of an OH band in the magnetite spectrum may be due to trace of water during its preparation. The appearance of characteristic bands at 591 cm^{-1} and 580 cm^{-1} in the IR spectrum of magnetite and magnetic chitosan is due to Fe-O bond. The FTIR spectrum of chitosan was characterized by the following absorption bands; the (NH) of backbone polymer arose around 1650 cm^{-1} , (C-O) of primary alcoholic group at 1380 cm^{-1} , and the (C-H) at 2893 cm^{-1} . The shift of the Fe-O stretching frequency from 591 cm^{-1} in magnetite to 580 cm^{-1} in the magnetic chitosan and of the N-H bending vibration from 1650 cm^{-1} to 1612 cm^{-1} strongly suggested the binding of Fe to

the NH₂ groups of chitosan. The band appeared at 1386 cm⁻¹ in the IR of magnetic chitosan is assigned to the C-O of alcoholic group of chitosan. Further, the peak around 2925 cm⁻¹ which corresponded to the C-H stretching band of chitosan was observed in the IR spectra of both chitosan and magnetic chitosan, strongly suggesting the successful coating of the magnetite surface with chitosan. The IR spectrum of Fe₃O₄-CS-AuNPs showed similar absorption bands as the Fe₃O₄-CS, which probably suggesting that AuNPs had successfully anchored on the Fe₃O₄-CS layer. This observation is in agreement with the findings of Liu *et al* [12].

The XRD patterns of Fe₃O₄ and Fe₃O₄-CS were shown in Figures 2(a) and 2(b) respectively. Six characteristic peaks were observed in both samples for Fe₃O₄ (JCPDS file, PDF No. 65-3107) which corresponded to crystal planes of (220), (311), (400), (511), and (440). Weak and broad diffraction lines were observed in the diffractogram of both samples probably due to very low amount of samples and small crystallite size. Meanwhile, XRD analysis of Fe₃O₄-CS-AuNPs in Figure 2(c) showed five characteristics peaks of AuNPs (JCPDS file, PDF No. 04-0784), corresponding to crystal planes of (111), (200), (220), (311) and (222), which are consistent with literature values [13]. Besides, there were also distinctive peaks of Fe₃O₄ seen in the diffractogram of Fe₃O₄-CS-AuNPs, showing the existence of the magnetite as the core.

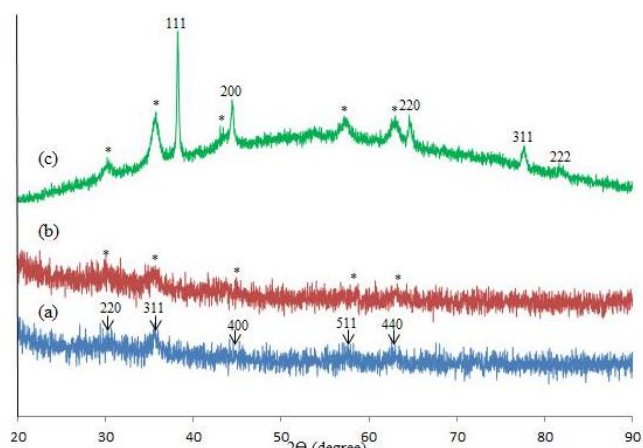


Figure 2 XRD pattern of (a)Fe₃O₄, (b)Fe₃O₄-CS and (c) Fe₃O₄-CS-AuNPs.

Meanwhile, TEM micrographs for Fe₃O₄ and Fe₃O₄-CS with same magnification were shown in Figure 3. The chitosan layer could be seen formed on the surface of the Fe₃O₄ nanoparticles as shown in Figure 3(b). In this figure, the parallel lines crossing the particle image represented the crystal planes of nanoparticles. Under careful examination, these lines did not extend till the edges of magnetite nanoparticles. The remaining area (bounded by the red circle) that did not include the crystal planes would correspond to an amorphous chitosan layer. Similar findings have been reported by Osuna *et al* in their work on chitosan coated magnetic nanoparticles [10].

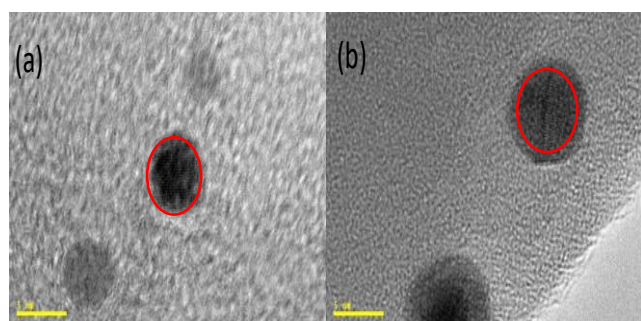


Figure 3 TEM images of (a) Fe₃O₄ and (b)Fe₃O₄-CS with same scale, 5 nm.

Table 1 CHN analysis of Fe₃O₄-CS.

Sample	Elements (%)		
	C	H	N
Fe ₃ O ₄ -CS	10.28	2.31	1.18

CHN elemental analytical data of Fe₃O₄-CS (Table 1), shows the existence of nitrogen element which further supports the coating of chitosan onto Fe₃O₄. Meanwhile Table 2 shows the AAS analytical data indicating the actual Au loading on the Fe₃O₄-CS surface of 5.4% which is in close agreement with the calculated values, suggesting that chitosan had acted as an effective support for Au(0) nanoparticles, binding through its -OH and -NH₂ groups.

Table 2 AAS analysis of Fe₃O₄-CS-AuNPs.

Au loading (%)	Calculated	Experimental
(%)	6.0	5.4

Figure 4 shows TEM image of Fe₃O₄-CS-AuNPs which showed good dispersity of the AuNPs which were mostly spherical with less agglomeration. This probably suggesting that the chitosan matrix is successful in preventing agglomeration of the AuNPs. Figure 4(b) shows that the d-spacing of AuNPs calculated was 0.23 nm which is in very good agreement with literature data [14]. Based on the imageJ software, the mean particle size calculated from 20 AuNPs (Figure 4(c)) was 7.3 nm.

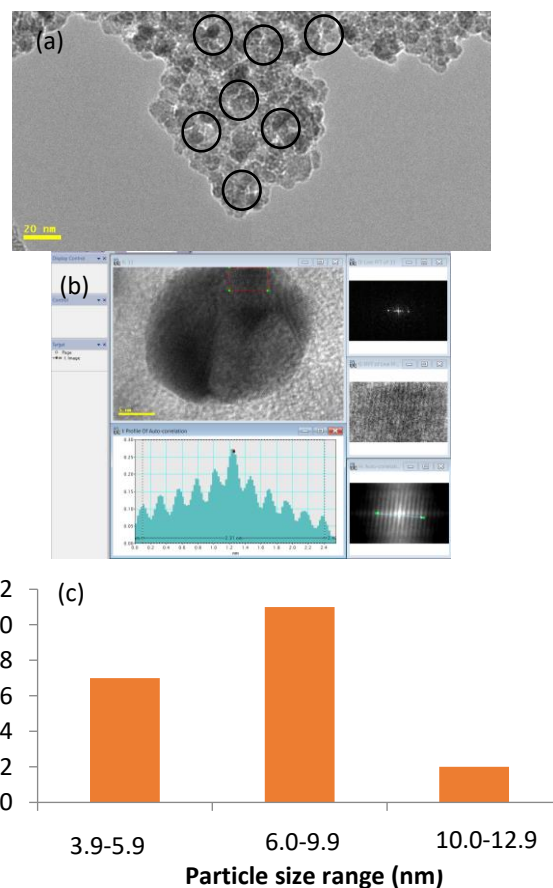


Figure 4 (a)TEM image of Fe₃O₄-CS-AuNPs with 20 nm scale (b) d-spacing of AuNPs (c) Particles size distribution of AuNPs.

The catalytic activity of Fe₃O₄-CS-AuNPs was investigated in the reduction of 4-nitrophenol in the presence of N₂H₄ and shown in Figure 5. The UV-visible spectrum of an aqueous solution of 4-nitrophenol (Figure 5(a)) showed a maximum absorption at 314 nm.

After the addition of hydrazine hydrate, a red shift was observed with the maximum absorption at 398 nm, indicating the formation of 4-nitrophenolate ions. After the addition of the $\text{Fe}_3\text{O}_4\text{-CS-AuNPs}$ catalyst (Figure 5(c)), a gradual decrease in the absorbance for the 4-nitrophenolate ions peak at 398 nm was observed along with gradual increase of a new absorbance at 313 nm, indicating the formation of 4-aminophenol. Complete reduction of 4-nitrophenol occurred within 40 min.

The reduction of 4-nitrophenol to 4-aminophenol was also monitored in the absence of $\text{Fe}_3\text{O}_4\text{-CS-AuNPs}$ catalyst. As can be seen in Figure 5(b), the peak at 398 nm showed almost no change in intensity after 40 min of reaction time, hence suggesting that no significant reduction has occurred without the presence of biostabilised $\text{Fe}_3\text{O}_4\text{-CS-AuNPs}$ catalyst.

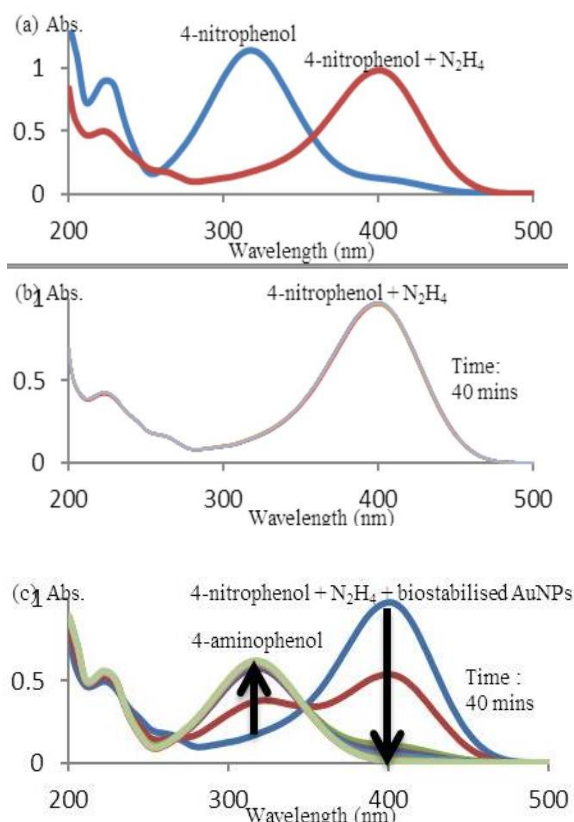


Figure 5 (a) UV-visible spectra of 4-nitrophenol and after the addition of N_2H_4 (b) UV-visible spectrum of (4-nitrophenol + N_2H_4) in 40 minutes time of reaction (c) UV-visible spectra of (4-nitrophenol + N_2H_4 + biostabilised AuNPs) in 40 minutes time of reaction.

The catalytic reaction was studied in excess of N_2H_4 concentration as compared to 4-nitrophenol. Thus, the concentration of N_2H_4 was considered as constant and the reaction rate constant (k_a) of the reduction was only depended on the 4-nitrophenol concentration. Hence, the reaction was assumed to follow the pseudo first-order kinetics and calculated by using the following kinetic equation:

$$-k_a t = \ln(C_t/C_0) = \ln(A_t/A_0) \quad (1)$$

where C_t and A_t are the concentration and absorption of 4-nitrophenol at time (t) while C_0 and A_0 are the concentration and absorption of 4-nitrophenol at the initial time of the reaction [15]. The rate constant (k_a) was calculated from the slope of the plot of $\ln(A_t/A_0)$ versus the reaction time (t) as displayed in Figure 6. The rate constant obtained from the reaction using 10 mg AuNPs and 5.4% Au loading of $\text{Fe}_3\text{O}_4\text{-CS-AuNPs}$ was 0.0046 s^{-1} .

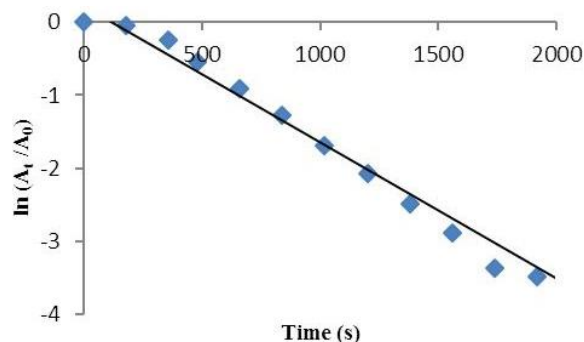


Figure 6 Plot of $\ln(A_t/A_0)$ versus time (t) for the reduction of 4-nitrophenol by using 10 mg AuNPs and 5.4% Au loading of $\text{Fe}_3\text{O}_4\text{-CS-AuNPs}$.

CONCLUSION

In summary, biostabilised AuNPs have been successfully supported onto the $\text{Fe}_3\text{O}_4\text{-CS}$ support. The use of $\text{Fe}_3\text{O}_4\text{-CS}$ as catalyst support provides a good dispersity of the AuNPs catalyst with less agglomeration. The biosynthesised AuNPs are mostly spherical with an average size of 7.3 nm. The magnetic chitosan supported biostabilised AuNPs showed good catalytic activity in the reduction of 4-nitrophenol to 4-aminophenol in the presence of N_2H_4 where the reduction was complete in 40 minutes with the rate of reaction of 0.0046 s^{-1} . The synthesised magnetic AuNPs catalyst can also be tested in the other organic transformations such catalytic oxidation of benzylic and allylic alcohols, reduction of nitroaromatics and epoxidations.

ACKNOWLEDGEMENT

This work was financially supported by Ministry of Higher Education Malaysia and Universiti Teknologi Malaysia under FRGS funding vot no. 4F779

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