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Production and Characterization of Magnetic Fe_3O_4 Nanoparticles Coated with PCL for Biomedical Applications

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Abstract

Currently, magnetic nanoparticles are widely studied with regard to their application in cancer treatment. This study aims to show a straightforward strategy for the production of Fe_3O_4 nanoparticles (NPs) with biocompatible surface modifications with polycaprolactone (PCL) for biomedical purposes. The effects of the polymer coating on the properties of magnetite were evaluated. Crystallinity, morphology, composition, hydrodynamic size and magnetic properties of the produced nanoparticles were analysed via X-ray diffractometry (XRD), Transmission Electron Microscopy (TEM), Fourier-Transform Infrared Spectroscopy (FTIR), Dynamic Light Scattering (DLS) and Vibrating Sample Magnetometry (VSM), respectively. The proposed method produced nanoparticles of magnetite with an average size between 9 and 11 nm, with spherical morphology and superparamagnetic properties. Magnetization values were not compromised even when the highest amount of polymer was used in the surface modification. On the other hand, the coating resulted in the decrease of the hydrodynamic size of the composites, indicating greater colloidal stability when the polymer was present. The obtained nanoparticles showed maintenance of significant superparamagneticbehavior, even in the presence of PCL on their surface. This phenomenon would allow for their application as a further optimized vector in hyperthermia cancer treatment, controlled drug delivery and resonance imaging.

Keywords: Nanoparticles; Magnetite;PCL; Cancer; Hyperthermia

Introduction

The term 'cancer' refers to a group of more than 100 diseases that are triggered by a disordered growth of cells, with the formation of tumours. Therefore, the treatment of this condition is based on the prevention the multiplication of these cells and/or the removal of the tumour; in order to improve the treatment, more than one tool can be combined. Many types of cancers can be reversed when caught early and treated effectively, with a possible decrease in the mortality rate of 30 to 50% [1,2,3,4]. Chemotherapy is one of the most common methods used for treating cancer. It consists of using chemical drugs systematically throughout the body to prevent the proliferation of cancer cells and to kill them. However, cancer cells are not the only ones with accelerated growth in the human body. Due to the unspecific delivery, healthy cells are also subjected to the effects of the drug as well. This phenomenon brings about several side effects, such as nausea, hair loss and immune suppression [5,6]. Besides these factors, the indirect delivery system decreases the amount of the remedy that actually gets to the tumor, reducing the therapeutic efficacy. To prevent this from happening, studies are being conducted in order to make drug delivery systems (DDS) that are cell-specific, optimizing the treatment [7]. These systems present several advantages: protecting the drug, releasing it in the location of interest, allowing the use of roughly 100 times less compound and reducing the side effects [8]. Another type of cancer treatment that has been widely studied is hyperthermia. Because cancer cells are more sensitive to higher

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temperatures, when the tissue containing the tumor is submitted to hyperthermia treatment, these specific cells suffer more damage or are killed [9]. Among the several nanoparticles that can be used for biomedical applications, magnetic materials are very attractive (MNP), especially those with superparamagnetic characteristics, as they are used in diagnosis, as contrasting agents for magnetic resonance imaging (MRI) and in the treatment of several conditions [10]. Iron(II) and (III) oxide (Fe_3O_4) MNPs appear to have the highest potential for use in cancer treatment as they are biocompatible, superparamagnetic and easy to functionalize. Furthermore, they can incorporate drugs and are easily separated from the solution by magnetic recovery, which prevents the degradation of the NP elements, including the drug. Moreover, this magnetic property allows for the use of these MNPs as targeted DDSs, using a magnetic field gradient to guide them to specific spots in the human body [11]. Their use as thermossed for hyperthermia is linked to their ability of generating local heat under alternating magnetic fields, as results of Néel and Brownian relaxations and hysteresis loss in this condition [9,12]. However, morphology, dispersibility and the hydrodynamic size of the nanoparticles are important and influence their use in biomedical applications [5,13]. Moreover, these MNPs have already been approved by the Food and Drug Administration of the United States [14]. For the purpose of biomedical applications, even though the MNPs of Fe3O4 (magnetite) have the previously aforementioned advantages, some modifications may be required for the particles to stay longer in the body and be more biostable. One of the most used approaches is the use of polymers as surface modifiers. Polycaprolactone (PCL) is a promising candidate for this function. It is a biocompatible and biodegradable polymer due to its being susceptible to enzymatic degradation, and is bioresorbable. PCL has already been approved by the FDA and it has good solubility in common organic solvents [15-18]. Withal, PCL has several advantages against other polymers as it is highly permeable to various drugs, can easily form copolymers and its degradation does not generate an acid environment in the body [19]. The greatest challenge remains in finding a cheap and large-scale synthesis method that produces these MNPs with satisfactory sizes with adequate surface modifications and which ensure stability under physiological conditions, more importantly, without compromising properties such as their magnetism, but having steps with minimal damage to the surface modifiers and active principles, thereby enabling them to be used as DDSs and vehicles for hyperthermia [4,8,20]. The present work aims to synthesize, through a simple method, magnetite MNPs with surface modification by PCL, with a smooth recovery process that alter as little as possible the surface polymer and possible drugs that may be used. This would improve their performance in physiological conditions without suffering significant loss in the magnetic properties.

Materials and Methods

Ferrous chloride tetrahydrate ($FeCl_2 \bullet 4H_2O$ ≥99%), ferric chloride hexahydrate ($FeCl_3 \bullet 6H_2O$ 97%) and PCL bovine serum albumin (BSA) were purchased from SIGMA-ALDRICH, ethyl acetate and isopropyl alcohol from NEON, acetone from REATEC and ammonium hydroxide from VETEC. All used chemicals were of AR grade unless otherwise specified, and all the aqueous solutions were prepared using deionized water from a MilliQ filtration system. Synthesis of Fe_3O_4@PCL NPs Magnetite nanoparticles were synthesized by co-precipitating Fe(II) and Fe(III) salt precursors in a basic medium. $FeCl_2 \bullet 4H_2O$ and $FeCl_3 \bullet 6H_2O$ (1:2 molar ratio of Fe_3+/Fe+2) were dissolved in 40 mL of deionized water. A solution of ammonium hydroxide (25% wt NH_4OH) was rapidly added dropwise until pH reached 11 under vigorous stirring and temperature of 60°C for 1 hour. The produced material was magnetically separated and washed with deionized water and isopropyl alcohol several times. The black rump was resuspended in deionized water. The PCL was dissolved in a stock solution of acetone and ethyl acetate (4:1) under vigorous stirring at 50°C. The MNPs were subjected to surface modification in four different amounts of the polymer (0, 20, 40, 60 and 80 mg, with the sample named after these amounts as Am0, Am20, Am40, Am60 and Am80) by an oil-in-water emulsion solvent-evaporation modified method. The solution carrying PCL was added dropwise in the water solution, holding the MNPs under vigorous stirring at 50°C for one hour for the polymer to interact with the nanoparticles and the solvents evaporate. Following this, the MNPs were collected with a magnet, washed several times with deionized water and isopropyl alcohol, resuspended in deionized water, submitted to ultrasound for 15 minutes, magnetically separated once again, and finally dried in an oven at 50°C for 24 hours.

Characterization

Powder X-ray diffractometry (XRD) was performed to analyse the formation of the spinel phase in an X'Pert MPD Phillips equipment with Cu-Kα radiation (1.54184 Å). Fourier transform infrared spectrometry (FTIR) was used to verify the presence of the polymer. Assays were performed using an IRAFFINITY 1 (Shimadzu) spectrometer. Spectra were recorded in the range between 4000 and 500 cm^{-1}, with samples diluted in KBr. The magnetic properties were characterized using a vibrating sample magnetometer (VSM, MicroSense) at room temperature. Transmission electron microscopy (TEM) was used to observe crystallite morphology and size. A Joel JEM device operating at 80kV was used, with the samples placed in a carbon-coated copper grid. Dynamic light scattering (DLS) measurements were performed to obtain the average hydrodynamic size of the nanoparticles using a Zetasizer Nano Zs (Malvern). The MNPs were diluted to a concentration of 0.1 mg.mL-1 with deionized water.

Result

The XRD results show the diffraction patterns of the produced MNPs, with the expected (220), (311), (400), (422), (440) and (511) reflections corresponding to the crystalline structure of Fe3O4 cubic spinel(Figure 1). Thus, the patterns confirm the successful production of the desired material. Furthermore, the

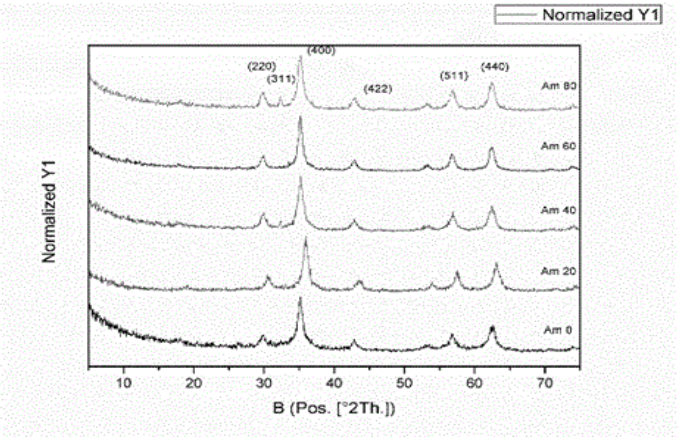

Figure 1: XRD patterns of Fe3O4 nanoparticles produced with and without PCL.

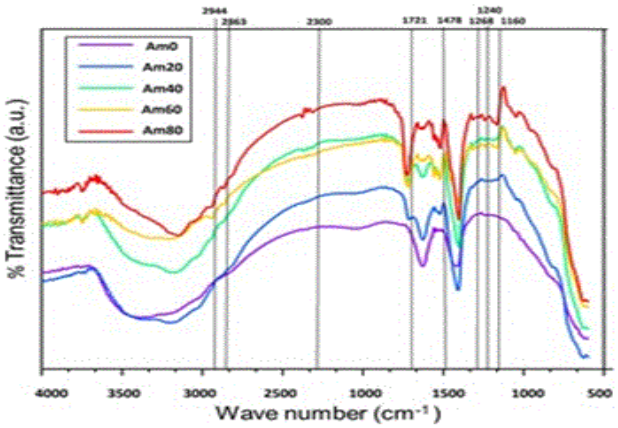

Figure 2: FTIR absorption spectra of the different produced MNPs, with stripes indicating important bands.

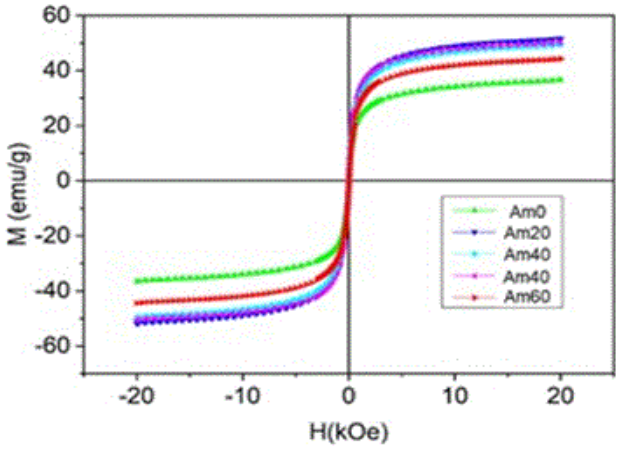

Figure 3: Magnetization curves of the MNPs with different amounts of polymer, measured via VSM.

addition of PCL did not affect the diffractograms, thus indicating that the crystalline structure of magnetite is maintained after the addition of the polymer.FTIR data shows the existence of absorption bands that correspond to the presence of the polymer(Figure 2). The most striking band is seen at approximately 1726 cm^{-1}, which corresponds to the stretching of the carbonyl (C=O) of the PCL ester group, confirming the surface modification by the polymer [20]. Beside this, other less expressive bands, such as those corresponding to the symmetrical and asymmetrical stretches of CH_2 bond (2944 and 2863 cm-1), OH bond stretch (2300 cm-1), CH group stretching in CH2 (1487 cm-1), the CO and CC double bonds (1268, 1160 cm-1) and COC asymmetric stretch (1240 cm-1) were also found [18]. It is worth mentioning that the markings were made next to each band in order not to hide them. The intensity of the bands is also directly linked to the added concentration of PCL, which could be linked to an increased presence of the polymer. The results of the VSM assays show the magnetic properties of treated and untreated MNPs(Figure 3). It is worth noting that all hysteresis curves display negligible remanence (Mr) and coercivity (Hc), proving that MNPs have superparamagneticbehavior[22]. Transmission electron microscopy was used to verify the morphology of the MNPs. (Figure 4) shows that the nanoparticles have spherical shapes with good crystallinity, in agreement with the XRD results and with the literature that also used polymers as a surface modifier [2,23-25]. A certain regularity among the NP sizes (around 10 nm) was also observed in (Figure 5). The hydrodynamic diameters of the MNPs are described in (Table 1). The values obtained by DLS showed a decrease in size of the particles containing the polymer, with a reduction of approximately 57% when comparing the bare NPs to the Am80 sample.

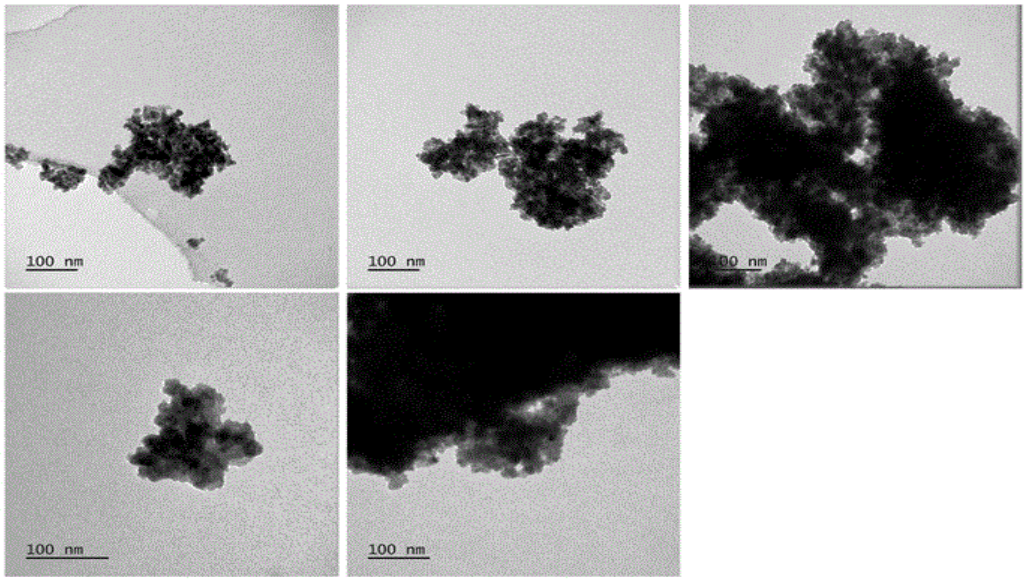

Figure 4: TEM images of a) Am0; b) Am20; c) Am40; d) Am60; e) Am80

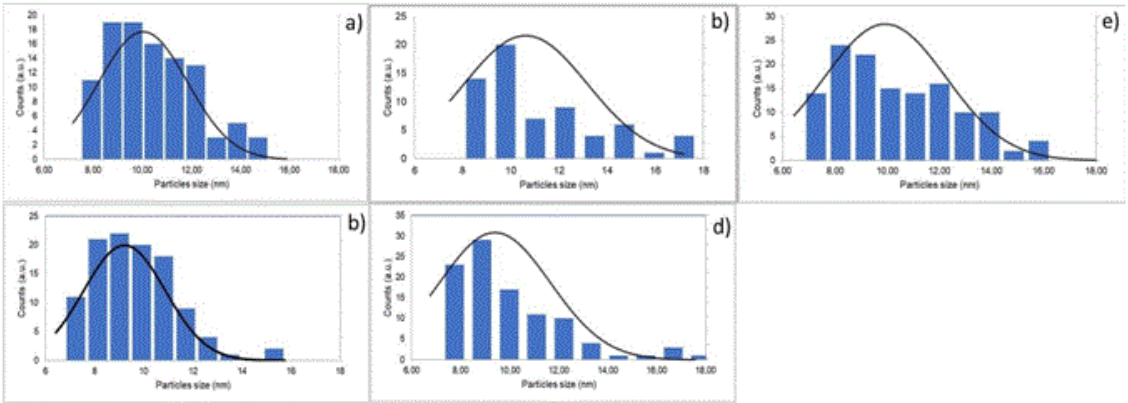

Figure 5: F Histogram data curve fitting on diameter of MNPs a) Am0; b) Am20; c) Am40;d) Am60; e) Am80

Table 1. Hydrodynamic sizes of the MNPs measured by DLS

Sample	Hydrodynamic size (nm)
Am0	466.50 ± 13.47
Am20	336.20 ± 31.48
Am40	290.18 ± 7.72
Am60	282.40 ± 10.33
Am80	267.78 ± 6.01

Discussion

As shown in the XRD results (Figure 1), the reflections corresponding to magnetite are all present, indicating the crystalline structure of Fe3O4 cubic spinel and thereby proving the effectiveness of the coprecipitation method in producing these nanoparticles [2,26]. As the present experiment was not carried out under nitrogen atmosphere, oxidation could occur, converting the material into maghemite. However, the black colour of the final material, as opposed to of an ochre colour, and the XRD reflections are good indications of the composition, consisting mostly of magnetite [4,27]. Unlike that which was reported by only magnetite reflections were observed in the XRD data, without clear signs of the polymer. This phenomenon may have occurred due to the lower concentrations of the polymer used to make the surface modification in this study, as the intention in the present work was to preserve as much as possible the magnetic properties for a possible further optimized hyperthermia. In the FTIR data (Figure 2), bands corresponding to the polymer bonds increased in intensity as the polymer concentration increased, while being absent in the sample of bare ferrite, AM0, proving that one can adapt the particle synthesis towards the desired composition via the proposed method [18]. The presence of these bands also indicates that the magnetic recovery used in the synthesis did not affect the polymer. The superparamagneticbehavior can be observed in their M-H curves, as in (Figure 3), proving that MNPs could be used for biomedical applications as thermoseeds for local cancer therapy and diagnosis. In (Figure 3), a slight increase in magnetization can be noticed in the samples that are surface-modified by PCL, contrary to the findings of some works, in which the presence of the polymer resulted in a decrease in the magnetization of the NPs [4,20]. However, the coating does not always limit the entire surface of the MNP to the point of reducing magnetization [26]. Sometimes, the modification may result in surface changes that facilitate the ordering of the moments, or even preserve the magnetite phase, reducing the oxidation to maghemite [27], events that may have occurred in this work. Either way, the maintenance of magnetic properties can be used to reduce the amount of MNPs that would be needed to induce a good response to the applied magnetic field, generating satisfactory heat production and targeting in the treatment of tumours by hyperthermia and controlled DDS, resulting in lower cytotoxic risks [9]. Moreover, a decrease in magnetization of the sample with the highest PCL concentration (AM80) is observed, which could be the combination of the result of the studies above, where it could still have a higher degree of magnetite and/or organized moments than the pure MNPs. The amount of polymer should result in a smaller fraction of magnetite in the final NP, decreasing the magnetization in relation to the other treated samples [26-28]. TEM images, (Figure 4), do not allow for the visualization of the polymeric layer, a fact that has also been reported by some authors who used PCL or even other polymers and organic coatings in low concentrations. Instead, the modification is confirmed by the FTIR data, where PCL bands could be easily discerned, as was also carried out in this work [29,2,23,26,25]. Nanoparticles with constant sizes, size distribution, and shape were observed, (Figure 5). This morphological uniformity is crucial considering that the successful application of these nanoparticles depends on their morphology [13]. Other characteristics that influence the biomedical applications of MNPs are their colloidal stability and their size when suspended [5]. The decrease in hydrodynamic size of those nanoparticles containing more polymer, presented in the DLS analysis in (Table1), evidences an increase in dispersibility caused by the surface modification with PCL, testifying the greater stability of the MNPs in the presence of the polymer. The difference between sizes found via DLS and TEM data likely occurs due to the presence of a hydration layer around the MNPs in the DLS configuration as this technique measures the size of particles in colloidal suspension combining the obtained results, it is clear that these MNPs are promising materials for biomedical purposes that utilize their magnetic responses in cancer treatment[1,2].

Conclusion

In this study, magnetite nanoparticles with surface modifications by PCL were successfully synthesized by a simple method. The magnetic separation used in the method did not cause damage to the polymer on the surface. The particles present spherical shapes, general size within the 10 nm range, and regular distribution. The bands of the polymer were stronger as the concentrations increased, as shown by the FTIR data. The hysteresis curves presented the superparamagneticbehavior of the MNPs, even in the presence of the polymer on the surface, indicating the possibility of them serving as thermoseeds for general hyperthermia. This character would also serve to target the MNPs using a magnetic field gradient for local hyperthermia and controlled drug release. The results allow for their use in these treatments without the need of higher concentrations of the nanoparticles in order to obtain a good response to the magnetic field.

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