Project Report

Surface Modification of Magnetic Iron Oxide Nanoparticles with Biodegradable Polymers for Plasma Protein Adsorption

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Abstract

Magnetic iron oxide nanoparticles have been proposed as one of the most popular and efficient drug carrying magnetic materials used in human treatment and diagnosis due to their non-toxicity, high stability and high magnetic responses. Magnetic iron oxide nanoparticles (MNP) were obtained by chemical co-precipitation of iron salts in the presence of ammonia. The prepared magnetic particles were modified with gallic acid to reduce aggregation of particles, maintain magnetic stability, and slowdown degrading process under physiological conditions. Magnetic iron oxide nanoparticles coated with gallic acid (MNPG) were obtained with small particle size ranging from 10 nm to 80 nm and retained magnetization properties. The magnetic nanoparticles were characterized by scanning electron microscope (SEM) coupled with an energy dispersive X-ray detector (EDX), Fourier transform infrared (FTIR), and powder X-ray diffraction (XRD). Surface functionalization of magnetic nanoparticles was evaluated via adsorption of protein bovine serum albumin (BSA) on nanoparticles. The highest adsorption of BSA was obtained from MNP as BSA adsorbed up to 70% within 30 min of incubation, while the adsorption of BSA by MNPG within 30 min of incubation was observed at 50% approximately and MNPG showed the lower BSA adsorption rate within 4 h of incubation. After 4 h of incubation, the result indicated similar adsorption profile of BSA by MNP and MNPG.
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1. BACKGROUND AND RATIONALE

Due to the unique combination of their high magnetization and paramagnetic behavior, magnetic iron oxide nanoparticles have gained the great interest in several medical applications such as magnetic-targeted drug delivery systems. With the development of imaging technology, a chemical compound known as a contrast agent has been used to improve the sensitivity and detectability of the imaging tools to enhance the visibility of the Magnetic Resonance Imaging (MRI). Because of their magnetic properties, non-toxicity and biocompatibility, magnetic iron oxide nanoparticles are considered as one of the group of powerful MRI contrast agents. Magnetic nanoparticles – based contrast agents have affected predominantly relaxation time T2 in Nuclear Magnetic Resonance (NMR) which result in T2-weighted images (negative contrast). Magnetic iron oxide nanoparticles are widely used in several medical applications such as magnetic-targeted drug delivery system. They could be served as nanocarriers for drugs and MRI contrast agents since they have been approved by Food and Drug Administration (FDA). The most commonly used magnetic nanoparticles-based contrast agents are monocristalline iron oxide nanocompounds such as superparamagnetic iron oxide (SPIO) approved by FDA (i.e. Combidex® and Feridex®)

In drug delivery systems, many colloidal nanoparticles have been developed to improve the site specificity of drug action to reach the targeted organ and reduce the systemic side effects. The ideal circulation time for the therapeutic nanoparticles requires sufficient half-life to remain in the affected area for image capture or drug delivery. Unfortunately, nanocarriers are rapidly removed from the blood circulation after intravenous administration mostly by the mononuclear phagocyte system (MPS). Concerns about the rapid uptake of nanoparticles by the MPS, the alternative surface modification strategies including substitute polymers and biomimetic surface functionalization could provide the nanoparticles with long circulation properties resulting in so-called stealth behaviors. Particularly, the stealth magnetic nanoparticles could be obtained by polymeric coating which require retaining their physiochemical features of nanocarriers such as particle size, surface properties, and particle shape, along with their magnetic properties.
2. INTRODUCTION

As nanovehicle systems, the magnetic iron oxide nanoparticles have been proposed as one of the most popular and efficient drug carrying magnetic materials used in human treatment and diagnosis due to their high stability, high magnetic responses and easy elimination. Due to its non-toxicity, iron oxide nanoparticles can be used in body via hemoglobin metabolism. After distribution in blood circulation and absorption in human body, the remaining iron oxide particles can be safely excreted from the body through kidney, bile, etc. Because of their unique magnetic properties, the major applications of iron oxide nanoparticles can be found in biomedical applications such as: cellular therapy such as cell labelling, targeting and as a tool for cell-biology research to separate and purify cell populations, tissue repair, magnetic field-guided carriers for localizing drugs or radioactive therapies, magnetic resonance imaging (MRI), and tumor hyperthermia. However, magnetic nanoparticles may found to be less stable due to the higher oxidation of iron. Magnetic nanoparticles can posse good stability with either desired size and shape, or coating with polymers. A major obstacle for synthesis of magnetic nanoparticles is to obtain uniform-sized and low aggregation of particles as well as low surface-to-volume ratio. Therefore, it is important to optimize the preparation procedure to achieve the narrow sized-distribution particles and prevent the coagulation of particles.

Because the customization of surface coating of nanoparticles results in a change in biocompatibility and protein-binding capacity, the surface modification of magnetic nanoparticles with biocompatible copolymers is purposed to be studied. The modification of the nanoparticles using suitable polymers such as dextran, chitosan, poly(ethyleneimine) (PEI), and poly(ethylene glycol) (PEG) with active functional groups have been studied for their biological and drug delivery applications. In this work the approach for preparation of magnetic iron oxide nanoparticles coated with gallic acid to obtain the magnetic nanoparticles with small particle size and size distribution as well as retained magnetization properties was investigated. The magnetic nanoparticles were characterized by scanning electron microscope (SEM) coupled with an energy dispersive X-ray detector (EDX), Fourier transform infrared (FTIR), and powder X-ray diffraction (XRD). The chemical composition of iron oxide nanoparticles and the amount of the protein adsorbed on the iron oxide nanoparticles to
observe the capture of magnetic drug carrier and bovine serum albumin were also discussed.

3. OBJECTIVES

1. To synthesize and characterize magnetic nanoparticles with uniform size and morphology, and low surface-to-volume ratio.
2. To increase functionalization of the particles by modification with biocompatible polymers.
3. To investigate protein-binding efficiency of the obtained magnetic nanoparticles in terms of adsorption capacity of the model protein.
4. RESEARCH METHODOLOGY

This research is based on scientific approaches conducted by an experimental design. The overview of the experimental design includes:

4.1 Preparation of magnetic nanoparticles from different ratio of Fe2+/Fe3+ through co-precipitation method by controlling the rate of addition of precipitating agents, pH of solution, stirring rate, reaction time and temperature.

4.2 Characterization of the resulting particles using various related characterization techniques.

4.3 Surface modification of the resulting particles by coating with biodegradable polymers to improve the functionality of the magnetic particles.

4.4 Study of the effect of plasma protein adsorption on the particle surface using bovine serum albumin as model protein and investigation of the in vitro plasma protein adsorption on different nanoparticle surface. An amount of plasma protein adsorption is determined by UV-VIS spectrophotometry.

4.5 Statistic analysis of samples is performed by one-way analysis of variance (ANOVA).
5. MATERIALS AND METHODS

5.1 Materials
Ferric chloride hexa-hydrate (FeCl₃·6H₂O) and ferrous chloride tetrahydrate (FeCl₂·4H₂O) used as the precursor material, gallic acid, and bovine serum albumin (BSA) were purchased from Sigma Aldrich (St Louis, MO, USA). Sodium hydroxide (NaOH) and aqueous ammonia (25%) in analytical grade were purchased from Merck Company (Darmstadt, Germany). All chemicals and reagents were used without further purification. Deionized water was used in all of the experiments.

5.2 Methods
5.2.1 Synthesis of magnetic nanoparticles
The magnetic nanoparticles used in this study were synthesized from FeCl₃·6H₂O and FeCl₂·4H₂O according to previous work. Briefly, 1.23 g of FeCl₃·6H₂O and 1.92 g of FeCl₂·4H₂O were dissolved in 200 mL deionized water under nitrogen atmosphere with vigorous stirring at room temperature for 30 min. Then 6 mL of aqueous ammonia solution (25%) was slowly added. The color of solution was turned from dark orange to the black immediately. The solution was continuously stirred for 2 hours and the magnetic nanoparticles were obtained. The precipitates were separated by permanent magnet and washed three times with deionized water and then with a mixture of acetone and methanol at a 1:1 ratio to remove electrolytes remaining in the solution. The prepared magnetic nanoparticles were dried in oven at 60°C for 3-4 hours, cool to room temperature, and stored at 4°C for the further study.

5.2.2 Gallic acid-coated magnetic nanoparticles
Magnetic nanoparticles were synthesized as previously described. After the magnet separation and purification, 20 mL of 1% gallic acid was added to the magnetic suspension with constant stirring for 24 hours at 25°C. Gallic acid-coated magnetic nanoparticles were separated from the electrolytes remaining in solution using a magnet and then washed three times with deionized water and then with a mixture of acetone and methanol at a 1:1 ratio. The prepared magnetic nanoparticles were dried in oven at 60°C for 3-4 hours and kept for the further study.
5.2.3 Characterization of magnetic nanoparticles

The particle size and morphology of the prepared magnetic nanoparticles were determined using scanning electron microscopy (SEM, S-4300, Hitachi, Tokyo, Japan). The elemental analysis for carbon, iron and oxygen was evaluated using scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM/EDX) based on the surface electrical properties of the different particles. The Fourier transformed infrared spectroscopy (FTIR) was applied for the physical characterization. FTIR of samples were recorded using FTIR spectrophotometer (Perkin Elmer) and FTIR spectra were obtained using the KBr pellet method. With the samples embedded in KBr pellets, the data were recorded in the spectrum range of 650–4,000 cm\(^{-1}\). The crystal structure of the Fe\(_3\)O\(_4\) nanoparticles was characterized using X-ray diffractometry (XRD) on a D8 Advance diffractometer (Bruker) with graphite-monochromatized Cu-Kα radiation (\(\lambda = 1.5406 \, \text{Å}\)).

5.2.4 Protein adsorption study

The prepared magnetic nanoparticles were suspended in 1 mL of deionized water. The same amount of BSA solution (100 mg/L) was added into the magnetic nanoparticles and incubated at 25°C for 30 min, 1 h, 2 h, 3 h, 4 h, 24 h, 48 h, and 72 h. At each time interval, the samples were centrifuged and magnetic nanoparticles were isolated from the supernatant. Subsequently, the unbound BSA in the supernatant was determined by Lowry method. The amount of BSA protein was quantified using UV-VIS spectrometer (Perkin Elmer) at 562 nm and the BSA concentration was quantified through predetermined standard concentration.

5.2.5 Statistical analysis

Data were reported as mean ± SD determined by at least three independent experiments. The statistical analysis of samples was performed using SigmaPlot 11.0 by one-way analysis of variance (ANOVA) using Student-Newman-Keuls. The differences were considered to be statistically significant at * \(p < 0.05\) and ** \(p < 0.01\).
6. RESULTS AND DISCUSSION

6.1 Preparation of magnetic nanoparticles (MNP and MNPG)

Magnetic particles ranging are being used in a number of medical applications. The important properties of magnetic nanoparticles for medical applications are nontoxicity, biocompatibility, and high-level accumulation in the target tissue or organ. Due to their small size, nanoparticles can efficiently penetrate across barriers through small capillaries into individual cells allowing efficient drug accumulation at the target site. Therefore, the unwanted side effects of the therapeutic agent are reduced and the therapeutic efficacy is enhanced.

Magnetic nanoparticles can be synthesized by physical, chemical and biological methods: (i) physical methods, such as gas-phase deposition and electron beam lithography; (ii) wet chemical preparation methods, such as sol–gel synthesis, oxidation method, chemical coprecipitation, hydrothermal reactions, flow injection synthesis, electrochemical method, aerosol/vapor phase method, sonochemical decomposition reactions, supercritical fluid method, synthesis using nanoreactors and (iii) microbial methods (Table 1).
Table 1. Comparative methods for synthesis of magnetic nanoparticles

<table>
<thead>
<tr>
<th>Methods</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>physical methods</td>
<td></td>
<td></td>
</tr>
<tr>
<td>gas-phase deposition</td>
<td>easy to perform</td>
<td>difficult to control the particle size</td>
</tr>
<tr>
<td>electron beam lithography</td>
<td>well controlled inter-particle spacing</td>
<td>expensive and highly complex machines requiring</td>
</tr>
<tr>
<td>sol-gel synthesis</td>
<td>precisely controlled in size, aspect ratio, and internal structure</td>
<td>weak bonding, low wear-resistance, high permeability</td>
</tr>
<tr>
<td>oxidation method</td>
<td>uniform size and narrow size distribution</td>
<td>small-sized ferrite colloids</td>
</tr>
<tr>
<td>chemical coprecipitation</td>
<td>simple and efficient</td>
<td>not suitable for the preparation of high pure, accurate stoichiometric phase</td>
</tr>
<tr>
<td>hydrothermal reactions</td>
<td>easy to control particle size and shapes</td>
<td>high reaction temperature, high pressure</td>
</tr>
<tr>
<td>wet chemical preparation methods</td>
<td>good reproducibility and high mixing homogeneity together with a precise control of the process</td>
<td>need continuous or segmented mixing of reagents under a laminar flow regime in a capillary reactor</td>
</tr>
<tr>
<td>flow injection synthesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>electrochemical method</td>
<td>easy to control particle size</td>
<td>reproducibility</td>
</tr>
<tr>
<td>aerosol/vapor phase method</td>
<td>high yields</td>
<td>extremely high temperatures</td>
</tr>
<tr>
<td>sonochemical decomposition reactions</td>
<td>narrow particle size distribution</td>
<td>mechanism not still understood</td>
</tr>
<tr>
<td>supercritical fluid method</td>
<td>efficient control of the particle size, no organic solvents involved</td>
<td>critical pressure and temperature</td>
</tr>
<tr>
<td>synthesis using nanoreactors</td>
<td>the possibility to precisely control the NP size</td>
<td>complex condition</td>
</tr>
<tr>
<td>microbial methods</td>
<td>microbial incubation</td>
<td>high yield, good reproducibility, and good scalability, low cost</td>
</tr>
<tr>
<td></td>
<td></td>
<td>time-consuming</td>
</tr>
</tbody>
</table>

The magnetic nanoparticles were prepared by chemical coprecipitation technique which might be the simplest and most efficient way to obtain iron magnetic nanoparticles.

The magnetic nanoparticles were produced in an aqueous medium through the reaction:

$$Fe^{2+} + 2Fe^{3+} + 8OH^- \rightarrow Fe_3O_4 + 4H_2O$$

In the presence of oxygen in air, magnetic nanoparticles are very sensitive and they may undergo oxidation or phase transition. Therefore, the synthesis of magnetic nanoparticles was done under nitrogen atmosphere. Another difficulty in preparing magnetic nanoparticles by co-precipitation is the tendency to agglomeration of the particles. The surface modification is carried out to solve this problem. In strong acidic solutions, iron magnetic nanoparticles are unstable and undergo leaching limiting the reusability and the lifetime of such materials.
Various surfactants and polymers such as oleic acid, lauric acid, polyethylene glycol (PEG), polyvinylpyrrolidone (PVP), and polyvinyl alcohol (PVA) have been used as coating materials in aqueous suspension. In this study, gallic acid is employed for coating in aqueous medium. Gallic acid is an organic acid, also known as 3, 4, 5-trihydroxybenzoic acid (C$_6$H$_2$(OH)$_3$CO$_2$H) and the structure of gallic acid is shown in Figure 1. It is commonly used in the pharmaceutical industry. Several studies have showed that gallic acid has anti-cancer properties against leukemia, certain prostate, colon and lung cancer cells. It also has anti-viral and anti-fungal properties. As powerful antioxidant, it helps to prevent oxidative damage. Due to its ability to inhibit histamine release and the expression of pro-inflammatory cytokine, gallic acid also has therapeutic applications for inflammatory allergic diseases such as asthma and allergic rhinitis.

![Figure 1. Chemical structure of gallic acid](image)

In this study, the magnetic nanoparticles were prepared as naked magnetic nanoparticles (MNP) and modified magnetic nanoparticles with gallic acid (MNPG). Both MNP and MNPG have magnetic properties which can be observed from magnet separation (Figure 2). The samples are immediately attracted to an external magnet within 10 seconds.

![Figure 2. The separation of magnetic nanoparticles using permanent magnet.](image)
After purification and drying, the MNP and MNPG were black powder as shown in Figure 3. There was no difference visually detected between the magnetic nanoparticles prepared with and without gallic acid. However, the particle size, physical and chemical characteristics of the materials were then analyzed by modern instrumental techniques.

Figure 3. Characteristics of MNP (a) and MNPG (b)

6.2 Characterization of magnetic nanoparticles (MNP and MNPG)

6.2.1 Characterization of magnetic nanoparticles by SEM

The further analysis of particle size and morphology were measured by SEM technique. SEM images showed the average size of the prepared magnetic nanoparticles ranging from 10 nm to 80 nm (Figure 4). The MNP and MNPG were generally well-structured, spherical in shape and uniform size. It was observed that the particle size of magnetic particles in this study were slightly larger than previous works reported in the synthesis of magnetic nanoparticles by coprecipitation method, which was found to be 5-10 nm.24
6.2.2 Characterization of magnetic nanoparticles by particle insight

Besides, the particle shape analyzer created by particle insight 2.55 revealed the equivalent circular area (ECA) diameter of MNP and MNPG which was less than 60 nm (Figure 5). There was no significant difference in a statistic analysis of the average particle size detected by particle insight and that of observed in SEM ($p > 0.05$). It was found that the diameter size of MNP was slightly smaller than that of MNPG as a result of gallic acid coating. However, the diameter size of nanoparticles for drug delivery applications should be more than 100 nm and less than 1000 nm. Therefore, the obtained MNP and MNPG could be suitable for a number of medical and biopharmaceutical applications.

Figure 5. Particle size analysis of (a) MNP and (b) MNPG.
6.2.3 Characterization of magnetic nanoparticles by SEM-EDX

According to further investigate the distribution of iron particles, the elemental analysis for carbon, oxygen and iron was measured by Scanning Electron Microscopy (SEM) with Energy Dispersive X-Ray Analysis (EDX) known as SEM-EDX mapping. As shown in Figure 6, the EDX mapping of iron indicated the homogeneous distribution of iron particles in MNP and MNPG (Fig. 6d and 6h). The EDX mapping for distribution of oxygen of MNP are similar to that of MNPG (Fig. 6c and 6g) while SEM-EDX images of carbon content demonstrated that the carbon content of MNPG was slightly increased compared to that of MNP (Fig 6b and 6f). From Table 2, the average iron content of MNP and MNPG were 19.5% and 18.2%, respectively. It can be seen that the average oxygen content was 32-42%. Furthermore, elemental compositions for the carbon content of MNP and MNPG was increased from 37.5% to 49.0%, respectively, when the MNP was modified with gallic acid (Fig. 6i and 6j). This confirmed that the iron oxide particles had been successfully coated with gallic acid under mild conditions and obtained the homogeneous MNPG.

Table 2. Comparison of elemental analysis for C, O and Fe in MNP and MNPG

<table>
<thead>
<tr>
<th>Sample</th>
<th>%C</th>
<th>%O</th>
<th>%Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>MNP</td>
<td>37.5</td>
<td>42.8</td>
<td>19.5</td>
</tr>
<tr>
<td>MNPG</td>
<td>49.0</td>
<td>32.6</td>
<td>18.2</td>
</tr>
</tbody>
</table>
Figure 6. SEM-EDX images of MNP and MNPG: (a) SEM image of MNP shows surface characteristic before modification used for EDX mapping, (b) C mapping of MNP composite from (a), (c) O mapping of MNP composite from (a), (d) Fe mapping of MNP composite from (a), (e) SEM image of MNPG used for EDX mapping, (f) C mapping of MNPG composite from (b), (g) O mapping of MNPG composite from (b), (h) Fe mapping of MNPG composite from (b), (i) EDX spectra of MNP for the corresponding images a-d, and (j) EDX spectra of MNPG for the corresponding images: e-h.
6.2.4 Characterization of magnetic nanoparticles by XRD

In order to identify the chemical compositions of iron oxide particles, the surface electrical properties were measured by energy-dispersive X-ray spectroscopy (XRD). In general, can exist in different chemical compositions, such as magnetite (Fe3O4) or maghemite (γ-Fe2O3) or hematite (α-Fe2O3), or combination of the two compositions. Magnetite and maghemite are the common iron oxide particles most frequently used for biomedical applications. As shown in Figure 7, the XRD results of MNP and MNPG exhibited the XRD pattern as characteristic peaks at 2 theta of 30.3°, 35.7°, 43.4°, 57.4° and 63.0°, corresponding to (220), (311), (400), (511), and (440) diffractions by the face-centered cubic structure of magnetite (Fe₃O₄, JCPDS No. 01-75-0449). This indicates that MNP and MNPG contained the magnetite (Fe₃O₄). It was consistent with the appearance of MNP and MNPG from SEM-EDX analyses, suggesting that the surface modification with gallic acid did not lead to phase change of magnetite (Fe₃O₄) as confirmed by the XRD patterns. For crystal structure, magnetite has a cubic inverse spinel structure with a face-centered cubic (fcc) unit cell (JCPDS No. 19-629) composed of 32 O anions, 16 Fe(III) cations and 8 Fe(II) cations. Half of the Fe(III) cations are tetrahedrally (tet) coordinated, while the other half and all of the Fe(II) ions are octahedrally (oct) coordinated. Magnetite can be subject to oxidation because of the reduced iron in the crystalline lattice. In the presence of oxygen, magnetite oxidizes to maghemite, thus Fe²⁺oct is oxidized to Fe³⁺oct, resulting in vacancies confined to the octahedral sites.

![Figure 7. XRD patterns of MNP and MNPG](image)
6.2.5 Characterization of magnetic nanoparticles by FT-IR

Fourier-transform infrared spectroscopy (FT-IR) spectra of MNP and MNPG are shown in Figure 8. The analysis indicated the strong absorption peak at 569 cm\(^{-1}\) for both sample MNP and MNPG corresponding to the Fe–O vibration related to the magnetite phase.\(^4\)\(^2\), \(^4\)\(^3\) Additionally, the absorption peak at 3758 cm\(^{-1}\) was attributed to the stretching vibrations of OH adsorbed on the surface of the iron oxide nanoparticles. Interestingly, the FTIR spectra of MNPG indicated the appearance of new bands at 1649 cm\(^{-1}\) corresponded to absorption band of the carboxyl groups. The intense absorption peak at 2345 cm\(^{-1}\) was due to the CH stretching vibrations and bending.\(^4\)\(^4\), \(^4\)\(^5\) This confirmed the binding of gallic acid molecules at the surface of magnetite.

![Figure 8. FTIR spectra of MNP and MNPG](image)

6.3 Protein adsorption study

To investigate the role of protein adsorption capability and the adsorption rate of gallic acid coated iron oxide nanoparticles, BSA, the most abundant protein in blood plasma, was used as a model protein. The amount of BSA uptake by unmodified MNP and modified MNPG were quantified through the predetermined standard concentration-intensity calibration curve.

The amount of BSA uptake in the presence of MNP or MNPG is illustrated in Figure 9. For MNP, BSA was rapidly adsorbed to the surface of MNP up to 70% within 30 min of incubation showing the initial fast uptake, then about 30% of BSA was significantly desorbed up to 4 h of incubation and after followed the steady state. This indicated the relevant interaction triggered by plasma protein BSA to induce protein
The hydrophobicity of individual BSA molecules and nanoparticles enhanced their mutual interaction and adsorption behavior. Moreover, the electrostatic interaction between nanoparticles with carboxylate anion and ammonium cation of BSA could also play an important role in surface adsorption phenomena. Compared with MNP, the MNPG showed the lower BSA adsorption within 4 h of exposure and then remained steady. The lower BSA adsorption capacity in first 4 h might result from the lower driving force for BSA molecules to functionalized MNPG. This confirmed the stabilizing effect between BSA and active sites on the surface of MNPG caused higher stericity due to surface modifying of MNP with gallic acid. After 4 h of incubation, the result indicated similar adsorption profile of BSA by MNP and MNPG suggesting that the higher concentration of gallic acid may require to increase the molar ratio of gallic acid and magnetic particles. However, protein adsorption on iron oxide nanoparticles depend not only on interactions between plasma proteins and nanoparticles related initial surface coating, but also the concentration of plasma proteins in the suspension.

Figure 9. Uptake of BSA by MNP and MNPG with incubation time
Because the cell uptake was correlated with amount of proteins bound in the nanoparticle corona, it is important to understand the protein adsorption phenomena for different nanoparticles including isolated nanoparticles, protein-induced clustering of nanoparticles, and surface functionalized nanoparticles, as all could result in different cell response. These findings indicated that BSA uptake by MNPG was relatively low as compared to unmodified MNP up to 4 h. Therefore, coating of MNP with gallic acid could prevent both adsorption of plasma protein BSA and rapid capture by macrophages. As a consequence, gallic acid-coated MNP could circulate in bloodstream for a longer time than naked MNP before promoting phagocytosis and removal from the blood circulation by mononuclear phagocytic system of liver and spleen.
The MNP and MNPG have been synthesized by one-step coprecipitation method. The results obtained from various techniques revealed that the average size of both iron oxide particles was about 10–60 nm with uniform size, spherical morphology and magnetic properties. The XRD results confirmed the chemical composition of MNP and MNPG having the magnetite (Fe₃O₄) which was suitable for biomedical applications. The resulting gallic acid-coated MNP was analyzed by FTIR to observe the binding of gallic acid. Compared with naked MNP, the gallic acid-coated MNP showed a lower BSA adsorption up to 4 h of incubation. The change in adsorption behavior of BSA by coating with gallic acid could make modified MNPG possible to possess different requirements for improving its biodistribution and diagnostic efficiency.

8. REFERENCES


27. Jeong, C. J.; Sharker, S. M.; In, I.; Park, S. Y., Iron Oxide@PEDOT-Based recyclable Photothermal Nanoparticles with Poly(vinylpyrrolidone) SulfoBetaines for Rapid and Effective Antibacterial Activity. *ACS Appl Mater Interfaces* 2015, 7 (18), 9469-78.
38. Fei, X.; Je, I. G.; Shin, T. Y.; Kim, S. H.; Seo, S. Y., Synthesis of Gallic Acid Analogs as Histamine and Pro-Inflammatory Cytokine Inhibitors for


