A COMPARITIVE STUDY OF THE CYTOTOXICITY OF SPINEL FERRITES

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Abstract. Spinel ferrites have been extensively used for various biological applications including magnetic fluid hyperthermia, drug delivery and as contrast agents for magnetic resonance imaging (MRI). This paper investigates three of the most frequently used spinel ferrites, namely magnetite, cobalt ferrite and manganese ferrite. The study focuses on the comparison of the cytotoxic effects for three species. Structural analysis using X-ray diffraction is presented along with the cytotoxic effects on human fibroblast (WI38) cell line using the MTT essay.

Key words: spinel ferrites, coprecipitation, magnetic nanoparticles, cytotoxicity.

INTRODUCTION

Magnetic ferrofluids based on spinel ferrites are currently a subject of intense research. Ferrofluids are utilized in theranostics [2, 14, 21, 22 23]. Applications include, magnetic fluid hyperthermia [7, 18, 20], MRI contrast agents [26] and drug delivery [3].

Spinel ferrites represent a class of magnetic materials with the general formula MFe_2O_4, where M is a divalent cation [$Fe^{2+}, Mn^{2+}, Co^{2+}$, etc.]. Spinel ferrites exhibit a face centered cubic structure (FCC). Oxygen ions are large in diameter compared to the divalent and trivalent cations, they thus form a close packed structure [17, 19]. The interstitials or voids between the oxygen ions form octahedral and tetrahedral sites. The cations occupy either the tetrahedral or octahedral sites. This distribution and the magnetic moment per atom determine the magnetic properties of the particles. In normal spinel, the divalent cations are all located in the tetrahedral sites as is the case of zinc ferrite. On the other hand, if the tetrahedral sites are occupied by the trivalent cation and the octahedral sites are filled by both trivalent and divalent cations, an inverse spinel ferrite is produced. Other distributions of cations result in intermediate spinel ferrites.

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Magnetite has been extensively used in magnetic fluid hyperthermia, it is considered biocompatible, easy to prepare but unfortunately suffers problems regarding oxidation and formation of different phases [17]. On the other hand, cobalt ferrite is very easy to prepare and has a remarkable chemical stability. The cytotoxicity of the latter two ferrites is still under investigation. Manganese ferrite and cobalt ferrite are intermediate spinel ferrites whereas, magnetite is an inverse spinel ferrite.

The aim of this study is to prepare three samples of the MNPs with a particle size small enough to guarantee that the particles exhibit superparamagnetic behavior at room temperature. Therefore, the coprecipitation method is the selected synthesis root [1, 5, 6, 12]. The cytotoxicity of the particles is another determinant factor regarding the feasibility of utilizing the particles. Cytotoxic effect [4, 8, 11, 15, 16] of the coprecipitated nanoparticles is studied using MTT assay. The used cell line is normal human lung fibroblast (WI38). The lethal dose 50 % (LD50) is determined for each of the three used samples.

MATERIALS AND METHODS

MATERIALS

The used chemicals include ferric chloride hexahydrate, ferrous chloride, cobalt chloride hexahydrate, manganese (ii) chloride tetra hydrate, sodium hydroxide, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) reagent, DMSO (dimethyl sulfoxide) and RPMI culture medium. All used chemicals are analytical grade.

SYNTHESIS OF SAMPLES

The coprecipitation method generally requires the use of soluble salts of each of the divalent and trivalent cations with a ratio of 1:2. These salts form insoluble hydroxides, upon the addition of a strong base (sodium hydroxide in this work), the two hydroxides precipitate simultaneously, thus the name coprecipitation, with further heating, condensation reaction takes place and the metal oxide phase (spinel ferrite) is formed. The reaction proceeds as follows:

$$M(Cl)_2 + 2Fe(Cl)_3 + 8NaOH \rightarrow M(OH)_2 + 2Fe(OH)_3 + 8\,NaCl$$

$$\xrightarrow{90^oC,\ pH12.8} MFe_2O_4 + 8NaCl + 4H_2O \quad (1)$$

STRUTURAL ANALYSIS

The structure of the prepared samples was determined using X-ray powder diffraction (XRD). X-ray powder diffraction patterns of the samples are collected on a Philips diffractometer (X'pert MPD) with Cu-K_α radiation. The samples are scanned with 2θ in the range 10–80° with step size 0.02° and counting time of 2 seconds. The crystallite size is calculated using the Scherrer equation [9]:

$$D = \frac{K\lambda}{\beta \cos\theta} \quad (2)$$

where D is the mean size of the crystallite, λ is the incident wavelength of the X-ray source, θ is the diffraction angle, and β is the full width at half maximum. K is set to 0.829 for the cubic isotropic crystals.

CYTOTOXICITY ASSAY

Cells are seeded in 96 well plate (1×10^5 cells/mL) then incubated at 37 °C for 24 hours till the developing of a complete monolayer sheet. The growth medium is then disposed and two-fold dilutions of the sample are made in RPMI medium with 2 % serum. 0.1 mL of each dilution is tested in different wells leaving 3 wells as control. Plate is incubated and checked for any physical signs of toxicity. 20 µL MTT are added to each well and shaken (150 rpm for 5 minutes). To allow the metabolism of MTT, the mixture is then incubated at 37 °C and 5 % CO_2 for 1–5 hours followed by dumping of the medium. The metabolite; formazan is resuspended in 200 µL DMSO and shaken (150 rpm for 5 minutes). Finally, optical density at 560 nm is read; background at 620 nm is subtracted and correlated to viable cells. Six concentrations of every ferrite species are tested with 1:2 serial dilutions starting from 10000 µg/mL to 312.5 µg/mL.

RESULTS

This section discusses the characterization of the prepared samples followed by an assessment of the cytotoxicity.

STRUCTURAL ANALYSIS

Figure 1 shows the X-ray diffraction patterns of the three as-prepared samples. XRD peaks confirm the formation of ferrites from their precursors. All samples exist

in face centered cubic phase and no other phases are detected. The characteristic peaks are indexed as shown in Figure 1 [17]. According to Scherrer equation, it is found that the crystallite sizes are 10.2 nm, 11.1 nm and 10.5 nm for magnetite, cobalt ferrite and manganese ferrite respectively.

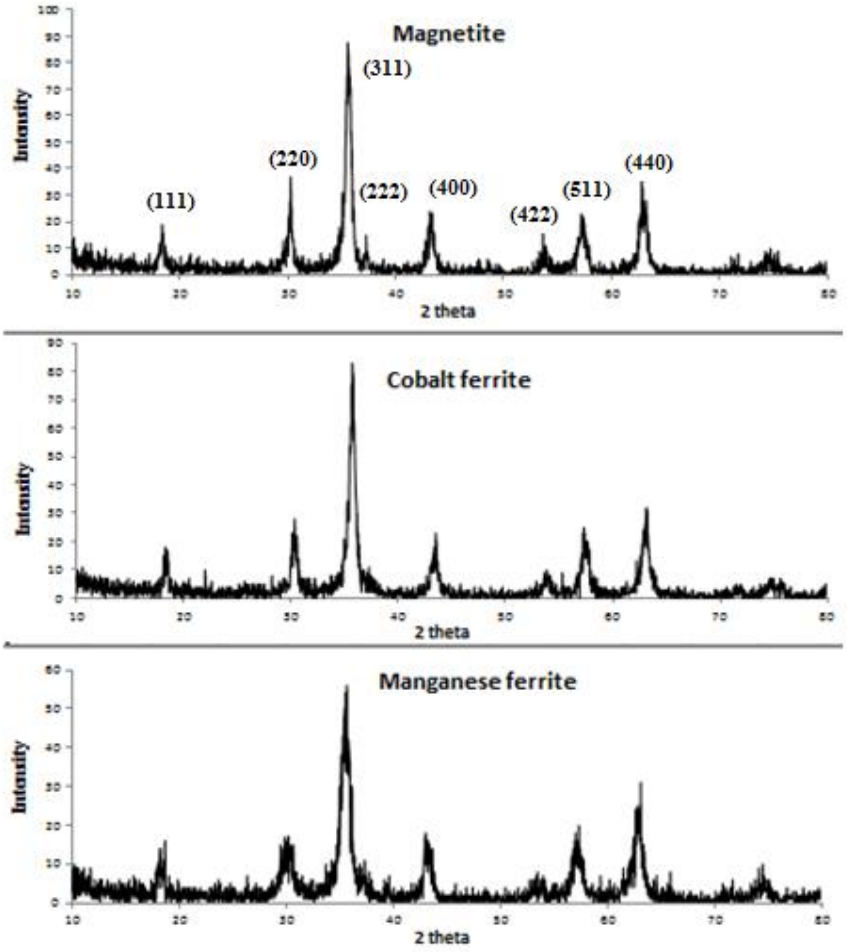

Fig. 1. XRD diffraction pattern for magnetite, cobalt ferrite and manganese ferrite.

CYTOTOXICITY ASSAY

Figure 2 shows photomicrographs for the WI38 cells exposed to two moderate concentrations of each of the ferrite species.

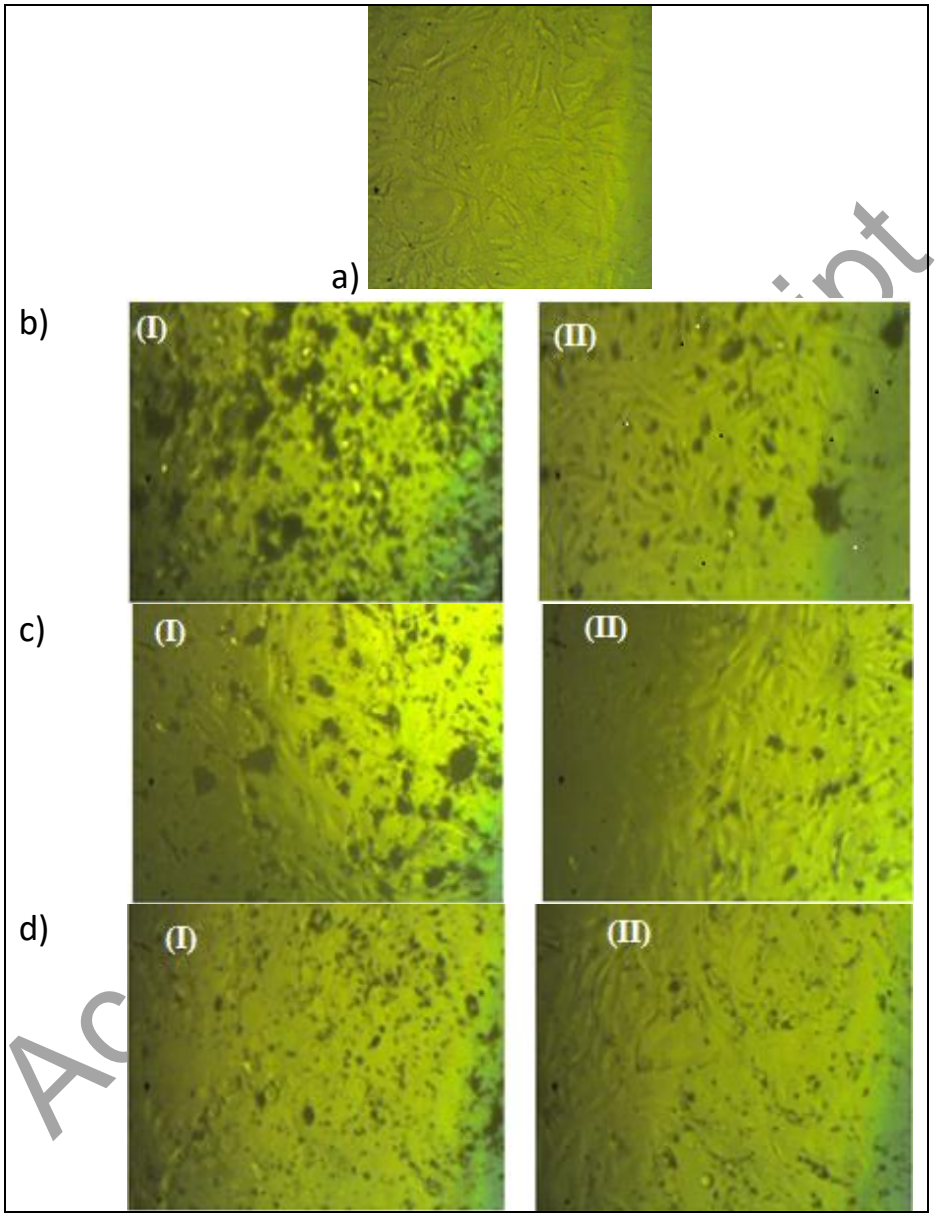

Fig. 2. Photomicrographs of normal WI38 cells (a), and WI38 cells with 5000 μg/mL (I) and 625 μg/mL (II) of magnetite (b), cobalt ferrite (c), and manganese ferrite (d).

Figure 3 shows an enlarged view for WI38 cells exposed to 5000 μg/mL. The figures show the aggregation of the ferrite particles in the biological medium as is expected for uncoated particles. The particles adhere to the cell membranes as depicted by the figures.

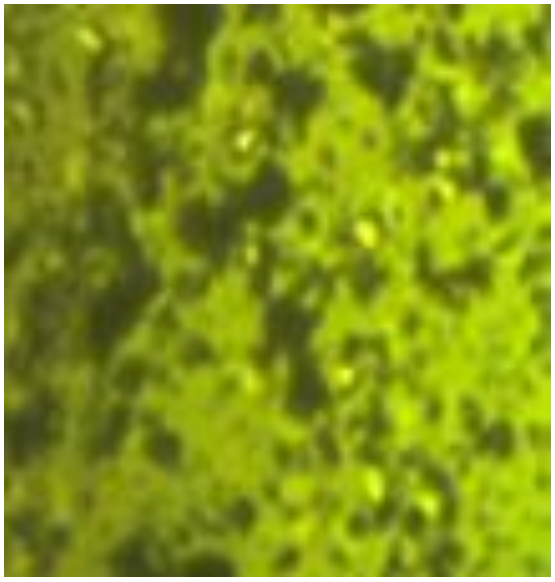

Fig. 3. Enlarged photomicrographs for WI38 cell exposed to 5000 μg/mL of magnetite.

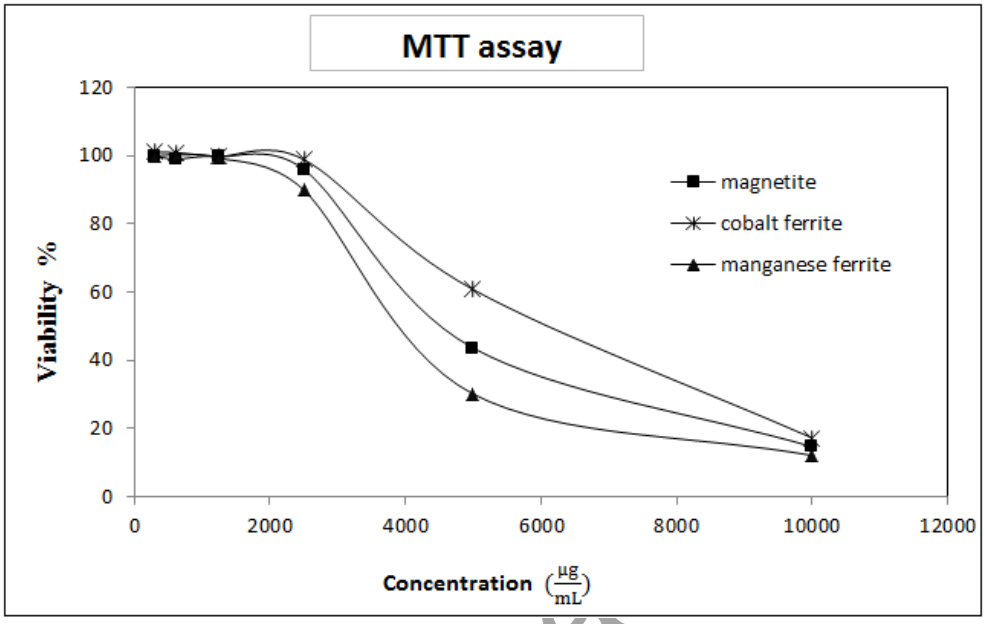

Fig. 4. Percentage of viable cells for different concentrations of the three ferrite samples.

The viability and toxicity data of WI38 cells as a function of the concentration of the three tested species of NPs is shown in Table 1. The viability in each case is graphically represented in Figure 4. The lethal 50 dose (LD50) is determined to be 5900 μg/mL in the case of magnetite nanoparticles, 6600 μg/mL with cobalt ferrites and 4100 μg/mL with manganese ferrite. The results reveal that cobalt ferrite from the biocompatibility perspective can be considered as a promising candidate for various biomedical applications.

Table 1

Viability and toxicity of WI38 cells with concentration of the three ferrite species

ID	Conc. (μg/mL)	Mean optical density	Viability (%)	Toxicity (%)	LD50 (μg/mL)
WI38	1:2	0.33	100	0	
Fe_3O_4	10000	0.04	14.58	85.41	
	5000	0.14	43.55	56.44	
	2500	0.32	96.03	3.96	5985.44
	1250	0.33	99.70	0.29	
	625	0.33	99.00	0.99	
	312.5	0.33	99.80	0.19	
	10000	0.05	17.16	82.83	
	5000	0.20	60.71	39.28	

$CoFe_2O_4$	2500	0.33	98.90	1.09	6603
	1250	0.33	99.80	0.19	
	625	0.33	100.89	0	
	312.5	0.33	100.99	0	
$Mn\ Fe_2O_4$	10000	0.04	12.00	87.99	4107.25
	5000	0.10	30.05	69.94	
	2500	0.30	89.98	10.01	
	1250	0.33	99.30	0.69	
	625	0.33	100.19	0	
	312.5	0.33	100	0	

Comparing the obtained results with previous work is quite challenging. This is a consequence of the wide variety of cations and cation distributions used in the synthesis of ferrites, in addition to the various size ranges of the particles and the vast variety of cell lines used. Nevertheless, a sample of these results is listed in this section. Magnesium ferrite $MgFe_2O_4$ (20 nm) was tested on (MCF-7) human breast cancer [10], and the viability was 34 % for a concentration of 800 $\mu g/mL$. The cytotoxic effect of 44 nm zinc ferrite was tested on three types of cell lines [2]; human lung epithelial cell lines (A549), skin epithelial cell lines (A431),and liver (HepG2) for various concentrations between 10–40 $\mu g/mL$. For the 40 $\mu g/mL$ dose, the cell viability for the cells was 25 %, 67 % and 48 % for (A431), (A549), and (HepG2) respectively. Another study investigating $Mg_{9.5}Co_{0.5}Fe_2O_4$ showed a cell viability that exceeded 60 % with both human embryonic kidney (HEK 292) cells and cervical carcinoma (HeLa) cells at a concentration of 200 $\mu g/mL$ [16]. Citrate coated cobalt ferrite nanoparticles (13 ± 1 nm) of concentration 8000 $\mu g/mL$ did not elicit any decrease in cell viability in Ehrlich ascites cell suspension [7].

Both $MnFe_2O_4$ and $ZnFe_2O_4$ of 12 nm were tested on four cell lines [8] namely; human retinal pigment epithelial cells (D407), human lung carcinoma cells (A549), human melanoma cells (MW35) and mouse melanoma cells (B16F10).The results showed that for a concentration of 100 $\mu g/mL$ manganese ferrite resulted in viability of (98–91 %) whereas zinc ferrite resulted in viability of (91–73 %) for the four cell lines. Authors also studied the hemolytic effect of $Mn_{0.5}Ga_{0.5}Fe_2O_4$ magnetic nanoparticles [22]. The results showed that even at a concentration of 10000 $\mu g/mL$ the hemolysis was less than 1 %; a result that renders the magnetic species not hemolytic.

Magnetite is the most intensively studied ferrite in literature. The cell viability of human leucocytes (L929) exposed to 18 nm magnetite nanoparticles concentrations between 322–322 $\mu g/mL$ was 42–23 % [15]. Magnetite particles (250 nm) stabilized by citrate groups were tested on human nasopharyngeal epidermal carcinoma (KB cells). For concentrations up to 2000 $\mu g/mL$, cell viability remained high (80 %) [13]. Concentrations of magnetite (40 nm) up to 1000 $\mu g/mL$ were tested on mouse embryonic stem cells (mouse ESCs) without any considerable

loss in viability [24]. A similar result was obtained for 10 nm magnetite on human breast cancer cell line MDA-MB-468 [25].

CONCLUSIONS

In this work, magnetite, cobalt ferrite and manganese ferrite were prepared using the coprecipitation method. XRD analysis showed that the spinel structure is formed in the as-prepared three samples without the need for any further post synthetic treatment. The obtained sizes of the three samples were 10.2 nm, 11.1 nm and 10.5 nm. This implies that they all samples exhibit superparamagnetic behavior at room temperature. The cytotoxicity of the samples was then tested. The assay showed that cobalt ferrite has the least toxicity, even less than magnetite, whereas manganese ferrite shows the highest toxicity. Based on the obtained results, both cobalt ferrite and magnetite are considered as promising candidate for biomedical applications.

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