NANOSTRUCTURED MAGNETIC MATERIALS USED IN CANCER TREATMENT

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Abstract. *Nanostructured biomaterials present a series of advantages which have greatly contributed to the advancements made in the biomedical field. Magnetic nanoparticles are often used as a core, which is covered by a biocompatible layer, determining a core-shell structure that can be used in the transport of bioactive components. Magnetite is a mineral that belongs to the iron oxide category and differs from the rest of the iron oxides by the fact that it contains both divalent and trivalent iron ions. The present study is focused on the development of magnetite based nanostructures functionalized with cytostatic by co-precipitation method, starting from $FeCl_3$ and $FeSO_4$, in an alkaline environment. For characterizing the obtained materials, a set of techniques were used, among which FT-IR spectrometry, Scanning electron microscopy, Transmission electron microscopy etc.*

Keywords: magnetite, chitosan, cannabidiol, co-precipitation, cancer

1. Introduction

Cancer is a major health problem throughout the world, which is accentuated by changing environmental conditions and especially lifestyle [1]. Lung cancer is one of the most common forms of cancer and an important cause of death in adults. Pulmonary neoplasms are often diagnosed late, in advanced stages of cancer, the large size of the lung allowing the tumor to grow and develop long before the first symptoms appear [2, 3]. Hence over one million deaths occur annually worldwide following a diagnosis of lung cancer. The current treatment methods have a multimodal approach, which combines the benefits of surgery,

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radiation and chemotherapy in a measure dependent on the stage of the disease, the tumor capacity to be eliminated and the general health condition of the patient [4].

Chemotherapy is currently known as an essential component of cancer treatment, used also for patients with fully resected tumors. Moreover, for patients diagnosed with primary tumor metastases (55% of cases), chemotherapy forms the basis of treatment and critically influences the patient's chances of survival and quality of life. However, chemotherapy presents the main disadvantage of affecting both cancer and healthy cells, the inability of traditional antitumor drugs to differentiate between sick and healthy cells causing significant toxicity and inducing unavoidable side effects. Non-specific activity of the usual antitumor active substances and their inadequate transport to the affected tissue also limit the dose of drug that can be used [5, 6].

In order to improve the therapeutic effect of the drugs, specialists have focused their efforts on the development of new transport media that will ensure a targeted delivery of the active substance. Various forms of transport systems have been created, including the use of inorganic materials (gold nanoparticles, carbon materials, mesoporous silica), composite inorganic-polymer materials or even liposomes. Although these new supports have some advantages, such as improving the solubility of the drug and prolonging its maintenance in circulating form, their use in the clinical context has some limitations, such as lack of targeted action at the tumor process, low cellular internalization and bioaccumulation in healthy tissues, that attract important side effects themselves [7].

Magnetite (Fe_3O_4) can be successfully used in tissue engineering, as a controlled release system for active bio-substances, in magnetic resonance imaging or in hyperthermia therapy, especially due to the susceptibility of this material to be magnetically guided in the area of interest, but also due to its positive influence on certain cell types [8, 9].

Coating the magnetic particles with large molecules, such as polymers (e.g. chitosan) or surface active agents, gives them better stability, facilitating their dispersion and thus avoiding particle agglomeration. Chitosan (CS) is a natural polymer, being the major component in the exoskeleton of arthropods and cell walls of fungi. The main properties that have determined the use of chitosan in the biomedical field are: biocompatibility, biodegradability, lack of toxicity. This is a material with hemostatic, analgesic, antifungal and antibacterial properties, usually used as a wound dressing, as it does not induce inflammation or allergic reactions in clinical practice [10, 11].

Natural products that cause cancer cell apoptosis are important instruments in cancer suppression. Due to the extensive availability of raw materials, loaded with essential oils (cannabidiol - CBD) is one of the handiest solutions. Besides the fact

that they are natural ingredients that have no adverse effects on the human body, synthesizing herbal oils is a very cheap process [12-15].

The current situation requires the development of locally controlled drug release systems that can ensure increased efficiency and implicitly the use of a minimal amount of active substances materialized in minimal systemic toxicity, which is the subject of this experimental study. Each component of the chosen system for this paper plays a very important role in various medical applications, even when used individually.

2. Materials and methods

Iron (II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), iron (III) chloride ($FeCl_3$), ammonium hydroxide solution 25% (NH_4OH), acetic acid ($C_2H_4O_2$), chitosan low molecular weight – CS and cannabidiol solution – CBD ($C_{21}H_{30}O_2$) were of analytical purity and purchased from Merck (Sigma-Aldrich).

In order to obtain 1 g of magnetite, first 1,2 g of $FeSO_4 \cdot 7H_2O$ and 1,4 g of $FeCl_3$ were dissolved in 200 mL distilled water under continuous magnetic stirring, and the resulted mixture was named Solution I. Simultaneously, another solution - Solution II, was obtained by adding 15 mL NH_4OH in 100 mL distilled water under continuous magnetic stirring, thus giving an alkaline pH to the reaction medium, necessary for the co-precipitation process. Solution I was added dropwise to Solution II, maintaining constant the flow by means of a peristaltic pump. The reaction is performed at room temperature (25 °C) and a pH value of 10. Because ammonia is a volatile substance, and its volatilization implies a decrease in pH during synthesis, in order to maintain a fixed pH, 2 mL of it is added every 10 minutes to the reaction medium.

After the dripping process is completed, the accelerated decantation of the magnetite nanoparticles is realized by placing a 100 kgf magnet underneath the beaker. The resulted black precipitate was washed with ultrapure water in order to remove the reaction by-products and possible excess of iron containing precursors or NH_4OH, until neutral pH. After washing, the nanoparticles are dried at 60°C for 24 hours and the obtained powder is forward referenced as Fe_3O_4.

In order to obtain the magnetite nanoparticles coated with chitosan, the first step in the synthesis was to obtain a 5% acetic acid solution in which the chitosan will be solubilized. Thus, 2g CS were added in 300 mL solution 5% $C_2H_4O_2$ and allowed to stir for 3 hours at room temperature, until complete solubilization. Ferric chloride and ferrous sulfate heptahydrate, in the stoichiometric proportions described in the previous synthesis, were added in the solution containing chitosan, afterwards following the same steps as previously described for Fe_3O_4. The obtained powder is forward referenced as Fe_3O_4@CS.

For the synthesis of a drug delivery system with minimal systemic toxicity, based on magnetite and natural essential oil with antitumor effect, a similar procedure was followed. To obtain chitosan coated magnetite nanoparticles with 20% (% wt.) of bioactive substance with antitumor effect (CBD), the equivalent mass of drug related to 1 g of magnetite was added to the alkaline solution of NH_4OH and the resulting mixture was subjected to homogenization until solubilized. In the precursor solution of Fe ions (II and III) prepared under the same conditions as in the case of Fe_3O_4, was added the solution of chitosan in acetic acid. After decantation, washing and drying processes, the obtained powder will be further referred to as *Fe_3O_4@CS+CBD.*

3. Results and discussion

Fourier Transform Infrared Spectroscopy (FT-IR) was performed using the Nicolet iS50R spectrometer. The measurements were performed at room temperature, using the Total Reflection Attenuation Module (ATR), with 32 scans of the samples between 4000 cm^{-1} and 400 cm^{-1} being performed, at a resolution of 4 cm^{-1}. Spectral data recording was possible by connecting the spectrometer to a data collection and processing unit, through the Omnic work program. The aim of this technique was to demonstrate the functionalization of the surface of magnetite nanoparticles by adding chitosan and biologically active substance (cannabidiol).

The FT-IR spectroscopy results for Fe_3O_4, Fe_3O_4@CS and Fe_3O_4@CS+CBD are presented simultaneously in Figure 1. It is observed in the characteristic spectrum of Fe_3O_4 the presence of the band from 551 cm^{-1}, specific to the Fe-O bond in the magnetite structure, which decreases slightly in absorbance with the introduction of the polymer. Also, after the addition of chitosan, a series of bands characteristic to it can be identified, especially at 1622 cm^{-1} and 1520 cm^{-1} attributed to the amide I and amide II groups, which demonstrates the successful coating of the oxide particles with the polymer layer. In addition, absorbance bands were identified at 1062 cm^{-1} specific to the C-O bond in the structure of saccharides, while at 1374 cm^{-1} the deformation of the -CH_3 group was found. The bandwidth in the region 3000 cm^{-1} - 3500 cm^{-1} corresponds to the stretching vibration of the N-H and O-H bonds, and the band from 2900 cm^{-1} corresponds to the stretching vibration of the C-H bond.

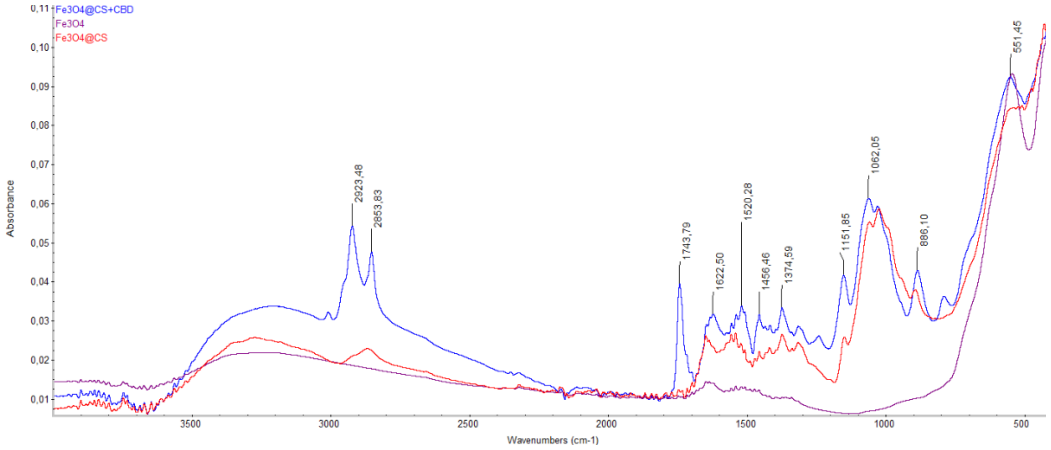

Fig. 1 FT-IR spectra of Fe_3O_4, Fe_3O_4@CS and Fe_3O_4@CS+CBD

After the introduction of the natural oil, an increase in absorbance of the bands corresponding to the stretching vibration of the O-H bonds is observed, as well as the appearance of some new bands, specific to it. Thus we notice the 2 bands from 1622 cm^{-1}, respectively 1520 cm^{-1}, which characterize the stretching vibration of the C = C bond in the benzene nucleus, as well as the 2 bands from 2853 cm^{-1}, respectively 2923 cm^{-1}, specific to the methylene group from CBD structure.

In this paper, scanning electron microscopy (SEM) was used to highlight aspects related to the morphology of the synthesized samples, respectively their particle size. The micrographs acquisition was possible using a high resolution scanning electron microscope, Inspect F50, at 30 KeV, at various magnifications.

Figure 2 shows the SEM images of Fe_3O_4, Fe_3O_4@CS and Fe_3O_4@CS+CBD nanoparticles. It can be observed that Fe_3O_4 nanoparticles are present in all samples, mostly disposed in agglomerates, with a spherical morphology and the diameter between 3 nm and 10 nm for the standard sample, better observed by TEM analysis. Analyzing the Fe_3O_4@CS powder, the formation of magnetite nanoparticles with a diameter between 3 nm and 7 nm is observed. Coating the nanoparticles with a protective polymeric layer determined a smaller particle size compared to the standard sample, with a lower tendency to aggregate, as a result of the decrease in surface tensions and magnetization.

Transmission electron microscopy images – TEM (Figure 3 and Figure 4) were acquired with the help of a high resolution transmission electron microscope Tecnai model G2 F30 S-TWIN equipped with Selected Area Electron Diffraction (SAED) detector, produced by FEI company. The microscope works in transmission mode at a voltage of 300 kV, with guaranteed point and line resolution of 2 Å and 1 Å respectively.

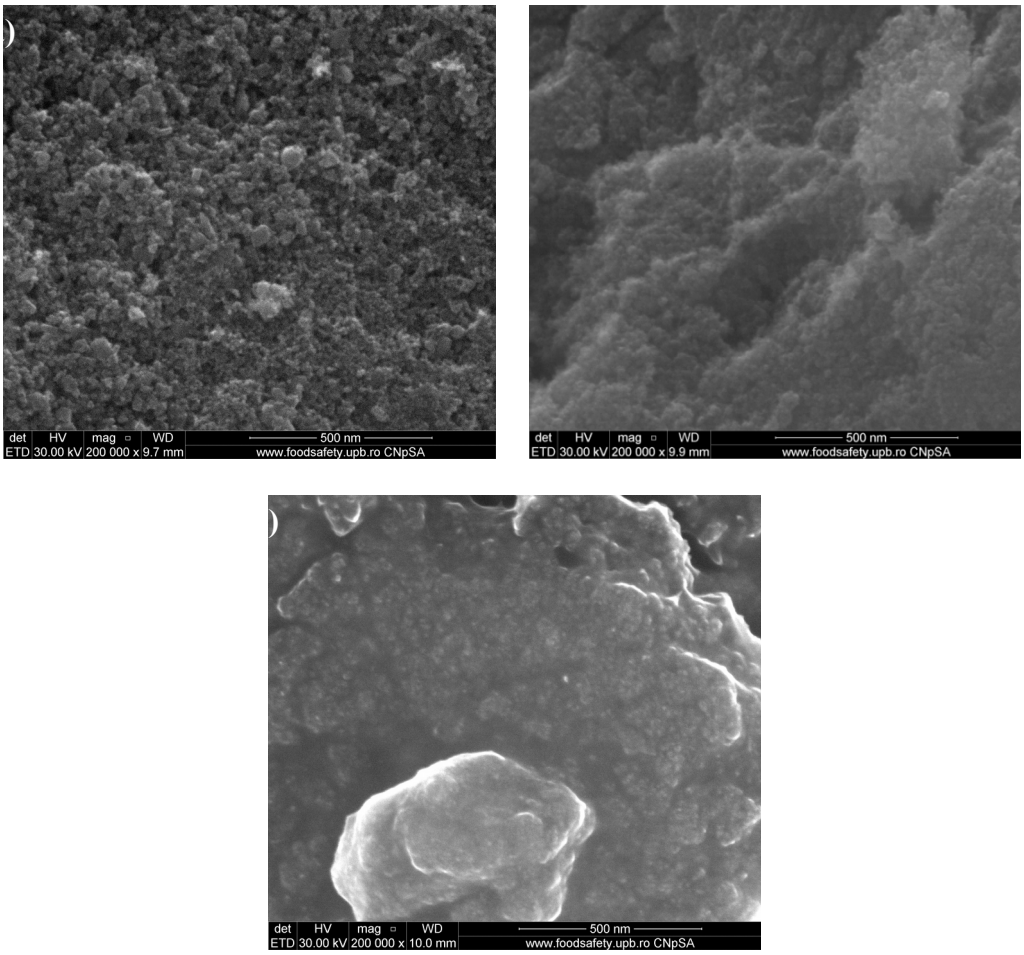

Fig. 2 SEM images of (a) Fe_3O_4, (b) Fe_3O_4@ CS and (c) Fe_3O_4@CS+CBD

From the presented micrographs, the nanostructured character of the obtained particles can be noted, with dimensions between 5 nm - 20 nm and polyhedral shape. It is observed that in the case of the simple magnetite sample, the particles have a high tendency to agglomerate, which decreases after the addition of the polymer. This fact again highlights the changes that have taken place on the surface of Fe_3O_4 and which are meant to give it more stability in contact with the physiological fluids and oxygen in the blood.

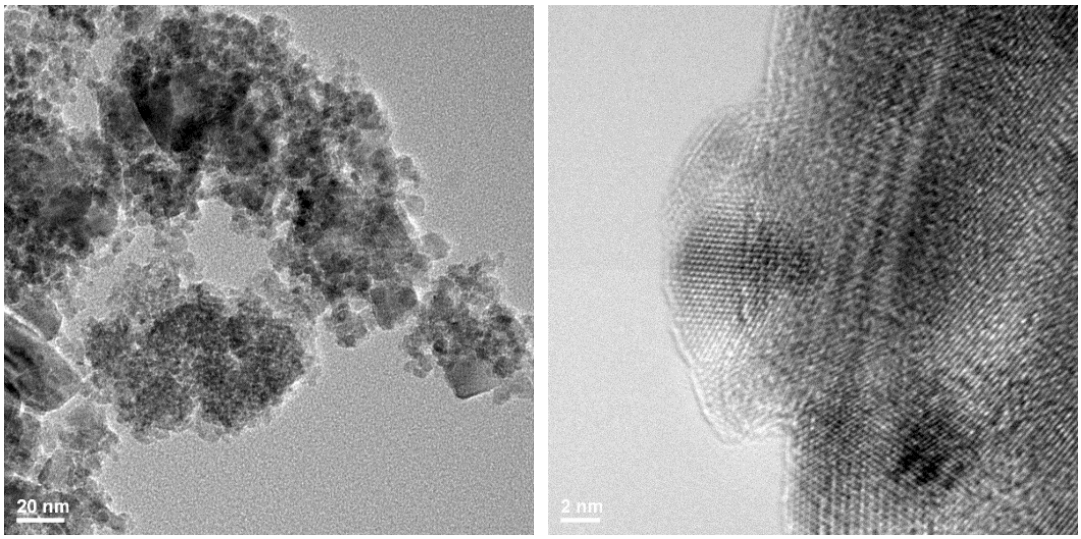

Fig. 3 TEM images of Fe_3O_4

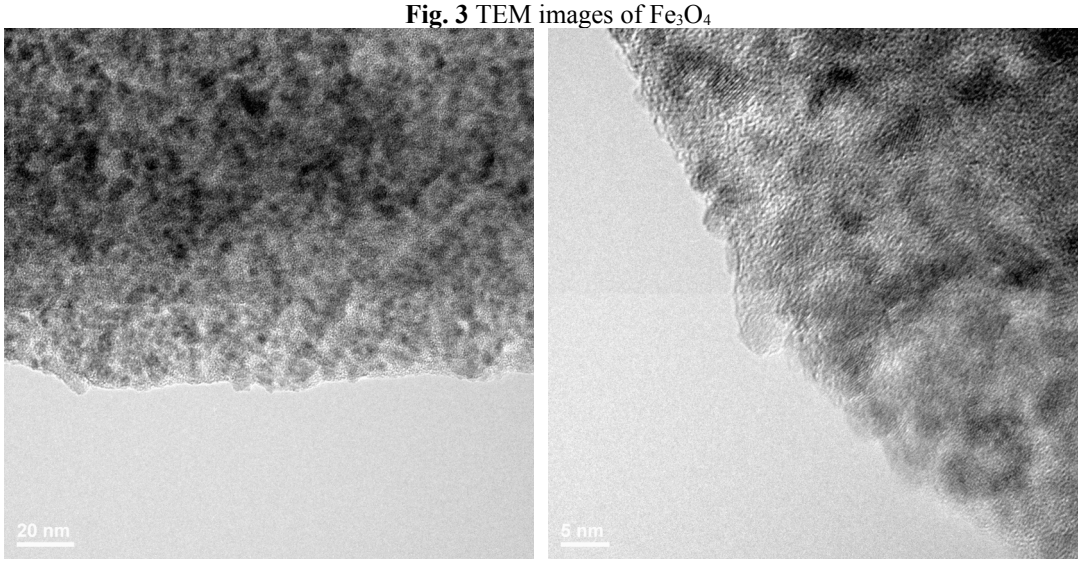

Fig. 4 TEM images of Fe_3O_4@ CS

The antitumor character and implicitly the *in-vitro* qualitative assessment of cell viability (Figure 5) for the obtained nanosystems was evaluated using the MTT assay. For this purpose, the SW-403 line of cancer cells was used, which were incubated in the presence of the materials to be analyzed. To each well were added 10 μL of MTT solution and 90 μL of culture medium specific for each cell line (MTT is added to the medium at a final concentration of 0,5 mg/mL). The plate was incubated at 37 °C (constant temperature) for 4 hours or until the intracellular crystals of violet formazan were visible under the microscope.

After the incubation period, the cell lysing solution was added to each well. The plate was then covered and incubated in the dark until the cells were lysed and the purple formazan crystals dissolved. The absorbance was afterwards read at a wavelength between 550 nm - 600 nm.

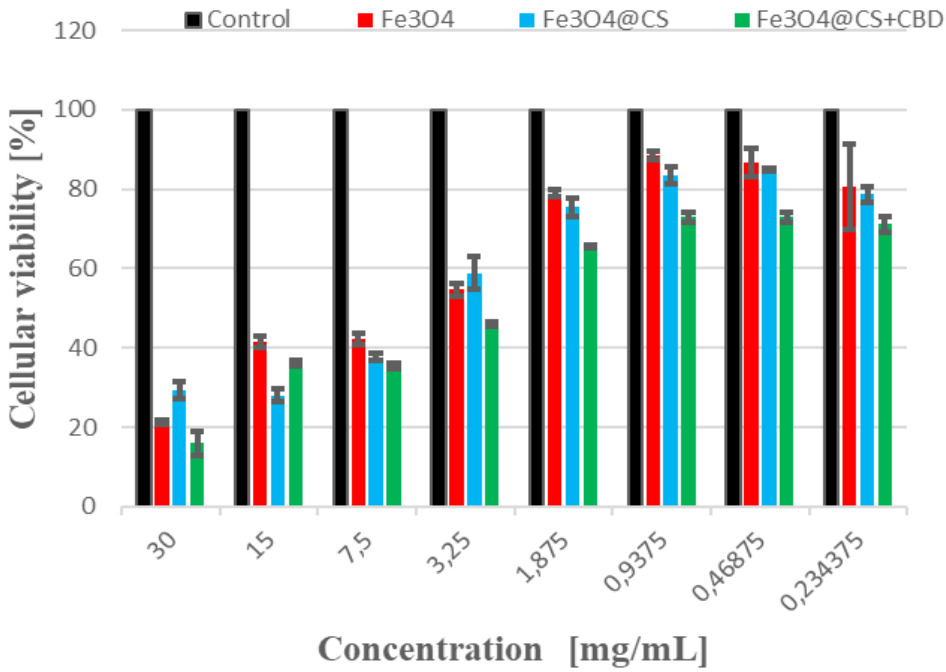

Fig. 5 *In-vitro* evaluation of cellular viability for SW-403 cancer cell line

The samples synthesized in this work were added at different concentrations in MTT / SW - 403 medium to evaluate the optimal concentration at which the tumor cells have the lowest cell viability. It is observed that, with the increase in the concentration of samples, in general, the cell viability of SW-403 cells decreases. At the highest concentration used (30 mg/mL), after 36 hours of powder dispersion in the incubation environment, only about 25% of the initial cells are still alive, proving the antitumor effect of the obtained nanosystems.

Conclusions

Magnetite nanoparticles (Fe_3O_4) were obtained in this work by co-precipitation, in the presence of a polymer (chitosan) and a biologically active substance (natural oil - cannabidiol) with antitumor properties was loaded. It has been shown that the obtained materials are nanostructured, with small dimensions (around 10 nm), with an antitumor potential demonstrated on the SW-403 tumor line, and with potential applications in targeted chemotherapy. In this regard, we

intend to continue the study by *in-vitro* evaluation of the system's capacity to release the active substance in a controlled manner, as well as to modify the synthesis parameters in order to achieve a controlled, slow release, after the cellular internalization takes place.

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