MAGNETITE-ALGINATE PARTICLES FOR MAGNETIC HYPERTHERMIA APPLICATION

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Abstract: Although magnetic nanoparticles have been studied as hyperthermia agents for decades, concerns remain regarding the possible toxicity and lower stability of pure Fe_3O_4, which could limit its effectiveness in therapeutic applications. However, their superparamagnetic nature enables the direction and localization of these particles to specific therapeutic targets only in the presence of an applied magnetic field. In this context, the gap in this research is to investigate new approaches to improve the stability and minimize the toxicity of pure Fe_3O_4 for application in magnetic hyperthermia. Therefore, the aim of this work is to develop magnetite particles coated with alginate for use in magnetic hyperthermia. The spherical particles were obtained using different parameters and characterized in terms of structure, morphology, hyperthermia behavior, swelling, and cell viability. The results showed that magnetite particles coated with alginate are predominantly spherical, with a rough surface and superparamagnetic behavior with almost zero remnant magnetization. The results indicate that these coated particles represent a promising new perspective for magnetic hyperthermia.

Keywords: Magnetic Nanoparticles, Hyperthermia, Alginate.

1. INTODUCTION.

Hyperthermia is a promising cancer treatment, in which the local temperature around the cancer cells are increased up to 41 – 45 ºC. With this, the cancer cells are more sensible to the heat cytotoxic effect, resulting in cell death with no harm to the health cells [1]. There are several advantages of the magnetic oxide nanoparticles in biomedical applications, for example, ability to interact with specific biological entities due to the size control and magnetic properties, and the great reason area/volume allowing the surface functionalization [2].

The only magnetic nanoparticles approved by FDA (Food and Drug Administration) for in vivo use is the iron oxides (Fe_3O_4 magnetite and γ-Fe_2O_3 maghemite). These nanoparticles present biocompatibility, low toxicity and superparamagnetic behavior (the particle do not have residual magnetic field or null remnant) [3]. The main challenge for in vivo application is to ensure that the nanoparticles reach a specific place inside the body, be present at the body fluids and have reduced toxicity. For this reason, it is essential to design particles that transport and stabilize the magnetite nanoparticles for the precision administration, by chirurgic or endoscopic method, at the tumor region allowing the hyperthermia [4]. Coating these materials with biopolymer is a promising alternative for the effective magnetic hyperthermia application and alginate is a biopolymer normally used due to the non-toxic benefits [5]. This is a natural polysaccharide extracted from brow seaweed, with empirical chemical formula NaCHO, usually presented as a salt. After coating, a high water content can be retained and, for this reason, this polysaccharide is commonly used in the food industry, also as coating for pharmaceutical, biomedical and agricultural materials [6].

Also after coating, magnetite particles become stable in aqueous medium, biodegradable and without toxic effects, causing no harm to blood cells, only low inflammatory reactions. In this context, the main objective of this research work is to design magnetite-alginate coating core-shell particles for magnetic hyperthermia application.

2. EXPERIMENTAL PROCEDURE.

2.1 Magnetic nanoparticles production and characterization

The sol-gel synthesis method was used according to Polla at al. [7], in which the reagents $Fe(NO_3)_3.9H_2O$ (Neon, 98% purity) and $C_6H_8O_7$ (Synth, 99,5% purity) were separated diluted in 25 mL of deionized water. After homogenization, the solution was kept at 85 ± ºC for 2 h (Velp Scientifica, F20500162) in water bath and the obtained gel was dried (CienLab, Ce 220/100) for 24 h. The xerogel was heated in vacuum stove (Splabor, SP-104/27) at 150 ºC during 4 h and, subsequently, the magnetic nanoparticle was washed with acetone for impurity remove.

Crystalline structure was confirmed using an X-ray diffractometer (D-5000 Bruker AXS) with Cu-Kα radiation and ICSD database was used to identify the crystalline phases. Crystallite sizes and net parameters were obtained after refining using Rietveld method and the Scherrer equation was also used [8, 9]. Magnetic

parameters were obtained from the hysteresis loop using a vibratory sample magnetometer (EZ9 model, Microsense).

2.2 Alginate-magnetite core-shell production and characterization

Magnetite nanoparticles were coated with sodium alginate (NaHCO, Êxodo Científica, 90.8% purity), dripped in 15 mL of a calcium chloride ($CaCl_2$, Êxodo Científica, 96% purity) solution. Magnetite suspension with 4 – 10 mg/mL was prepared with deionized water (pH = 9) and homogenized in ice bath in ultrasound (Ultronique, Desruptor) for 15 min. Alginate solution with 5 – 250 mg/mL was prepared with deionized water and shaken at 1200 rpm. The mixed solution (magnetite + alginate) was homogenized at ultrasound for 15 min in ice bath, frozen with liquid nitrogen and then vacuum liofilized (K105, LIOTO) at -50 ºC.

The chlorides solution acts as a reticulation solution and the dripping stage was made with 0 and 300 rpm, resulting in rounded particles. Literature indicates that this is the best shape for biomedical applications [10]. The core-shell particles were produced using a needle with 0.7 mm gauge (Descarpack, 3 mL, 25x0.7 mm) without its beveled tip.

Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) (SDT Analyser Q600, TA Instruments) measurements were performed at a heating rate of 10 °C/min in synthetic air in the temperature range of 25–400 °C.

Magnetic hyperthermia of the core-shell particles was evaluated in a 1 mL deionized water dispersion, using a coil exposed to alternated field to magnetic alternated field generation. Particles temperature was verified with an alcohol thermometer and the equipment parameters were: 50.7 V, 3.2 A and 160 W.

The swelling degree was verified after lyophilization the core-shell particles, adding 2 mL of deionized water and oven dried for 24 h at 37 ºC, as described by Cavalcanti [11]. After this time, the water excess was removed and the proportional weight difference correspond to this swelling degree.

3. RESULTS AND DISCUSSION.

The characterization of the magnetite nanoparticles was already verified in another research work [7], so this is briefly explained.

Figure 1a shows the X-ray diffractogram of the magnetite nanoparticles and this crystalline phase (Fe_3O_4) can be verified, despite the low temperature used in the heat treatment. This formation can be associated with the reduction gases liberation during the self-ignition process [7]. After Rietveld refinement, the parameters verified were: crystallite diameter = 5.9 nm, net parameter a = 8.347 Å, unity cell volume = 581,5 $Å^3$ and good of fit = 1.53.

Figure 1b presents the magnetization curve as a function of time, presenting a hysteresis curve quite narrow with low values for remnant magnetization (2.33 emu/g) and coercive field (11.1). This is a characteristic for a superparamagnetic material, which is a non-permanent magnet. The saturation magnetization for this material was 21.97 emu/g and the reason with the remnant magnetization resulted in a value of 0.011, confirming that the obtained nanoparticles are superparamagnetic with null hysteresis. Besides, the superparamagnetic particles do not keep any remnant magnetization after magnetic field removal, which is really interesting for in vivo hyperthermia applications [12].

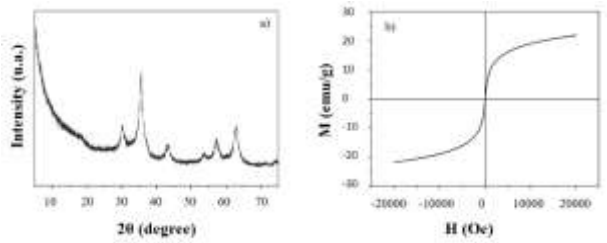

Figure 1. Magnetite nanoparticles characterization: a) X-ray diffractogram after thermal cycle, b) Magnetization curve. Reference: [7]

In Figure 2 it is possible to verify the thermal gravimetric variation (TG, blue curve) and differential thermal analysis (DTA, black curve) for the core-shell particles, with different concentrations. For all the samples, a mass loss with heat absorption was verified between 0 to 100 ºC related to water evaporation. Two events of mass loss, between 135 and 269 ºC and between 269 and 298 ºC can be associated with desorption and preliminary alginate degradation (first event) and additional alginate degradation (second event). They are also associated with a small heat absorption (endothermic event) [12].

Two of the concentrations presented similar mass loss, as verified in the Figures 2a and 2c, and this is associated with the similar concentration studied. Both presented mass loss around 85% between 30 and 110 ºC, with an endothermic event associated. These TG curves are characteristics of a sample with mass decomposition in one stage. As the decomposition starts in ambient temperature, the first mass loss can be associated with the humidity. The end of the event is up to 100 ºC and can be related with a bounded water molecule with the polymer [13, 14]. With the highest magnetite concentration (Figure 2b), only a small mass loss (around 2.5%) is verified between 30 and 110 ºC, associated with an endothermic event (humidity loss or gas desorption).

Table 1 shows the swelling degree of the particles obtained with different parameters. Considering the velocity used during the mixing the suspension for the dripping stage, increasing the velocity resulted in decrease the value for the higher magnetite concentration and opposite behavior for the smaller magnetite concentrations. The water absorption content by the polymers are usually related with the hydrophilicity of the chains and the density of the crosslinking agents used during the synthesis [15].

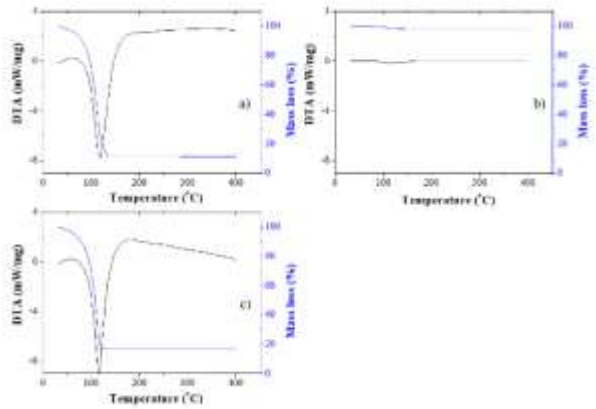

Figure 2. Thermal analyses (TG and DTA) of the core-shell particles with different concentrations: a) Alginate 4 mg/mL + magnetite 5 mg/mL, b) Alginate 10 mg/mL + magnetite 250 mg/mL, c) Alginate 4 mg/mL + magnetite 10 mg/mL

Table 1. Swelling degree with different concentrations.

Dripping (rpm)	Alginate	Nano magnetite	Swelling degree
0	4	5	87.88
	10	250	73.95
	4	10	71.52
300	4	5	89.36
	10	250	69.46
	4	10	91.62

Figure 3 presents the particles characterizations. As verified in Figure 3a, the scanning electron microscopy of the core-shell nanoparticle presented spherical morphology, with rough external surface, without fissures or apparent porosity, which facilitates the incorporation of compounds with pharmacological and/or industrial interests.

In Figures 3b and 3c are possible to verify the result of the magnetic energy transformation in thermal energy for possible hyperthermia application. For comparison, a pure magnetite (pink line in Figure 3b and green line in Figure 3c) and a calibration sample using pure water (blue line) are also presented. At 0 rpm (Figure 3b), the best results are verified with the super concentrate magnetite content (250 mg/mL), around to 40.5 ºC. Similar result is verified at 300 rpm (Figure 3c) with 10 mg/mL of magnetite. Pure magnetite presents a temperature of 43.5 ºC and calibration sample of about 37.5 ºC. In general, increasing the velocity the verified temperature also increases due to the sample agglomeration and stronger particle magnetic field.

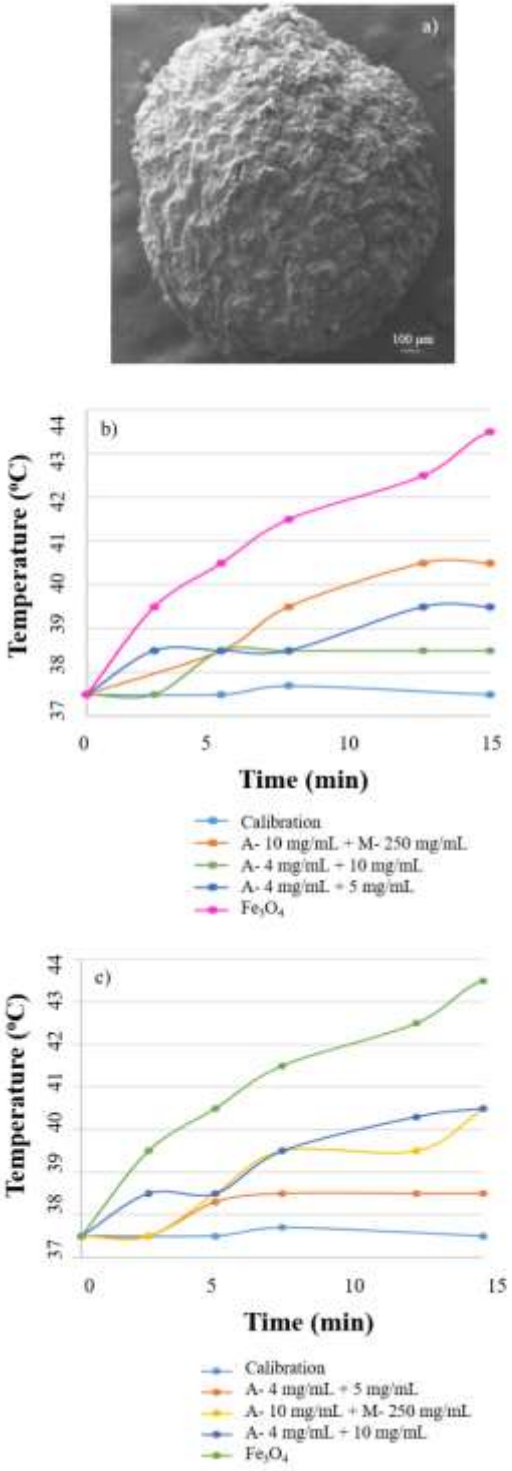

Figure 3. Characterization of the obtained core-shell particles a) SEM 100x , b) Magnetic hyperthermia at 0 rpm in the dripping stage, c) Magnetic hyperthermia at 300 rpm in the dripping stage

4. CONCLUSIONS.

Superparamagnetic particles were successfully produced with alginate coated magnetite like as 0.7 needle diameter, alginate concentration from 4 to 10 mg/mL, magnetite concentration from 5 to 250 mg/mL The obtained particles presented a crystallite size of ~5.9 nm, magnetic hyperthermia temperature around 40.5 ºC, high

swelling degree, non-toxicity and spherical shape. These results indicate the possibility to use in biomedical applications, for example, in hyperthermia application in cancer treatment. Besides, the results indicated that could be possible to increase the hyperthermia temperature increasing the magnetic nanoparticles concentration.

5. ACKNOWLEDGMENTS.

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