



## Recyclable silver–magnetite nanocomposite for antibacterial application



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### ABSTRACT

Surface modification of magnetite nanoparticle (MNP) with cysteine for efficient conjugation with silver nanoparticle (AgNP) is herein presented. This novel nanocomposite was prepared via a specific binding interaction between thiol groups of cysteine coated on the MNP and the surface of AgNP. Transmission electron microscopy indicated the formation of the nanocomposite with 18–24 nm silver nanoparticles and 8–12 nm magnetite nanoparticles. Energy dispersive spectrometry verified the presence of both Ag and Fe in the nanocomposite. After repetitive adsorption–desorption–washing process, the nanocomposite retained higher than 90% antibacterial efficiency against *Escherichia coli* K12 for at least five recycling cycles.

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### Introduction

Magnetite nanoparticle (MNP) has been long used in many industrial applications such as magnetic recording media and sensors. Recently, the applications of MNP have been more diversified in biomedicine such as targeted drug delivery, contrast agents for magnetic resonance imaging (MRI) [1–3], specific targeting and imaging of cancer cells [4], hyperthermia treatment of tumors [5], enzyme and protein immobilization [6,7] and RNA and DNA purification [8]. MNP for such biomedical applications should be well dispersible in its media, chemically stable, biocompatible and possesses active functions on its surface [9]. MNP with these properties can be enhanced by modifying its surface with long chain polymers to provide steric repulsion mechanism [10] or charge molecules to provide electrostatic repulsion mechanism [11] to the particles. Conjugation of MNP surface with bioentities such as amino acids [12,13], peptides [14], and antibodies [15] was an efficient method to obtain the particle with active functions for advanced and specific applications.

Synthesis of nanocomposite containing MNP and silver nanoparticle (AgNP) has been extensively studied by many groups [16–18]. AgNP has drawn a lot of attention particularly as an antibacterial agent because of its stability, durability and good antibacterial activity [19–24]. Therefore, nanocomposite of MNP and AgNP combined the functions of both components, magnetic responsiveness from MNP and antibacterial activity from AgNP. Many previous works reported on preparation of binary Ag–magnetite nanocomposite for antibacterial applications [25–28]. However, aggregation of these nanocomposite due to magnetic and electrostatic attractive forces was a major limitation in the synthesis process and particularly when applying it in practice [29,30]. To overcome these problems, coating the nanocomposite with polymer matrix provided good stabilization and good dispersibility in a media. Nonetheless, this method might limit application potential of the nanocomposite from a restriction of its possible transport due to a large size of the polymer matrix [31], and also reduced its magnetic responsiveness and antibacterial effectiveness.

In this work, surface modification of MNP with cysteine to obtain specific binding interaction with AgNP is presented. Cysteine, a water-soluble and sulfur-containing amino acid [32,33], was chosen for coating MNP surface in this work because the thiol groups in cysteine molecules can provide strong binding interactions with AgNP [34–37]. In addition, the existence of the

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NH<sub>2</sub> and COOH groups in cysteine molecules will enable the cysteine-coated MNP to have good biocompatibility and good dispersibility in aqueous media. Unlike cysteine, the other amino acid containing a thiol group such as methionine is classified as nonpolar and hydrophobic because it has a straight side chain that possesses an S-methyl thioether. In the present work, AgNP, known to have extraordinary inhibitory and bactericidal properties, was conjugated to MNP surface using cysteine as a linker to form magnetic AgNP-containing nanocomposite. It was hypothesized that silver ions interacted with sulfhydryl groups, or thiol groups (-SH), on the membranes of bacteria, causing disruption of their permeability and thereby leading to microbial cell death [38–40]. The novelty of this work is that this is the first report on coating MNP surface with cysteine and its use to effectively bind AgNP on the surface. In addition, taking advantages of MNP, the nanocomposite was efficiently used as a recyclable antibacterial agent.

In this research, amino-coated MNP reacted with glycidyl methacrylate as a linker to obtain the MNP coated with methacrylate functional groups. The MNP surface was then further modified *via* a Michael addition reaction between the methacrylate and cysteine to form cysteine-coated MNP (Fig. 1). The functional groups of the MNP were studied *via* Fourier transform infrared spectroscopy (FTIR) and the hydrodynamic size and zeta potential values were characterized *via* photo-correlation spectroscopy (PCS). AgNP was separately synthesized *via* a chemical reduction method and subsequently conjugated with cysteine-coated MNP. Transmission electron microscopy (TEM) and energy dispersive spectrometry (EDS) techniques were used to verify the successful conjugation of the MNP with AgNP. In addition, the magnetic properties and composition of the nanocomposite were investigated using vibrating sample magnetometry (VSM) and thermogravimetric analysis (TGA), respectively. Antibacterial activity and recyclable efficiency of Ag-magnetite nanocomposite were also discussed.

## Experimental

### Materials

Unless otherwise stated, all reagents were used as received; iron(III) chloride anhydrous (FeCl<sub>3</sub>) (Carlo Erba), iron(II) chloride tetrahydrate (FeCl<sub>2</sub>·4H<sub>2</sub>O) (Carlo Erba), ammonium hydroxide (28–30%, J.T. Baker), oleic acid (Fluka), 3-aminopropyl triethoxysilane (99%, Acros), glycidyl methacrylate (97%, Sigma), L-cysteine (99%, Acros), sodium borohydride (Fisher Scientific) and silver nitrate (99.8%, Merck). Triethylamine (97%, Carlo Erba) and toluene were stirred under CaH<sub>2</sub> and distilled prior to use.

### Syntheses

#### Synthesis of MNP and methacrylate-coated MNP (MA-coated MNP)

A FeCl<sub>3</sub> solution (1.66 g in 20 ml deionized water) and a FeCl<sub>2</sub>·4H<sub>2</sub>O solution (1.00 g in 20 ml deionized water) were mixed together with stirring, followed by adding 25% NH<sub>4</sub>OH (20 ml). After 30 min stirring, the mixture was centrifuged for 20 min to precipitate large aggregate and the aqueous supernatant was discarded. An oleic acid solution in hexane (2 ml in 20 ml hexane, 10%v/v) was then added into the MNP dispersion with stirring. The dispersion was concentrated by evaporating hexane to obtain concentrated MNP in hexane. The MNP was then re-suspended in toluene in the presence of triethylamine (1.2 ml, 0.0086 mol), followed by adding 3-aminopropyl triethoxysilane (1.2 ml, 0.0068 mol). After stirring at room temperature for 24 h, the dispersion was precipitated in ethanol and washed with toluene to obtain amino-coated MNP. To prepare MA-coated MNP, the amino-coated MNP was stirred in toluene for 15 min, following by adding triethylamine (1.39 ml, 0.0099 mol) and glycidyl methacrylate (1.33 ml, 0.01 mol). After stirring at 60 °C overnight under nitrogen atmosphere, MA-coated MNP was retrieved by a magnet, repeatedly washed with ethanol and toluene, and dried under reduced pressure.

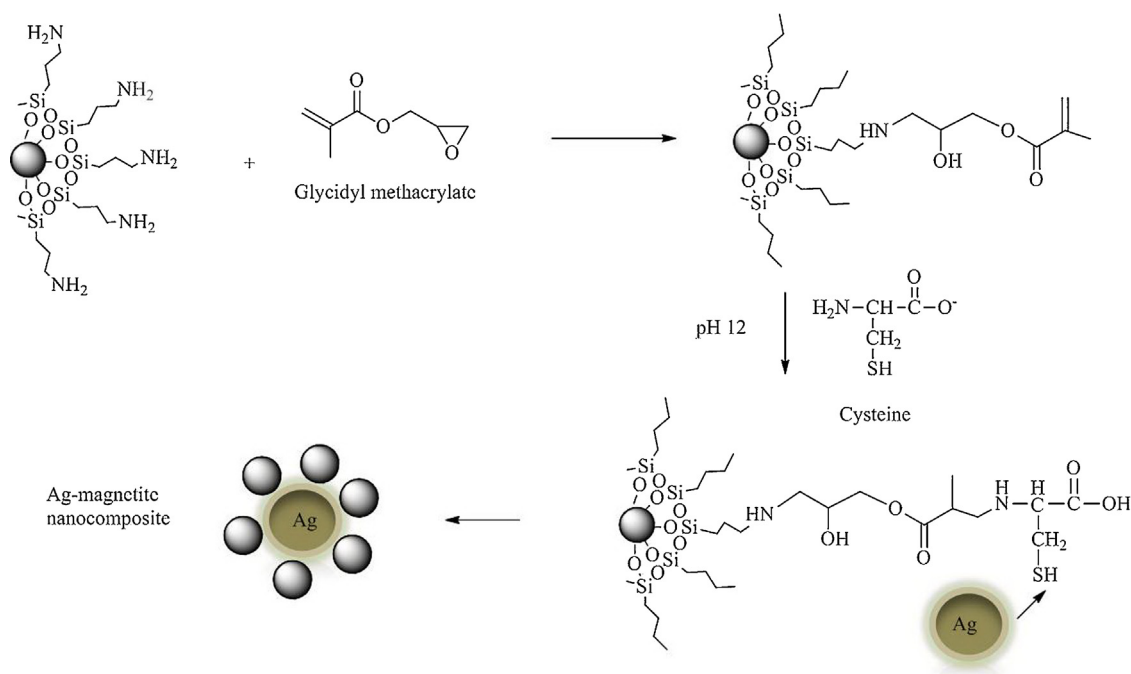


Fig. 1. Schematic diagram for the synthesis of Ag-magnetite nanocomposite.

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