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Adsorption of hemoglobin, fatty acid and glucose to iron nanoparticles as a mean for drug delivery

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Abstract

Recent research in biomedicine has documented the application of iron-based nano-particles in supplying the human body with essential nutrients or in drug delivery systems.

In this work, the possibility of using iron nanoparticles sorption of biomaterial, such as hemoglobin, cholesterol, triglyceride, serum albumin, and glucose, to cure some specific syndromes have been studies. Bradford assay was used for protein adsorption measurement in supernatant, and FTIR was used for studying the ligands attached to iron nanoparticle. Cholesterol kit and Triglyceride kit were used to measure the cholesterol and triglyceride in supernatant. The result showed that glucose was adsorbed, and the remaining polysaccharide left in supernatant was 42%. Also sorption of triglyceride, cholesterol and serum albumin by nanoparticles was 69%, 73.3% and 87% respectively. FTIR showed hemoglobin being adsorbed to nanoparticles as a ligand. These results indicate that iron nano particles have the capability of being used as a delivery system for biomaterials.

Key words: FTIR, Hemoglobin and biomaterials adsorption, Iron nanoparticles, Bradford assay.

Introduction

The rapid development of nanotechnology promises to have great advantages for biological applications (Oberdorster et al. 2007). Nanomedicine is a rapidly growing field that uses nanoparticles to ease the analysis and treatment of diseases. Notable early successes in the clinic comprise the use of super paramagnetic nanoparticles as a differentiating agent in MRI and nanoparticle-based treatment systems (Desai et al. 2006; Weissleder et al. 1995).

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Nanomaterials are engineered structures with at least one dimension of 100 nanometers or less (Nel et al. 2006). Various types of metals and metal oxide nanoparticles have been used based on their antimicrobial activities. Silver nanoparticles are among those that are used to decrease infections and to synthesize the polymer/nanoparticle composites with effective antibacterial activity (Furno et al. 2004; Sambhy et al. 2006; Bayston et al. 2010).

Iron and iron oxide are two alternative nanoparticles which have been generally used in biomedical research because of its biocompatibility and magnetic properties (Gupta and Gupta 2005; Berry and Curtis 2003). Researchers have established that the use of magnetic nanopartiles leads to an optimal drug delivery system which can be developed by using an external magnetic field to direct such nanoparticles to the desired sites for treatment (Pareta et al. 2008; Petri-Fink et al. 2005).

The outstanding properties of nanaoparticles such as fast diffusion, high specific surface areas, enhanced reactivity in liquid or gas phase, and a size near to bio-macromolecules, may cause some problems in case of an uncontrolled exposure to the environment (Thill et al. 2006). However, biocompatibility of some nonoparticles such as iron or iron oxide nanoparticles may be of great help in exploiting them for specific purposes such as drug delivery.

In this work we report the results of our investigation on the adsorption of biomaterials to the iron nanoparticles and also the toxicity of these particles to the blood cells and bacteria.

Materials and methods

Characterization of iron oxide nanoparticles

Iron nanoparticles were obtained from German PLASMA CHEM Company and analyzed by X-ray diffraction (XRD), Fourier transform infrared (FT-IR) spectroscopy, scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) spectrometry.

X-ray diffraction (XRD) pattern of dry sediments was determined by Bruker (D8ADVANCE, Germany) X-ray diffractometer (Cu Kα

radiation, λ = 1.5406 Å, V= 40 v) using a continuous scan mode, and 2θ data were collected from 20 to 80.

FT-IR spectra for bacterial MIONs were recorded in the transmission mode on a FT-IR Spectrophotometer (JASCO PLUS-680, Japan) as KBr pellets in 4000 to 400 cm^{-1} range. To study the morphology and chemistry of iron oxide nanoparticles, the SEM and EDX was performed using the AIS2100 SEM-EDS instrument (Seron Company, Korea). The samples were prepared by gold sputter-coating to improve the conductivity and were observed in 20 kV.

Toxicity effect of iron ion and nanoparticles on bacteria and blood cells

Gram negative and positive bacteria were cultured on Nutrient Agar as lawn. Then ferric ion and nanoparticles adjusted on the well of Nutrient Agar and the zones of inhibition were studied. The number of red and white blood cells and hemoglobin content of treated samples with iron nanoparticles was also evaluated by Sysmex instrument.

Assay of hemoglobin adsorption

For preparation of hemoglobin solution, fresh venous blood samples were collected in EDTA vacutainerm tubes (Becton Dickinson, Rutherford, NJ) and RBCs were separated from plasma by centrifugation at 2,000 g at 5 °C for 10 min. Then the RBCs were washed three times with 5 mL of 0.9% normal saline. Each washing step contained the addition of normal saline, slow dispersion of RBCs by mixing with a plastic pipette, and centrifugation at 650 g at 10 °C for 10 min. The supernatant was then aspirated and discarded. The final centrifugation was carried out at 1500 g at 10 °C for 10 min to pack the RBC sample more tightly and to minimize the volume of saline. In order to lyses RBCs they were suspended in de-ionized distilled water (pH 7.4) and incubated for 24 h. The cellular debris was then removed by centrifugation at 1500 g for 20 min.

To assay the adsorption of hemoglobin, 1000 µL of 1% hemoglobin was used with 10 µL of 0.1% nanoparticles in micro sample tubes. The micro tubes were then placed on top of the ND magnet to separate them from solution. The hemoglobin in supernatant was assayed for hem adsorption by nanoparticles.

Assay of cholesterol and triglyceride adsorption

For assessing the cholesterol and triglyceride adsorption by nanoparticles the cholesterol and triglyceride kits (with enzymatic method of biology-chemistry Company) were used. The cholesterol kit functions on the basis of the hydrolysis of cholesterol esters and release of hydrogen peroxide. The function of the triglyceride kit is also based on the release of hydrogen peroxide resulting from the hydrolysis of triglyceride followed by a catalytic reaction. The hydrogen peroxide is measured by the red color produced in the Trinder reaction, the reaction between hydrogen peroxide, 4-aminoantipyrine and phenol, which yields the red quinoneimine using UV-vis spectrophotometer (Deyhimi et al. 2006). The intensity of the color measured in the 325-576 and 495-530 nm regions is proportional to the density of the cholesterol and triglyceride in the sample respectively.

Human serum was used to assess the extent of adsorption of cholesterol and triglyceride by the iron nanoparticles. 10 µL of 0.1% nanoparticles was transferred into a micro sample tube and vortexed in 1 mL of human serum and the sample tube was placed on top of the ND magnet for a few days. The aforementioned spectroscopic method was used to determine the extent of cholesterol adsorption. A similar experiment was carried out for triglyceride measurement; however, the sample, standard, and blank agent (control) of triglyceride were kept in a single 37 °C water bath for 10 min prior to the adsorption reading.

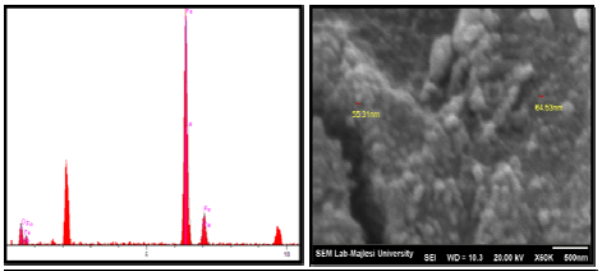
Assay of glucose adsorption

To assay the glucose adsorption Anthrone agent was used. 0.4 g of Anthrone powder was dissolved in 100 mL of 98% sulfuric acid. The resulting solution was used as a blank (control) and the intensity of green color was measured at 600 nm.
10 µL of 0.1% iron nanoparticle was vortexed with 1 mL of 1% glucose solution and the mixture was kept in a micro tube on the top of the ND magnet for a few days. 1 mL of the aforementioned Anthrone solution was then added and the adsorption of the mixture was assessed by using UV-vis spectroscopy (Morris 1948).

Result

Identification of iron oxide nanoparticles

Iron oxide nanoparticles were identified by different analytical methods. The crystallographic analysis by XRD gave the pattern with peaks corresponding to iron oxide nanoparticles. The size of the nanoparticles, determined by SEM and EDX analysis, was less than 55 nm (Fig. 1). These results were also confirmed by the vibrational bands in 550-650 cm^{-1} region, assigned to Fe–O stretching, in the FT-IR spectrum of the nanoparticles (*vide infra*, Fig. 5).

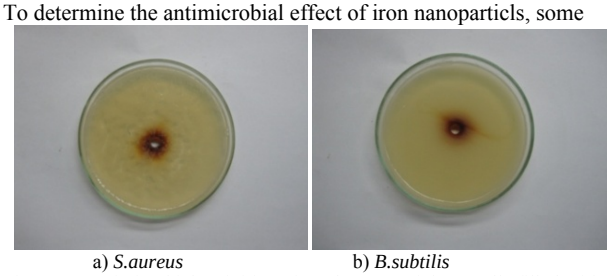


	Units	Conc	Error sig-2	Intensity (c/s)	Line	Element
	%.wt	13.211	3.529	31.15	Ka	O
	%.wt	86.789	15.129	572.36	Ka	Fe
Total	%.wt	100.000				

Figure 1: SEM and EDX obtained for iron oxide nanoparticles.

Effects of iron nanoparticles on bacteria

To determine the antimicrobial effect of iron nanoparticls, some

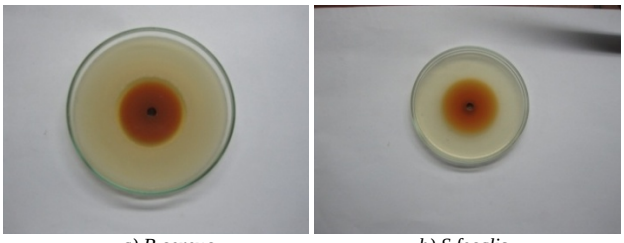


a) *S.aureus* b) *B.subtilis*

Figure 2: Antimicrobial activities of used nanoparticles. Wells filled with 20 µL of 10% iron nanoparticle, showing no antimicrobial activity against a) *S.aureus* and b) *B.subtilis*

species of bacteria were grown on the Nutrient Agar plates with wells in the middle of the media. After a 24 h incubation period, bacteria grew all around the plates with no zone of growth inhibition around the wells, indicating that the iron nanoparticles have no antimicrobial activity against *S.aureus* and *B.subtilis* growth (Fig. 2).

In order to compare the toxicity of iron nanoparticles (containing Fe^{3+} ions in oxide form) with free Fe^{3+} ions a similar experiment was carried out using 0.9% $FeCl_3$ aqueous solution. The observation of zone of growth inhibition of 4.9 cm for *B.cereus* and 5.3 cm for *S.fecalis* indicated that contrary to the iron oxide nanoparticles, the free Fe^{3+} ions have antimicrobial activity against bacteria (Fig. 3).



a) B.cereuc *b) S.fecalis*

Figure 3: Toxicity of free Fe^{3+} ions, showing antimicrobial activity against *a) B.cereus* and b) *S.fecalis*

Effect of iron oxide nanoparticles on blood cells

To investigate the effect of iron oxide nanoparticles on blood cells, blood samples from healthy donors and individuals with minor thalassemia phenotype were obtained. RBCs were extracted and treated with aliquots of aqueous magnetic fluids with final concentrations of 0, 25, 50, 100, 200, .400, 500 and 1000 µg/mL and then incubated at 37 °C for 3 h. The number of red and white

Table 1. The effects of iron nanoparticles on blood cells

	Iron nanoparticle's concentration	WBC	RBC	HGB
Healthy	control	6.9×10^3	5.28×10^3	18.4
	25 µg/mL	6.9×10^3	5.34×10^3	18.2
	200 µg/mL	6.6×10^3	5.26×10^3	18
	500 µg/mL	6.3×10^3	5×10^3	17.2
Minor thalassemia	Control	8.1×10^3	6.71×10^3	15.3
	25 µg/mL	8.1×10^3	6.4×10^6	15.2
	200 µg/mL	7.9×10^3	6.4×10^6	14.6
	500 µg/mL	7.3×10^3	6×10^6	13.6
RBC	Control	-	2.5×10^6	8
	25 µg/mL	-	2.5×10^6	7.9
	200 µg/mL	-	-	-
	500 µg/ mL	-	-	-

blood cells and hemoglobin contents of the treated samples was evaluated by Sysmex instrument. No change was observed in WBC, RBC, and HGB of healthy blood samples, however, meaningful reduction of HGB was observed in minor thalassemia samples (Table 1).

Adsorption of biomaterial to iron nanoparticles

The adsorption of glucose, cholesterol, triglyceride, serum albumin and hemoglobin to iron nanoparticles were examined by UV-vis and FT-IR spectroscopy. The results showed that glucose, left in supernatant was 42%. Adsorption of triglyceride, cholesterol and serum albumin by nanoparticled was 69%, 73.3% and 87% respectively (Table 2).

Table 2. Adsorption of biomaterial to iron nanoparticles.

	Standard	Sample+Nanoparticle	% of adsorption
Glucose[(a)]	0.062	0.026	58%
Serum albumin	0.322	0.042	87%
Human serum[(b)]	0.628	0.168	73.3%
Human serum[(c)]	0.371	0.115	69%

a) Antron agent was used to assess glucose, b) Cholesterol kit was used to assess cholesterol, and c) Triglyceride kit was used to assess triglyceride.

The ability of iron nanoparticles to adsorb hemoglobin was investigated by comparing the vibrational spectrum of pure nanoparticles with the spectrum of nanoparticles that have adsorbed hemoglobin from the sample solution. Hemoglobin adsorption by nanoparticles is evidenced from the vibrational bands at 3292, 1649, 1528, and 1440 cm^{-1}, which are shifted relative to the corresponding bands (1663, 1544, 1451 cm^{-1}) in the FT-IR spectrum of pure hemoglobin (Wood et al. 2001; Wood and McNaughton 2002) (Figs. 4 and 5).

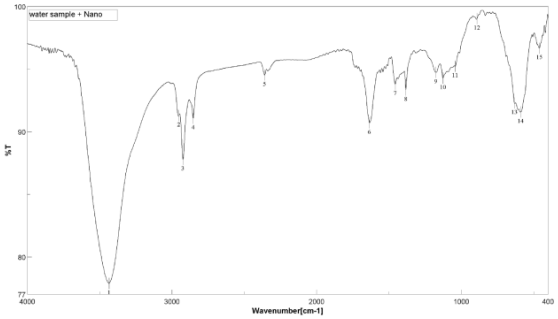


Figure 4: FT-IR spectrum of pure nanopartciles

Discussion

Due to the low toxicity and stable magnetic properties of iron oxide nanoparticles, these particles have been extensively investigated for their possible applications in bio-separation, bio-sensing, drug delivery, magnetic fluid hyperthermia, and magnetic resonance imaging (MRI) contrast enhancement (Xie et al. 2006; Xu and Sun 2007; McNeil 2005). These magnetic nanoparticles also can be used to increase the tissue contrast in MRI, to advance the efficiency in anticancer drug delivery and to remove tumor cells by magnetic fluid hyperthermia (Xu et al. 2008).Also the tissue repair, immunoassay, detoxification of biological fluids, and hyperthermia using iron nanoparticles have been reported (Pankhurst et al. 2003; Gupta and Gupta 2005).

In this paper we present the results of our investigation on the capability of non-toxic iron nanoparticles as a delivery system for hemoglobin and other bio-materials such as fatty acids and carbohydrate. However In an alternative report , Nhiem Tran *et al.* have demonstrated that the ratio of live/dead bacteria is significantly

lower in the solution with a high dose (3 mg/ml) of iron oxide nanoparticles compared with the control sample as well as the

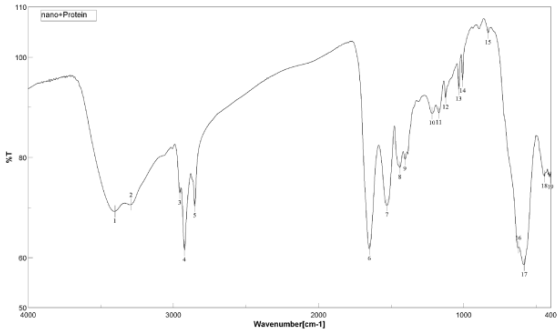


Figure 5: FT-IR spectrum of iron nanoparticles with adsorbed hemoglobin.

samples containing low and medium doses of iron oxide nanoparticles (Tran et al. 2010).

We also demonstrate the toxicity of iron free ions against various species of bacteria and the result indicated that although the iron ion is toxic to Gram positive and negative bacteria, the iron oxide nanoparticles were not toxic however, this has been demonstrated by C.W.K. Chow *et al.* that *Cyanobacteria* grows better in the presence of free Fe^{3+} ions (Chow et al. 1998).

Conclusion

In this research we have established that the iron oxide magnetic nanopartiles are not toxic and carry biomaterials. Therefore they can be used as a promising optimal drug delivery system. Also nanoscale iron particles provide a new alternative for environmental remediation technologies which can response in cleaning up the environment.

Acknowledgement

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