BIOSYNTHESIZED GOLD NANOPARTICLES SUPPORTED ON MAGNETIC CHITOSAN MATRIX AS CATALYST FOR REDUCTION OF 4-NITROPHENOL

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DEDICATION

For my beloved husband, Mohd Aidil bin Elias, thanks for your endless support in all aspects and encouragement for me during this journey. For my parents, Saffee bin Haji Lebar and Mon binti Kasiran, thanks for your never ending pray and concern for me to reach this level. For my son, Muhammad Fateh Iman bin Mohd Aidil, thanks for your good behaviour that ease me to accomplish this research thesis. For my siblings, family in-law and friends who involve in my Master journey, thanks for everything. May all of us always showered by Allah's bless and keep thankful in each step of our life.

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ABSTRACT

The design and synthesis of environmentally-safe magnetically recoverable solid-supported metal nanoparticles with remarkable stability and catalytic performance has attracted significant interest. In the present study, an inexpensive bioinspired approach for assembling gold nanoparticles (AuNPs) in magnetic chitosan (CS) and carboxylmethylchitosan (CMC) network under green, mild and scalable condition is reported. AuNPs were well loaded on the surface of the magnetic support due to the presence of hydroxyl (-OH) and amino ($-NH_2$) groups in chitosan molecules that provided the driving force for the complexation reaction with the Au(III) ions. Reduction of the Au(III) ion to Au(0) is achieved by using *Melicope ptelefolia* aqueous leaf extract. The synthesized magnetic chitosan supported biosynthesized Au nanocatalyst was characterized using Fourier transform infrared spectroscopy (FTIR), carbon, hydrogen and nitrogen analysis (CHN), transmission electron microscopy (TEM), X-ray diffraction (XRD) and atomic absorption spectroscopy (AAS). FTIR spectrum of magnetic chitosan showed peaks at 1570 cm^{-1} indicative of N-H bending vibration and at 577 cm^{-1} which designated the Fe-O bond. CHN analytical data further supported the coating of chitosan onto the magnetite. 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). In addition, XRD analysis of the catalyst showed characteristic peaks of AuNPs at 2θ (38.21°, 44.38°, 62.2°, 77.32° and 80.76°) which corresponded to (111), (200), (220), (311) and (222) lattice plane (JCPDS file, PDF No.04- 0784). TEM analysis showed an amorphous layer around the magnetite core which supported the coating of chitosan on the magnetite surface and the average particle size of AuNPs calculated was 7.34 ± 2.19 nm. 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 optimum conditions of Fe_3O_4-CS-AuNPs were 12% of Au loading and 15 mg of catalyst amount. Both the prepared magnetic chitosan supported AuNPs catalysts, Fe_3O_4-CS-AuNPs and Fe_3O_4-CMC-AuNPs showed good performance as catalyst for the reduction of 4-nitrophenol which gave rate constant of 0.0055 s^{-1} and 0.0104 s^{-1}, respectively. Fe_3O_4-CS-AuNPs also gave good recyclability which at least four times without significant loss of activity.

ABSTRAK

Reka bentuk dan sintesis nanopartikel logam berpenyokong pepejal yang terkembalikan semula secara magnetik mesra alam sekitar yang memiliki kestabilan dan prestasi pemangkinan luar biasa telah menarik minat yang ketara. Dalam kajian ini, satu pendekatan bioinspirasi yang murah untuk menghimpun nanopartikel emas (AuNP) dalam rangkaian kitosan (CS) dan karboksilmetilkitosan (CMC) magnetik dalam keadaan hijau, mudah dan boleh berskala dilaporkan. AuNP mudah dimuat di atas permukaan penyokong magnetik kerana kehadiran kumpulan hidroksil (-OH) dan amino ($-NH_2$) di dalam molekul kitosan yang memberikan daya pendorong bagi tindak balas pengkompleksan dengan ion Au(III). Tindak balas penurunan ion Au(III) menjadi Au(0) dicapai dengan menggunakan ekstrak akueus daun *Melicope ptelefolia*. Nanomangkin Au terbiosintesis Au berpenyokong kitosan magnetik telah dicirikan menggunakan spektroskopi inframerah transformasi Fourier (FTIR), analisis karbon, hidrogen dan nitrogen (CHN), mikroskopi elektron penghantaran (TEM), pembelauan sinar-X (XRD) dan spektroskopi penyerapan atom (AAS). Spektrum FTIR kitosan magnetik menunjukkan puncak pada 1570 cm^{-1} yang menandakan getaran bengkokan N-H dan pada 577 cm^{-1} yang menunjukkan ikatan Fe-O. Data analisis CHN selanjutnya menyokong penyalutan kitosan di atas magnetit. Analisis XRD menunjukkan enam puncak cirian bagi magnetit yang sepadan dengan satah kekisi (220), (311), (400), (422), (511) dan (440) di dalam kedua-dua sampel magnetit dan kitosan magnetik (fail JCPDS, PDF No. 65- 3107). Tambahan lagi, analisis XRD mangkin menunjukkan puncak cirian bagi AuNP pada 2θ (38.21°, 44.38°, 62.20°, 77.32° dan 80.76°) yang sepadan dengan satah kekisi (111), (200), (220), (311) dan (222) (fail JCPDS, PDF No.04-0784). Analisis TEM menunjukkan lapisan amorfus di sekitar teras magnetit yang menyokong lapisan kitosan pada permukaan magnetit dan saiz purata zarah AuNP yang dikira adalah 7.34 ± 2.19 nm. Analisis AAS menunjukkan muatan AuNP adalah 5.4%. Pemalar kadar yang dicapai bagi penurunan 4-nitrofenol menjadi 4-aminofenol dengan kehadiran hidrazin hidrat menggunakan 10 mg mangkin adalah 0.0046 s^{-1}. Keadaan optimum bagi Fe_3O_4-CS-AuNPs adalah 12% muatan AuNPs dan 15 mg jumlah mangkin. Kedua-dua mangkin AuNPs berpenyokong kitosan magnetik, Fe_3O_4-CS-AuNP dan Fe_3O_4-CMC-AuNP menunjukkan prestasi yang baik sebagai mangkin penurunan 4-nitrofenol untuk menghasilkan pemalar kadar masing-masing 0.0055 s^{-1} dan 0.0104 s^{-1}. Fe_3O_4-CS-AuNP juga boleh dikitar semula dengan baik sekurang-kurangnya empat kali tanpa kehilangan aktiviti yang ketara.

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LIST OF ABBREVIATIONS

FTIR	-	Fourier Transform Infrared
TEM	-	Transmission Electron Microscopy
CHN	-	Carbon, Hydrogen and Nitrogen
AAS	-	Atomic Adsorption Spectroscopy
XRD	-	X-Ray Diffraction
UV-Vis	-	Ultraviolet-visible spectroscopy
CS	-	Chitosan
Fe_3O_4-CS	-	Magnetic Chitosan
AuNPs	-	Gold Nanoparticles
CMC	-	Carboxymethyl Chitosan
4-NP	-	4-nitrophenol
4-AP	-	4-aminophenol
JCPDS	-	The Joint Committee on Powder Diffraction Standards

LIST OF SYMBOLS

%	-	Percentage
% w/v	-	percent weight per volume
°C	-	degree Celcius
2Ө	-	Bragg angle
cm	-	centimeter
cm^{-1}	-	Frequency
g	-	Gram
h	-	hour(s)
K_a	-	rate constant
m	-	Meter
mg	-	Milligram
min	-	minute(s)
nm	-	Nanometer
°	-	degree angle
s	-	second(s)
s^{-1}	-	per second
wt%	-	weight percent
λ	-	Wavelength

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Nanotechnology is a very promising area of research which involves the production of nanomaterial as the basic strategy (Agrawal *et al*., 2014). It involves the synthesis, characterization and studying the properties of nanomaterial that have at least one spatial dimension in the range of 1-100 nm. This includes nanorods, nanowires and nanoparticles. Recently, there are wide applications of metal nanoparticles in many fields such as optics, optoelectronics, catalysis, photography, nanostructure fabrication, sensor and photonics (Bindhu *et al*., 2014). The performance of the nanomaterials is highly dependent on the size, shape, surface morphology and composition.

Gold (Au) is known to be highly resistant towards oxidation and corrosion as compared to other metals and is traditionally regarded as chemically inert and catalytically inactive (Thakor *et al*., 2011). However, when the size of Au is reduced to nanometres, the catalytic properties of Au are revealed. Currently, Au nanoparticles (AuNPs) is considered the catalyst of choice for many reactions such as oxidation of alcohols, epoxidation of propylene, hydrogenation of nitrobenzenes, hydrochlorination of alkynes, carbon-carbon bond formation and C–N bond formation (Alshammari, 2019).

Commonly, AuNPs synthesis is usually carried out by physical and chemical methods (Alshammari, 2019). Although there are several variety of chemical synthesis methods are available for metal nanoparticles, many of the reactants and starting materials used in these reactions are toxic and potentially hazardous.

Meanwhile most physical techniques of metal nanoparticles suffer from high energy demand and the requirement of complex instrumentations. Recently, the biogenic metal nanoparticles synthesis method that employs biological entities, such as microorganisms and plant extracts, has been suggested as a valuable alternative to the physical and chemical synthesis routes. This green synthesis method that employs naturally occurring reducing agents could be a promising method to replace more complex physiochemical syntheses since the green synthesis is free from toxic chemicals and hazardous by-products and instead involves natural capping agents for the stabilization of metal nanoparticles (Bindhani and Panigrahi, 2014).

Melicope ptelefolia is a tree 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 vegetable salad and consumed raw by the Malay community. The plant has been reported to exhibit antioxidant, anti-proliferative and apoptosis induction activities due to the presence of high polyphenols content (Abd. Karim *et al*., 2011). This type of biomolecules has great potential to be used reducing agents in the synthesis of metal nanoparticles.

Generally, AuNPs are not stable and tend to form larger particles in order to minimise its high surface energy. Colloidal AuNPs when used directly as catalyst in the liquid phase will show a decrease in its activity with time due to agglomeration (Gulina *et al*., 2015). In addition, the very small size of AuNPs is very difficult to be separated from the reaction mixture by traditional filtration methods. Therefore, dispersion of AuNPs onto a solid support has attracted a lot of attention to avoid it from agglomeration and allow ease of separation.

Chitosan (CS) is a type of polyaminosaccharide synthesized from the deacetylation of chitin, a polysaccharide consisting predominantly of unbranched chains of β-(1 → 4)-2-acetoamido-2-deoxy-D-glucose (Ahmad *et al*., 2015). The amino and hydroxyl functional groups of CS are effective binding sites for metal ions. The use of CS as novel supporting material for heterogeneous catalysis is on the increase, mainly due to its high affinity to metal ions. The unique properties of

CS also enhanced its applicability for further functionalization and immobilization of metal complexes through covalent attachment (Lee *et al*., 2015).

In recent years, the use of magnetic support as a robust, highly efficient and rapid catalyst separation tool has become increasingly important. Energy is not required throughout the separation process while the mass lost is prevented and its operation time is reduced. By using a magnetic support, the catalyst can be separated from the reaction mixture with the use of an external magnet. Thus, magnetic separation is often the best choice in terms of efficiency.

1.2 Problem Statement

Preparation of metal nanoparticles can be carried out by physical, chemical and biological methods. Physical method is known to be an expensive technique as it normally will involve specialized equipment such as laser ablation and requires high energy consumption. Meanwhile, chemical method usually requires the use of toxic and hazardous chemicals and generates huge amount of solvent. Furthermore, this method may be difficult and costly to scale up for industry purposes. In this research, a biological approach employing plant extract is applied in order to synthesize AuNPs. This method is simple and low-cost as it does not require high energy consumption or expensive equipment. In addition, the use of plant extract as a source of reducing agent is cheap due to the readily available biomass usage. At the same time, this method is considered to be safe and environment friendly as it avoids the use of toxic and hazardous chemicals. Qiu *et al*., (2014) has reported the preparation of magnetic Au nanoparticle-chitosan catalysts without employing any toxic reductants or capping agents. Therefore, in this research, a locally available plant, *Melicope ptelefolia* leaf extract is used as reducing and capping agents to synthesise the AuNPs. Phenolic compounds such as oleanolic acid, O-geranylcoumaric acid and 2,4,6-trihydroxy-3-phenylacetophenone identified in the aqueous leaf extract of *Melicope ptelefolia* have potential as reducing and capping agents in the preparation of AuNPs (Abas *et al*., 2010)

AuNPs have been immobilised on various solid supports such as carbon, silica, metal oxides and zeolites to ease the catalyst recovery and enhance the catalyst stability (Bond & Thompson, 1999). However, the use of these traditional inorganic supports requires time and energy consuming workup procedures such as filtration and centrifugation to collect the supported AuNPs catalysts. In order to overcome this problem, the utilisation of magnetic nanoparticles as catalyst support material has appears as worthwhile alternative as their paramagnetism enables easy and efficient catalyst recovery (Karimi *et al*., 2015). In this research, magnetite nanoparticles (Fe_3O_4) were used as the catalyst support as it could facilitate the dispersion of AuNPs as well as can be easily recovered and separated from the reaction mixture by using an external magnetic field. The unique properties of the magnetite nanoparticles are high surface area per volume ratio, small size, surface modifiability, excellent magnetic properties and great biocompatibility (Sureshkumar *et al*, 2016).

Furthermore, the AuNPs tend to agglomerate and form larger particles which leads to loss of catalytic activity. Hence, the AuNPs has to be stabilised to prevent agglomeration. With the aim of addressing this problem, chelating ligand with strong metal affinity groups, such as hydroxyl (−OH) and amino ($-NH_2$) groups is desirable to be functionalised onto the magnetite surface to stabilise and disperse the AuNPs. In this research, CS, an abundant and natural biopolymer was surface functionalised onto magnetite to increase the dispersion and minimised the agglomeration of AuNPs.

4-aminophenol is an important intermediate produced in the synthesis of various chemicals including dyes, pharmaceutical and anti-corrosive lubricants industries. However, 4-nitrophenol is a common organic waste water pollutant and being used for the preparation of pesticides and explosive industries. Besides, this chemical also poses significant health risks due to its carcinogenic activities. Thus, the development of a method for efficient removal of 4-nitrophenol is important for public health and can help to restore impacted environments. In this research, an effective catalytic reduction of 4-nitrophenol to 4-aminophenol in aqueous solution and under mild condition by using the magnetic chitosan supported biosynthesised AuNPs as catalyst and clean reductant, hydrazine hydrate have been carried out.

1.3 Objectives of the Research

The objectives of this research were:

i. To synthesize and characterize magnetite coated chitosan (CS) and carboxymethyl chitosan (CMC) as support material via co-precipitation method.

ii. To immobilize Au(III) ions onto the magnetite coated CS and CMC supporting materials via wet impregnation technique.

iii. To reduce and characterize the immobilized Au(III) ions into Au(0) using *Melicope Ptelefolia* aqueous leaf extract as reducing agent.

iv. To evaluate the performance of the immobilized biosynthesised AuNPs supported onto magnetic chitosan (Fe_3O_4-CS-AuNPs) and carboxymethyl chitosan (Fe_3O_4-CMC-AuNPs) as catalyst in the reduction of 4-nitrophenol to 4-aminophenol in the presence of hydrazine hydrate.

1.4 Scope of the Study

This study involved the synthesis of magnetite, Fe_3O_4 nanoparticles via co-precipitation method of Fe(II) and Fe(III) ions using ammonium hydroxide as the precipitating agent. Then, the magnetite was coated with CS supporting materials by mixing them together under vigorous stirring and the final product was separated via magnetic decantation method. The CS coated magnetite obtained was characterized by using Fourier Transform Infrared (FTIR) spectroscopic, Transmission Electron

Microscopy (TEM), Carbon, Hydrogen and Nitrogen (CHN), Atomic Adsorption Spectroscopy (AAS) and X-Ray Diffraction (XRD) analytical data.

Immobilization of Au(III) ions onto the magnetic chitosan was carried out with the aid of a mechanical shaker using tetrachloroauric acid ($HAuCl_4$) as the precursor. The supported Au(III) ions were then reduced to Au(0) by using 10% w/v *Melicope Ptelefolia* aqueous leaf extract as the reducing agent.

The formation of the biostabilised Au(0) supported onto magnetic chitosan nanoparticles was characterised using UV-Visible (UV-Vis) spectroscopy, Atomic Absorption Spectroscopy (AAS), X-Ray Diffraction (XRD) , and Transmission Electron Microscopy (TEM) analytical techniques.

The catalytic activity of the magnetic chitosan supported biosynthesised gold nanoparticles was investigated in the reduction of 4-nitrophenol to 4-aminophenol in the presence of hydrazine hydrate. The catalytic conditions were optimized in terms of the percentage of Au loading (3, 6, 9 & 12%) and weight of catalyst (5, 10 & 15 mg).

1.5 Significance of the Study

This study applied a simple and green method in the synthesis of AuNPs using the *Melicope ptelefolia* aqueous leaf extract of as reducing agent. The use of plant extract as reducing agent offers several advantages as it is safe and environmentally friendly, simple laboratory set up and can be easily scale up and avoid the need of toxic and hazardous chemicals and high energy consumption. This study also introduced the use of a renewable and abundantly available biopolymer, chitosan, as an active catalyst support to stabilised and minimise the undesired agglomeration of the AuNPs. The developed protocol employed low catalyst loading and easy work-up for the synthesis. More importantly, the use of magnetic chitosan matrix as the catalyst support is an effective strategy to improve the efficiency and recyclability of the catalyst.

Environmental degradation is one of the major concerns of environmentalists. The toxic waste generated by the chemical industries is one of the major threats to human, animals and plant lives on the earth. The magnetic chitosan supported biostabilised Au catalyst synthesized in this study showed high activity in the reduction of 4-nitrophenol to 4-aminophenol and is reused at least for times without significant loss in the activity. Thus, development of efficient and recyclable heterogeneous Au -based catalytic system is an important objective as generation of huge amount of metal waste is against the principles of Green Chemistry. It is predicted that CS supported AuNPs catalysts can have potential applications in terms of efficiency and recyclability in the transformation of 4-nitrophenol to 4-aminophenol.

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LIST OF PUBLICATION

Norfazreen binti Saffee and Mustaffa Shamsuddin (2019), Biosynthesized Gold Nanoparticles Supported on Magnetic Chitosan Matrix as Catalyst for Reduction of 4-Nitrophenol, *Malaysian Journal of Fundamental and Applied Sciences*, 15(3), pp. 451–455