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Synthesis and characterization of magnetite nanoparticles by co-precipitation method coated with biocompatible compounds and evaluation of in-vitro cytotoxicity

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ABSTRACT

Recent advances in the use of magnetite nanoparticles for biomedical applications have led to special attention to these nanoparticles. The unique properties of magnetite nanoparticles such as superparamagnetism, low toxicity, and the ability to bond with biological molecules, are suitable for drug delivery, diagnostic methods and therapeutic approaches. The aim of this study was to synthesize magnetite nanoparticles with different biocompatible coatings and investigate their cytotoxicity. Magnetite nanoparticles were synthesized by co-precipitation method and the cytotoxicity of these nanoparticles was investigated with Hepatoma G2 cell using the MTT assay. Treated cells, did not showed any evident cell cycle arrest. The Fourier Transmission Infrared (FTIR) spectroscopy, X- ray powder Diffraction (XRD), Transmission Electron Microscopy (TEM) were evaluated. The results of XRD showed the coated magnetite nanoparticles were 10–12 nm and this size also achieved with TEM images. Synthesized magnetite nanoparticles with SiO_2 and oleic acid coatings had lower cytotoxicity than other coatings.

1. Introduction

Iron oxide nanoparticles have been highly regarded by researchers due to their many applications in drug delivery [1], Magnetic Resonance Imaging (MRI) [2] and absorption of environmental pollutants [3]. One of the most important iron oxide nanoparticles is magnetite, which is more widely used than other magnetic nanoparticles due to its biocompatibility [1].

Magnetite (Fe_3O_4) has cubic inverse spinel structure [4,5]. This structure gives it special properties so that it can be used in many medical, pharmaceutical and therapeutic applications. [6–10]. Although magnetite is chemically inert [1], surface modification using some polymers, metals, organic and inorganic molecules can expand its application [1,11]. In addition to preventing the agglomeration of magnetic nanoparticles, It is necessary to modify its surface with biocompatible coatings [12]. The coating of nanoparticles with biocompatible materials has been studied and applied in many researches [8,10,13–19].

Several methods have been reported for the preparation of magnetite, among which the co-precipitation method is the simplest method in which no organic solvents are required [20,21]. In this method, the reaction medium must be basic and the ratio of ferric salts to ferrous should be 2 to 1 [18,22–24]. Several studies have been carried out the effect of coating, temperature, stirring rate and pH on the size and properties of nanoparticles [25–28].

One of the important properties of metal oxide nanoparticles in medical application is their toxicity [29,30]. As mentioned in the articles, nanoparticles can cause toxicity in cell proliferation [31–33]. Iron oxide can have toxic effects on yeast and bacteria and can be used for inhibitory effect. Peng et al. were able to investigate the inhibitory effect

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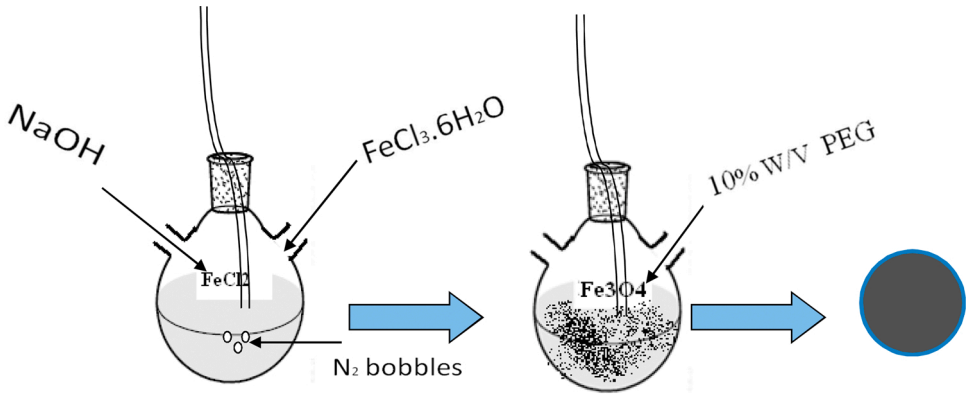

Fig. 1. Preparation and modification of Fe_3O_4 with Co-precipitation method.

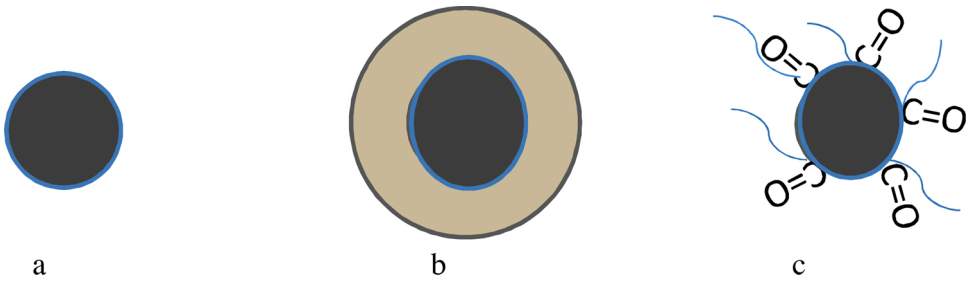

Fig. 2. a) Magnetite Nanoparticle coated with PEG, b) Magnetite core with SiO_2, c) Magnetite core with OA.

of uncoated iron oxide nanoparticles on yeast [34]. The biocompatible colloidal suspensions of magnetic iron oxide nanoparticles coated with oleic acid were prepared by Coricovac et al. and effects of these nanoparticles both *in vitro* on normal cell lines —human keratinocytes (HaCat cells) and *in vivo* by evaluating the acute dermal toxicity after topical application of the colloidal suspensions was investigated [35]. The *in vitro* evaluations of the Fe_3O_4 nanoparticles tested indicated a lack of toxicity on human keratinocytes cell viability, proliferation, and migration. Although, The *in vivo* acute dermal toxicity test showed different results [35].

However, magnetite-coated nanoparticles with biocompatible coatings have no toxicity and can be used in clinical research [36,37]. Souza et al. showed the silica-coated magnetite nanoparticles did not cause much toxicity to osteoblast cells and did not alter osteoblast collagen secretion [38]. The modification of nanoparticles with biocompatible coatings demonstrated none toxic effects at the experimental concentrations [39–41]. Although, Saranya et al. reported the relation of the exposure time, and concentration of iron oxide nanoparticles in their In-Vitro cytotoxicity studies [42]. They also mentioned iron oxide nanoparticles between 10−100 μg/μl showed better activity.

For the MRI application of magnetite nanoparticles, their size should be less than 20 nm and show very low toxicity [4,43]. These magnetite nanoparticles with special coatings are approved for Magnetic Resonance Imaging (MRI) [29,44]. The biocompatibility of magnetic nanoparticles with PEO triblock copolymer coating for intraocular targeting and tested their toxicity upon addition of the MNPs in a colorimetric MTT (3-[4,5-dimethylthiazol-2 yl]-2,5-diphenyltetrazolium bromide) assay were studied [44]. Further research showed that PVA coated iron oxide nanoparticles had very low toxicity on the cell line [45]. The cytotoxicity of SiO_2 nanoparticles has also been investigated [46]. Several studies demonstrated that silica-coated superparamagnetic iron oxide NPs (SiO_2-SPIONs), have a high potential in medicine [47].

The aim of this study is the synthesis of coated magnetite nanoparticles with organic, inorganic, and polymeric coatings and evaluation of the toxicity of these nanoparticles by the MTT assessment [37,48].

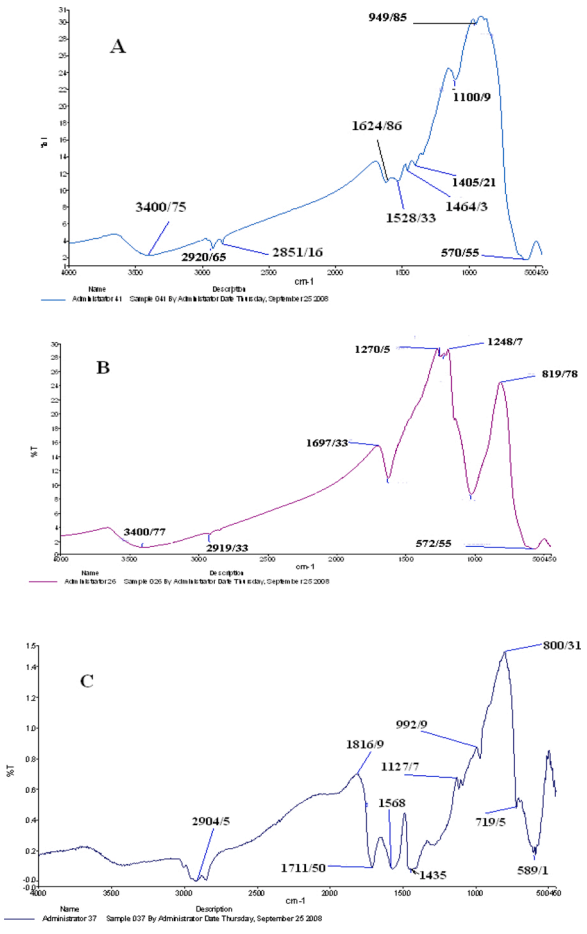

Fig. 3. The FT-IR spectra of A)IO-PEG, B) IO-PEG-SiO_2,and C) IO-OA.

The results of Fourier-transform Infrared Spectroscopy (FT-IR) and X-ray powder Diffraction (XRD) spectra, as well as, Transmission Electron Microscopy (TEM) images, revealed that these coatings were bonded to magnetite nanoparticles. Also, the MTT assay well showed the lack of cytotoxicity of these nanoparticles. The results showed SiO_2 and oleic acid had lower cytotoxicity than other coatings and magnetite nanoparticles with 10−40 μg/μl concentration can be used for clinical applications.

2. Materials and methods

2.1. Materials

Ferrous chloride tetrahydrate ($FeCl_2$ $0.4H_2O$), Ferric chloride ($FeCl_3.6H_2O$), Polyethylene Glycol (PEG)4000, Oleic Acid (OA), Tetra ethoxysilane (TEOS), Iso-butanol, Ammonia(NH_3) and Sodium hydroxide (NaOH) were purchased from (Merck. KGaA, Darmstadt, Ggermany).

2.2. Preparation of magnetite nanoparticles

The method for the preparation of magnetite nanoparticles was discussed in previous studies [22,28]. The salt of $FeCl_2.4H_2O$ was added to 100 mL of distilled water that was previously purged by nitrogen, and $FeCl_3.6H_2O$ was added after the good dissolution of salt. In this synthesis, the stoichiometry of $FeCl_2.4H_2O$/ $FeCl_3.6H_2O$ was 1/2. The NaOH was added to receive a pH of 11. For coating with PEG, 10 % of PEG 4000 solution was prepared and sonicated 5 min with ultrasonic bath (JINYUANBAO ULTRASONIC CLEANER, RoHS, Korea).

$$Fe^{2+}(aq) + Fe^{3+}(aq) + 8OH^{-}(aq) \rightarrow Fe_3O_4(s) + 4H_2O \quad (1)$$

After synthesizing magnetite nanoparticles, PEG solution was slowly added, and stirring was continuing with 400 and 700 rpm. The black solution was centrifuged three times with distilled water and two times with acetone. Then, the nanoparticles were put in an oven at 35 °C and the dried nanoparticles were obtained.

To coat with OA, 6cc oleic acid was added to the solution, and it was stirred 15 min after that. The solution was washed with ethanol then water and dried at 35 °C [2,10,49].

Coating with SiO_2 was prepared with the Sol-gel method. First, 0.16 g of PEG-magnetite nanoparticles was weighted and dispersed in 40 mL iso-butanol, 2 mL TEOS, 1 mL NH_3, and 4 mL distilled water [19,22,50]. The stirring was continuing for 12−16 h. In addition, the solution was washed with ethanol and dried at 35 °C.

2.3. Cytotoxicity assay

Cytotoxicity was measured using MTT assay. The basis of this cytotoxicity test is the conversion of MTT to purple of formazan which was performed by mitochondrial enzymes of living cells. The greater the amount of living cells in the environment, the more the color tends to be purple, and the less it is, the more likely it is in the color of MTT. In this part of the study, the viability of four samples of the synthesized samples on HepatomaG2 (HepG2) cells *in vitro* was investigated. These samples include (IO@PEG@SiO_2), (IO@OA), (IO@PEG), and IO@PEG in 700 rpm. The cells were cultured at 37 °C under atmosphere of CO_2, in Incubator, in Dulbecco's modified Eagle`s Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS). After 2 weeks, the HepG2 cells were seeded at an initial density of 2×10^3 cell/cm^2 on flat bottom 96-well plates. Then, to *in vitro* evaluate the cytotoxicity studies, 10 μL of MTT and 10, 20, 40, 80 and 160 μg/μl of mentioned samples was added. The medium was discarded and the formazan blue, which formed in the cells, was dissolved with 100 μL dimethylsulphoxide (DMSO). The absorbance was measured at 570 nm in a microplate reader (Synergy). The percentage of viability compared with the control wells was calculated by Microsoft office excel 2010. The IC50 value of the nanoparticles were then calculated using CalcuSyn version 2.9 software (BIOSOFT, UK).

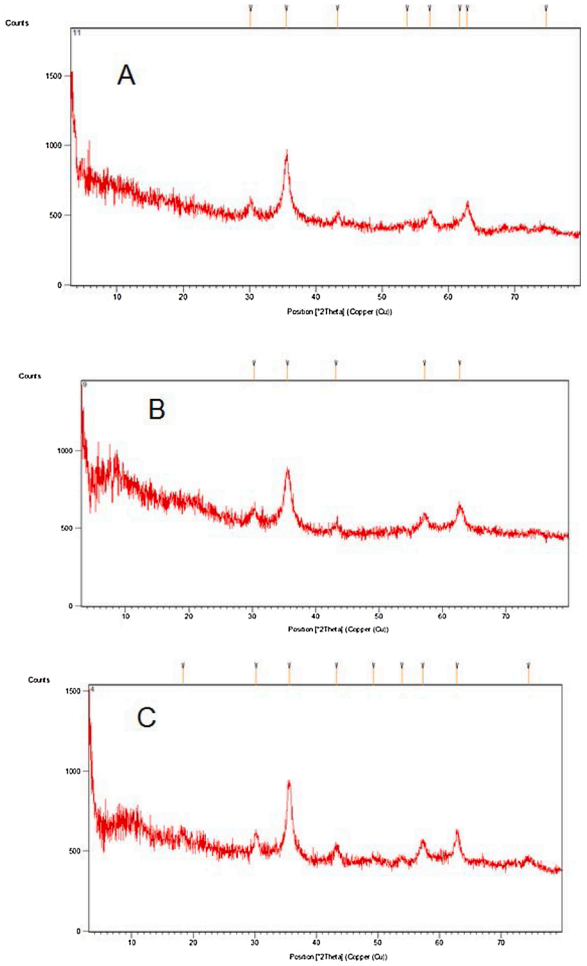

Fig. 4. The X-ray powder diffraction of magnetite nanoparticles with A) PEG, B) PEG-SiO_2 and C) OA coatings.

2.4. Characterization of magnetite nanoparticles

The synthesized magnetite nanoparticles were characterized by TEM, XRD, and FT-IR techniques. The TEM (ZEISS, EM-10C, Germany) operating at 100 kV was used for the size and morphology characterization. The XRD (Siemens, D5000, Germany) with Cu Kα radiation was utilized for the phase characterization. The average size of the nanoparticles was determined by using the Scherrer formula. The FT-IR characterizations of magnetite nanoparticles with different coatings were determined with PerkinElmer Spectrum II. FT-IR spectra were obtained with KBr pellets and the spectrum was taken from 4000–450 cm^{-1}.

3. Results and discussions

Fig. 3 displays the FT-IR spectra of magnetite nanoparticles with A) PEG, B) PEG -SiO_2, and C) OA coatings. It is worth mentioning that the peak at 570 cm^{-1} confirms the magnetite lattice absorption. These results are confirmed by comparing the IR spectrum with previous studies [20,51]. In fact, FT-IR spectra of magnetite exhibited strong bands in the low-frequency region (1000–500 cm − 1) due to the iron oxide skeleton [52]. It is obvious that the characteristic band of Fe-O at 572.95 cm − 1 appears in the FT-IR spectrum of Fe_3O_4 particles. The PEG has characteristic bonds in 3370 and, 1049 cm^{-1}(OH stretching), 2800 cm^{-1}(C–H

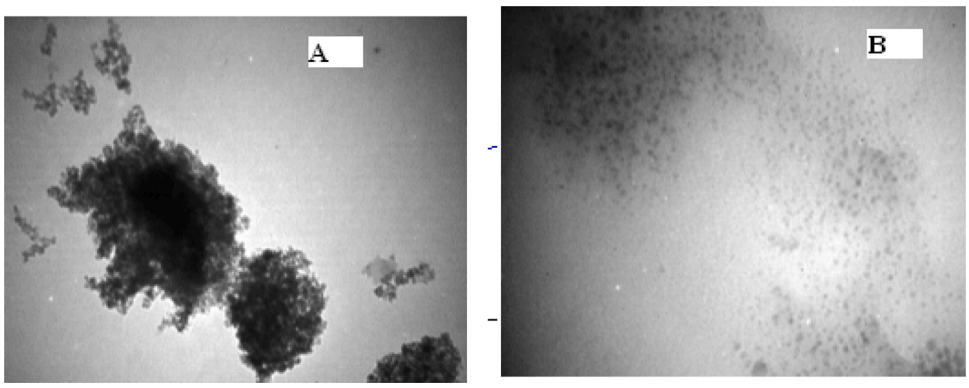

Fig. 5. Transmission electron microscopy of A) IO@PEG@SiO_2 and B) IO@OA nanoparticles.

stretching), 1279 cm^{-1}(COH—— stretching), 1360, and 1280 cm^{-1}(CO— stretching) as well as 1460 (CH_2 bending). When PEG was bonded with iron oxide nanoparticles, some peaks overlapped or shifted. The sample with polymer coating showed an OH peak at 3300−3500 cm^{-1} and stretching vibration of CH_2 at 1100 cm^{-1}. Polymer coating removed the band at 1080 cm^{-1} and shifted the 1250 cm^{-1} peak to 1528 cm^{-1}. As it was observed in the IO-PEG spectrum, removing or weakening of some peaks (3200, 2090, 1,800,830 cm^{-1}) was indicative of the polymer coating.

The FT-IR of the IO-PEG-SiO_2 (Fig. 1B) showed Si-O-Si asymmetric stretching at 1629 cm^{-1}, while in SiO_2, this peak was located at 1659 cm^{-1} and the shift of the peak represented SiO_2 coating of the IO. A lower wavelength shift was observed in symmetric stretching Si-O-Si (1029 cm^{-1}) relative to SiO_2 coating.

Oleic acid is a fatty acid that has the formula $CH_3(CH_2)_7CH = CH(CH_2)_7COOH$. The OA has indicator bands at 2854 and, 2924 cm^{-1} relative to symmetric and asymmetric stretching bands of CH_2. These peaks shifted to the lower wavelengths (2850 and, 2920 cm^{-1}) in IOO—A nanoparticles (Fig. 1C). The peaks around 1538 and 1438 cm^{-1} relative asymmetric and symmetric stretching of COO^{-1} (Fig. 2).

Fig. 4 shows XRD of the nanoparticles. It was notable that Fe_3O_4 nanoparticles showed XRD pattern with diffraction peaks at 2θ of 30.1°, 35.5°, 43.1°, 54.5° and 57.6° relative to the diffractions of 220°, 311°, 400°, 422°, 511°, and 440°crystal faces of Fe_3O_4 spinel structure. These diffraction peaks were observed in IO-PEG, IO-PEG-SiO_2, and IO-OA nanoparticles. The crystalline size of the nanoparticles was calculated by the Scherrer equation as follows:

$$D = K\lambda/\beta_{1/2}\cos\theta$$

Where Dis the average crystal size, K is a constant (here chosen as 1), λ is the wavelength of X-ray radiation (1.54056 A), β 1/2 is the half width of the diffraction peak, and θ (°) is Bragg's angle. The results of D values, using 311 planes for the samples were calculated at10 nm (IO-PEG), 10 nm (IO-PEG-SiO_2)and 6−7 nm(IO-OA).

The morphology and size of the magnetite nanoparticles were investigated by TEM. The TEM images of synthesized nanoparticles showed that the nanoparticles had spherical structure and the size of them was under 10 nm (Fig. 5), which confirmed the XRD results.

Biocompatibility of magnetite nanoparticles coated with PEG, PEG-SiO_2, and OA was demonstrated with MTT assay. The assay was yellow and water soluble tetrazolium salt. In this study, doses of 10, 20, 40, 80, and 160 μg/μl of iron oxide nanoparticles with PEG, SiO_2 and oleic acid coatings were studied to determine the survival rate of HepG2 cells. The tetrazolium salt was converted to a water insoluble insoluble and purple formazan derivative with active cells [45]. The Fig. 6(A–D) shows the toxicity test of the compounds. As the figures depict, the synthesized

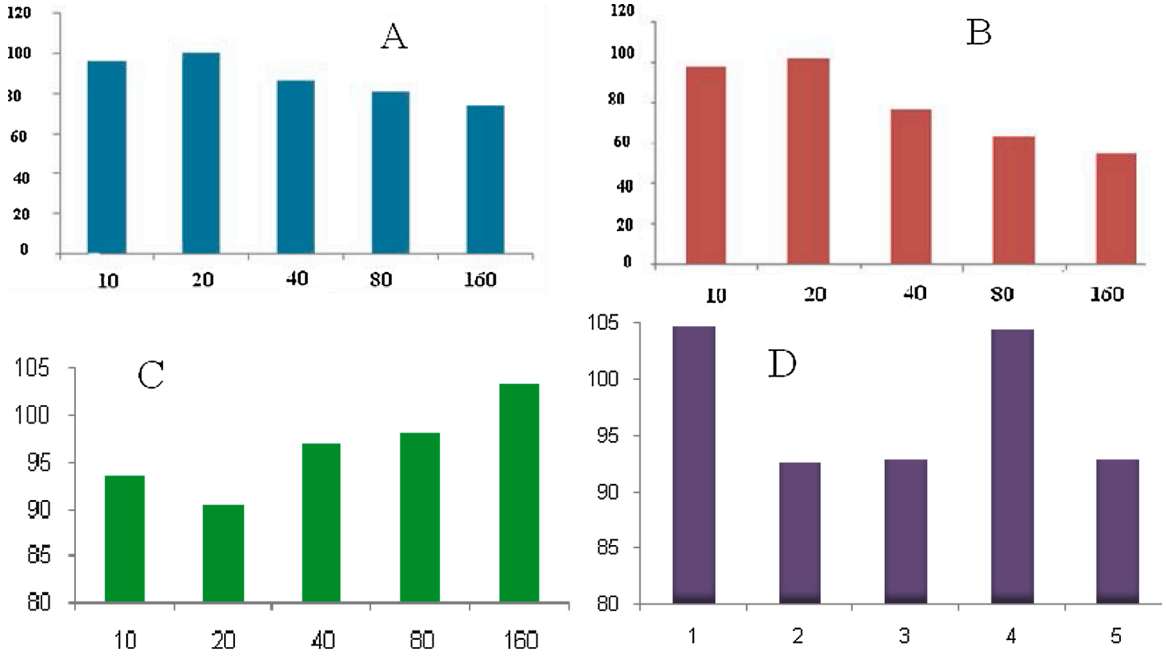

Fig. 6. The toxicity test of A) IO@PEG@SiO_2, B) IO@OA nanoparticles C) IO@PEG(400 rpm) and D) IO@PEG (700 rpm rate).

compounds are non-toxic and allow cell growth.

The results from FT- IR, XRD spectra, and TEM images showed well that magnetite nanoparticles were synthesized by co-precipitation and coated directly with biocompatible coatings investigated in this research. These results have been predictable according to previous articles [23]. The results of MTT method cell viability indicate the biocompatibility of magnetite nanoparticles with biocompatible coatings. Results from the MTT assay showed the magnetic nanoparticles with coatings investigated in this paper give a lack of toxicity in the MTT assay. Saranya et al. reported the relation of the exposure time, and concentration of iron oxide nanoparticles in their In-Vitro cytotoxicity studies [42]. They also mentioned iron oxide nanoparticles between 10−100 μg/μl showed better activity. Hafeli et al. determined biocompatibility of magnetic nanoparticles with PEO coating for intraocular targeting and tested their toxicity upon addition of the MNPs in a colorimetric MTT (3-[4,5-dimethylthiazol-2 yl]-2,5-diphenyltetra zolium bromide) assay [44]. Further research by Mahmoudi et al. [45] showed that PVA coated iron oxide nanoparticles had very low toxicity on the cell line. According to Kononenko results on ATII-like A549 human lung cancer cells, silica-coated superparamagnetic iron oxide NPs (SiO_2-SPIONs) have a high potential in medical application.

Although, iron oxide nanoparticles without no biocompatible coatings showed toxicity and can be used for inhibitory effects [34]. According to the cell viability results, it should be noted that the dose of magnetite nanoparticles can be effective in cell viability. Also, the type of coating can be effective in cell viability. Silica and oleic acid can provide highly biocompatibility of magnetite nanoparticles.

4. Conclusion

This study investigated the preparation of magnetite nanoparticles. Magnetite nanoparticles can be synthesized by co-precipitation method and directly coated with organic (OA), inorganic (SiO_2) and polymeric (PEG) coatings. The results of the FT-IR, XRD and TEM showed the successful synthesis of nanoparticles. Cell viability was assessed with MTT assay. The results from MTT method showed well that coated magnetite nanoparticles with silica and oleic acid have low toxicity. Doses between 10 and 40 μg/μl are also much more suitable for clinical researches, and higher doses can and higher doses can increase cytotoxicity. Therefore, these magnetite nanoparticles are very suitable for use in MRI. This investigation will be attributed to future studies regarding the MRI application of magnetite nanoparticles.

Declaration of Competing Interest

The authors declared no conflicts of interest

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