Functionalization of Magnetite (Fe₃O₄) Nanoparticles for Cancer Treatment

A. Herrera, H. Rodríguez, M. Torres-Lugo, and C. Rinaldi

Chemical Engineering Department, University of Puerto Rico-Mayagüez Campus, crinaldi@uprm.edu

ABSTRACT

We have studied the synthesis of magnetite (Fe₃O₄) nanoparticles functionalized with crosslinked dextran for the application of magnetic fluid hyperthermia. The CaCo-2 cell model was employed as the biological system. X-Ray diffraction was used to characterize the structure of the synthesized nanoparticles, showing a crystallite size of 9.0 nm. FTIR spectroscopy indicated the presence of dextran in the magnetite samples. SQUID magnetometry was employed to measure the DC magnetization response of the samples, demonstrating superparamagnetic behavior at room temperature, with a saturation magnetization of about 50 A/m, and a magnetic core diameter of about 7.0 nm. TEM measurements confirmed the average size of approximately 9.0 nm. Viability and apoptosis experiments in CaCo-2 cells in contact with 0.15 mg/ml of nanoparticles were determined at different contact times. No cytotoxic effects were observed. A decrease in cell viability of about 60 % was found upon the application of an AC magnetic field of 3.0 kA/m and 1.0 kHz for about 45 min.

Keywords: magnetite, dextran, magnetocytolysis, saturation magnetization, cytotoxicity

1 INTRODUCTION

Magnetic fluid hyperthermia is a form of cancer therapy, in which magnetic nanoparticles are internalized in a cancer tumor and, upon the application of an oscillating magnetic field, a localized temperature increase is obtained inducing tumor remission [1, 2]. In this treatment it is believed that an oscillating magnetic field induces rotational motion of the magnetic nanoparticles, which causes energy dissipation [3]. This phenomenon has not been amply analyzed. We have studied the synthesis of magnetite (Fe₃O₄) nanoparticles functionalized with crosslinked dextran for magnetic fluid hyperthermia using human colon cancer cells (CaCo-2) as a biological system.

2 METHODOLOGY

2.1 Materials

Iron (II) chloride tetrahydrate (FeCl₂, 4H₂O) 99%, iron (III) chloride hexahydrate (FeCl₃, 6H₂O) 97%, 9.26 kDa dextran, 10 kDa Fluorescein Isothiocyanate (FITC) dextran, ammonium hydroxide (NH₄OH) 29% v/v, and epichlorohydrin 99% were purchased from Sigma Aldrich. Deionized and degasified water was used in all preparations.

2.2 Synthesis of magnetite nanoparticles coated with dextran

Magnetite nanoparticles were synthesized through co-precipitation of aqueous solutions of ferric and ferrous chloride ions in the presence of 9.26 kDa dextran [4], used to improve dispersion of the nanoparticles in cell culture media. An aqueous solution of ferric chloride hexahydrate (0.36 M) was mixed with an aqueous solution of 9.26 kDa dextran (0.123 M), and cooled to 5 °C [5]. This mixture was vigorously stirred with a solution of ferrous chloride (0.18 M) previously cooled to 5 °C, in the presence of a nitrogen stream, in order to avoid oxidation of the iron ions [6]. Ammonium hydroxide at 5 °C was added to the iron-dextran solution. The product of this reaction quickly changed color from brown to black, which is indicative of magnetite formation. This solution was heated to 75 °C for about one hour under continuous stirring at pH 10. After cooling to room temperature, the solution was sonicated for 20 min and submitted to magnetic decantation overnight.

2.3 Crosslinking procedure

Crosslinking with epichlorohydrin (ECH) [7] was performed afterwards to avoid desorption of the dextran molecules from the nanoparticle surface. The reaction between ECH and dextran is carried out in the presence of NaOH, which allows the opening of the epoxy groups of the ECH molecules, forming free chlorohydrin fragments. Afterwards, a dehydrochlorination reaction occurs between two linear macromolecules of dextran and the free chlorohydrin fragments, forming a crosslinked structure. This crosslinked solution was dialyzed with a membrane with12,000 kDa cut off, using distilled water in order to remove uncrosslinked ECH monomers. Distilled water was changed every two hours, until conductivity < 0.1 μS/cm was obtained. Afterwards, the crosslinked solution was dried in a vacuum oven at 40 °C [7]. Magnetite nanoparticles functionalized with crosslinked dextran were suspended in Hank’s Balanced Salt Solution (HBSS) at pH 7.0, and submitted to a sterile filtration procedure for subsequent biological assays with the Caco-2 cell line.
2.4 Characterization

Fourier Transform Infrared (FTIR) spectra were acquired using a Digilab Variant FTS 1000 FTIR-ATR. X-Ray diffraction was used to determine ferrite structure using a Siemens B500 DACO-MP Diffractometer. A Quantum Design MPMS X17 SQUID magnetometer was used to measure the DC magnetization response of the nanoparticles. Sizes of the magnetite nanoparticles were determined using a ZEISS 922 Transmission Electron Microscope (TEM).

3 RESULTS AND DISCUSSION

3.1 X-ray diffraction

X-ray diffraction was used to determine the crystalline structure and crystallite size of the synthesized magnetic nanoparticles. Scherrer’s equation was used to estimate the size of the crystals [8]. Figure 1 shows the diffraction patterns obtained for magnetite nanoparticles coated with dextran, in which is observed peaks indicative a ferrite structure. An average crystal size of about 9.0 nm was determined using equation (3.1).

![Figure 1: X-ray diffraction of magnetite/ dextran nanoparticles, with an average diameter of 9.0 nm as determined by Scheerrer’s equation. Squares refer to magnetite standard diffraction.](image)

3.2 Fourier Transform Infrared (FTIR)

Fourier transform infrared (FTIR) spectra were acquired using a Digilab FTS 1000 FTIR-ATR, with a wavenumber range from 400 to 4000 cm\(^{-1}\). Figure 2 shows the FTIR spectra of the characteristic vibrational state of magnetite synthesized by the co-precipitation method, of magnetite coated with dextran, and of 9.26 kDa dextran.

In Figure 2 it is observed that the sample functionalized with dextran exhibits a vibrational mode at 580 cm\(^{-1}\) which is related to the Fe-O bond [9] present in magnetite. Moreover, this spectrum also presents vibrational modes characteristic of the organic structure of dextran, such as the \(\alpha\)-glucopyranose ring deformation modes at 770, and 845 - 915 cm\(^{-1}\) [10]; the presence of these vibrational modes is an important indication of surface functionalization.

Vibrational bands at 1600 and 3400 cm\(^{-1}\) are representative of adsorbed water in the samples [11]. Vibrational modes ranging from 1250 – 1460 cm\(^{-1}\) and 2900 cm\(^{-1}\) are distinctive of C-H bonds [10, 11], and were observed in the dextran spectrum, and the spectrum of magnetite nanoparticles functionalized with dextran. The vibrational bands for the C-O bond, ranging from 1040 to 1150 cm\(^{-1}\) [11], were also observed.

![Figure 2: FTIR spectra obtained for magnetite, 9.26 kDa dextran, and magnetite coated with dextran.](image)

3.3 Magnetic measurements

We measured the magnetization of liquid samples of magnetite nanoparticles coated with dextran and crosslinked dextran in the presence of a magnetic field, using a Quantum Design MPMS X17 SQUID magnetometer at 300 K. Due to the nature of our samples, which are dilute suspensions of nanoparticles in a buffer solution, we performed background subtraction of the diamagnetic signal of the sample holder filled with 0.15 ml of deionized water.

The diamagnetic signal of the sample holder is represented through a linear equation with intercept at the origin and negative slope. This negative slope is calculated using the magnetization response of the samples at high magnetic field. Figure 3 summarizes the results obtained from the measurement of magnetite nanoparticles coated with crosslinked dextran, and suspended in HBSS solution at pH 7.4, in which the saturation magnetization \(M_s\) is observed at about 50 A/m.

![Figure 3: Magnetic measurements results.](image)
At low fields, a linear relation was obtained between the magnetization of the liquid samples and the applied field, as described by the low field limit of the Langevin function [3]

$$X_i = \left[ \frac{dM}{dH} \right]_{H \to 0} = \frac{\pi \mu_0 M_d^3 \phi}{18kT}$$  \hspace{1cm} (3.2)

where $X_i$ is the initial susceptibility of the samples, $M$ is the magnetization, $H$ is the applied magnetic field, $k$ is Boltzmann’s constant ($1.38 \times 10^{-23}$ J/K), $T$ is the temperature of the system, $\mu_0$ is the permeability of free space ($4\pi \times 10^{-7}$ H/m), $M_d$ is the domain magnetization of magnetite (446 kAm$^{-1}$), $d$ the size of the magnetic core of the nanoparticles, and $\phi$ is the magnetic fraction, which can be expressed as a relationship between the saturation magnetization $M_s$ and the domain magnetization.

$$\phi = \frac{M_s}{M_d}$$  \hspace{1cm} (3.3)

Using equation (3.2) and the saturation magnetization obtained from the calculation of the magnetization of the liquid samples, it was possible to determine the size of the magnetic core of the nanoparticles, and their magnetic fractions. Table 1 summarizes these results.

### 3.4 Transmission Electron Microscopy (TEM)

We used a ZEISS 922 TEM to determine the size of the synthesized magnetite/dextran nanoparticles. A drop of liquid sample was deposited on a copper grid with a carbon support film (3 nm thick), and dried at room temperature. Some preliminary results of these measurements are shown in Figure 4, from which we observe an average size of about 9.0 nm for magnetite/dextran nanoparticles.

### 3.5 Cytotoxicity analyses performed on CaCo-2 cell culture

The viability effects and possible apoptosis induction in CaCo-2 cells due to the presence of magnetite nanoparticles coated with crosslinked dextran and suspended in HBSS buffer solution at pH 7.0 were determined at contact times of 2, 24, and 48 h. These experiments were performed using a fluorescent assay (Cell Titer Blue and Apo-ONE Promega). No cytotoxic effects were observed from these measurements, as shown in Figure 5.
Table 1: SQUID measurements of magnetic core diameter and volume fractions of magnetite nanoparticles functionalized with 9.26 kDa dextran and crosslinked dextran. Magnetite nanoparticles were suspended in HBSS solution at neutral pH for biological assays using the Caco-2 cell lines.

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>Ms (A/m)</th>
<th>φ</th>
<th>χ₀</th>
<th>d (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnetite / dextran in HBSS</td>
<td>7.2</td>
<td>55.35</td>
<td>1.2×10⁻⁴</td>
<td>2×10⁻³</td>
<td>7.0</td>
</tr>
<tr>
<td>Magnetite / dextran crosslinked in HBSS</td>
<td>7.4</td>
<td>50.0</td>
<td>1.1×10⁻⁴</td>
<td>7×10⁻⁴</td>
<td>7.0</td>
</tr>
</tbody>
</table>

4. SUMMARY

We have synthesized magnetic nanoparticles coated with 9.26 kDa dextran. X-ray diffraction indicated the presence of a phase with crystalline structure consistent with that of a ferrite, with a crystal size of about 9.0 nm. FTIR confirmed the functionalization of magnetite nanoparticles with dextran, showing characteristic vibrational modes of these polysaccharide molecules in samples of magnetite synthesized in the presence of dextran; such as vibration bands of α-glucopyranose ring deformation at 770, and 845 - 915 cm⁻¹.

We have determined, by SQUID measurements, the size of the magnetic core of the magnetite nanoparticles coated with dextran and crosslinked dextran at about 7.0 nm, using the Langevin function at low fields. These measurements were performed on liquid samples of functionalized nanoparticles suspended in buffer solution of HBSS at pH 7.0 previously submitted to a sterile filtration.

Preliminary results of TEM measurements show an average size of about 9.0 nm for magnetite/dextran nanoparticles. Magnetite nanoparticles synthesized with dextran and crosslinked dextran were determined to be non cytotoxic from analyses performed in CaCo-2 cell culture media. Application of 1.0 kHz, 3.0 kA/m magnetic field resulted in a reduction in cell viability of 60 %, after 45 min of field application.

ACKNOWLEDGMENTS

This work was supported by the Puerto Rico NSF-EPSCoR (EPS-0223152) and the NIH MBRS. We acknowledge the Center for Nanoscale Materials, financed by NASA-NCC3-1034, for the use of the High Resolution Transmission Electron Microscope.

BIBLIOGRAPHY