MAGNETITE-BASED DRUG DELIVERY SYSTEMS FOR THE CONTROLLED RELEASE OF CYTOSTATIC AGENTS

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In this study, the potential of magnetite particles as the nanostructured support for the loading and controlled release of two cytostatic agents, namely 5-fluorouracil and irinotecan, was investigated. Magnetite particles were obtained through the solvothermal reduction method, while drug loading was performed through the dispersion of particles into drug solutions. The obtained systems were analyzed through a series of characterization techniques, namely X-ray diffraction (XRD) to assess the mineralogical phases within the nanostructured supports, scanning electron microscopy (SEM) for investigating their morphological features, Fourier-transform infrared spectroscopy (FT-IR) for confirming the loading of the chemotherapeutics on the surface of the particles, thermogravimetry and differential scanning calorimetry (TG-DSC) and UV–Vis spectrophotometry for determining the bioactive compound loading efficiency and cumulative release. The obtained results proved the suitability of magnetite particles for the loading of irinotecan but not of 5-fluorouracil, and for its sustained release over time.

Keywords: magnetite, cancer treatment, 5-fluorouracil, irinotecan, drug delivery systems

1. Introduction

Magnetite particles (Fe_3O_4) are characterized by excellent structural and magnetic properties, showing great promise for applications in the biomedical area owing to a lack of cytotoxicity and increased chemical stability [1]. However, in order to ensure their suitability for applications involving targeted drug delivery and controlled release, hyperthermia, and enhanced magnetic resonance imaging (Fig. 1), magnetite particles should possess some key physico-chemical and structural characteristics, such as reduced size with narrow size distribution and superparamagnetic character with high saturation magnetization values [2].

Selective drug delivery refers to the use of particles for the transport of drugs to targeted cells, tissues, and organs via local or systemic blood circulation, which further allows for the drugs to be released and act directly on the disease-affected areas by generating their therapeutic effects. This selective delivery method stimulates the activity of therapeutic molecules at the targeted sites while also reducing the associated adverse effects in healthy areas, thus maintaining a

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minimal systemic effect [3, 4]. In this context, current scientific research focuses on the application of micro and nanoparticles as drug or other bioactive therapeutic molecule carriers to improve therapeutic efficacy and delivery, as well as to reduce side effects. Among these nano/microparticles, Fe_3O_4 nanoparticles are widely used in a variety of fields, such as biosensing, separation techniques, biotechnology, magnetic fluids, energy storage, and catalysis [5, 6].

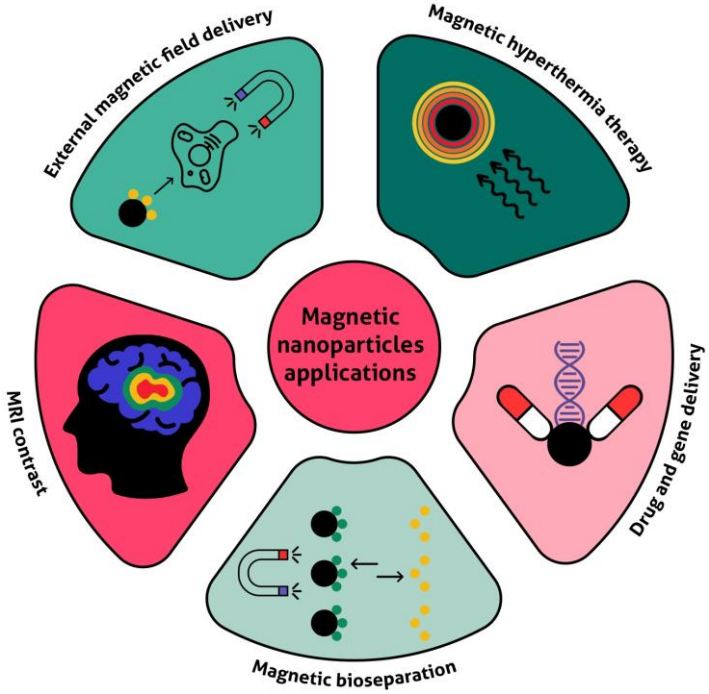

Fig. 1 The main applications of magnetic nanoparticles (reprinted from an open access source [7])

Owing to their unique magnetic characteristics, considerably reduced toxicity, suitable biodegradability, and reactive surface due to the hydroxyl groups present that allows for easy modification with biocompatible coatings, magnetite micro/nanoparticles are particularly beneficial in selective drug delivery [8]. In addition, they exhibit superparamagnetic behavior characterized by the lack of residual magnetization following the external magnetic field removal. In this manner, coagulation can be avoided, thus reducing agglomeration inside the body. Additionally, applying an external magnetic field at the tumor tissue site, magnetite micro/nanoparticles can release drugs and concomitantly generate heat for thermal therapy [9].

Therefore, within the present study, the loading and controlled release of cytostatic agents, namely 5-fluorouracil and irinotecan, from the surface of magnetite particles were investigated. In this manner, the cytostatic drug therapeutic dosage and the associated adverse reactions could be reduced.

2. Materials and methods

The materials used included sodium acetate trihydrate (NaAc), which was purchased from Silal Trading (Bucharest, Romania); anhydrous ferric chloride, anhydrous ethylene glycol (EG), polyethylene glycol (PEG), ethanol, 5-fluorouracil, and irinotecan, from Sigma-Aldrich Merck (Darmstadt, Germany); and phosphate buffer saline (PBS), from Carl Roth (Karlsruhe, Baden-Württemberg, Germany).

2.1. Magnetite particle synthesis

Magnetite particles were obtained according to the procedure developed by Deng et al. [10] and further described in our previous study [11], where anhydrous ferric chloride (7.2 g) was dissolved in EG (360 ml) under magnetic stirring until complete dissolution. Afterwards, NaAc (51.6 g) and PEG (9 g) were added and left under magnetic stirring for 30 minutes. The mixture was transferred to a 500 mL capacity autoclave and maintained for 12 h at 200 °C. The precipitate was magnetically separated, washed under ultrasound sonication with water and ethanol to remove the solvent and the unreacted reagents, and placed inside the laboratory oven at 50 °C for 12-h drying.

2.2. Drug loading

The synthesized magnetite particles were further used for 5-fluorouracil (Fe_3O_4@5-FU) and irinotecan (Fe_3O_4@Iri) loading. Initially, 250 mg were degassed using a vacuum pump, and dispersed in 25 mL drug solutions of 1 mg/mL concentration. Dispersions were maintained under constant stirring for 48 h at 37 °C using an orbital shaker, followed by centrifuging at 10,000 rpm and drying at 50 °C for 12 h.

2.3. Physico-chemical and morpho-structural characterization

The powder was investigated through XRD using a Malvern PANalytical Empyrean diffractometer with Bragg-Brentano geometry (Malvern PANalytical, Almelo, The Netherlands) provided with a CuKα radiation with a 1.541874 Å wavelength to confirm that magnetite is the single crystalline phase present.

The morphological properties of the obtained particles were evaluated by SEM using a Thermo Fisher high-resolution microscope operated at 30 KeV (Thermo Fisher—former FEI, Eindhoven, The Netherlands). The secondary electron mode was used for acquiring the micrographs at different magnifications.

To determine the encapsulation of 5-fluorouracil and irinotecan, UV-Vis measurements were carried out using a Thermo Evolution UV–Vis spectrophotometer with a double beam and equipped with a standard 1 cm glass cuvette (Thermo Fischer Scientific, Waltham, MA, USA). The dispersions containing magnetite particles and drug molecules were magnetically separated and 1 mL of supernatant was withdrawn after 24, 48, and 72 h and analyzed by UV-Vis spectroscopy at the 220 nm wavelength.

The release profile of irinotecan was obtained as follows: 50 mg of irinotecan-loaded particles were immersed in 25 mL of PBS solution (pH value of

7.4), and the mixture was gently stirred at 37 °C. 500 µL of the released supernatant medium was withdrawn from the solution at predetermined time intervals. Meanwhile, after each sampling, fresh pre-warmed buffer (500 µL) from the original supernatant medium was added.

FT-IR was applied for confirming cytostatic drug presence within the Fe_3O_4@5-FU and Fe_3O_4@Iri samples. FT-IR spectra were obtained in the 4000-400 cm^{-1} wavenumber interval, with 64 scans for each measurement, and a 4 cm^{-1} resolution. The equipment used was a Nicolet iS50 FT-IR spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

The TG-DSC technique was carried out on the STA TG/DSC Netzsch Jupiter apparatus (Selb, Germany). The thermal treatment was performed in a dynamic atmosphere of 50 mL/min and it was ranged in the 25-900 °C interval at a heating rate of 10 K/min.

3. Results and discussions

3.1. Magnetite particles analysis

As it can be seen in Fig. 2, the diffraction pattern of F_3O_4 shows nine diffraction peaks. According to JCPDS 04-008-4511, these diffraction planes are representative for F_3O_4 in the cubic crystal system (Fd-3m space group) [11, 12]. Moreover, the diffractogram shows a considerably high intensity and a reduced width of the diffraction peaks, which could be indicate the presence of large crystallites within the structure of the particles and, consequently, a high degree of crystallinity. In this context, the calculated unit cell parameters include a = b = c = 8.40 Å and α = β = γ = 90°, and an average crystallite size of 114.79 ± 19.45 nm, thus confirming the previous observations.

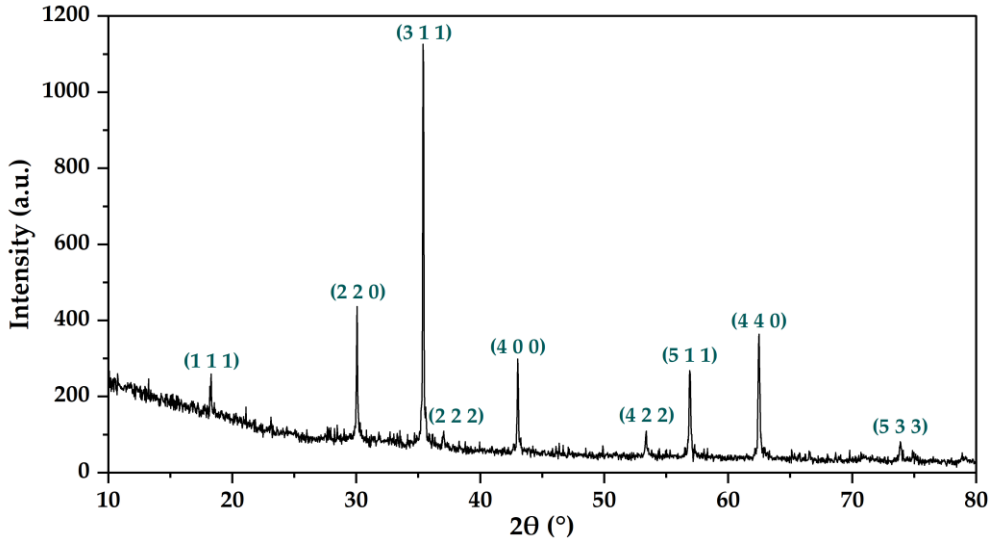

Fig. 2 XRD pattern for the synthesized magnetite particles and the associated Miller indices.

Fig. 3A, B, C depicts the SEM images obtained for the synthesized magnetite particles. It can be seen the particles present a uniform spherical morphology with an agglomeration tendency significantly lower than other

studies reported in the literature [13-15] due to higher particle sizes. Furthermore, the size distribution of the particles is monomodal, with an average 228 nm diameter (Fig. 3D).

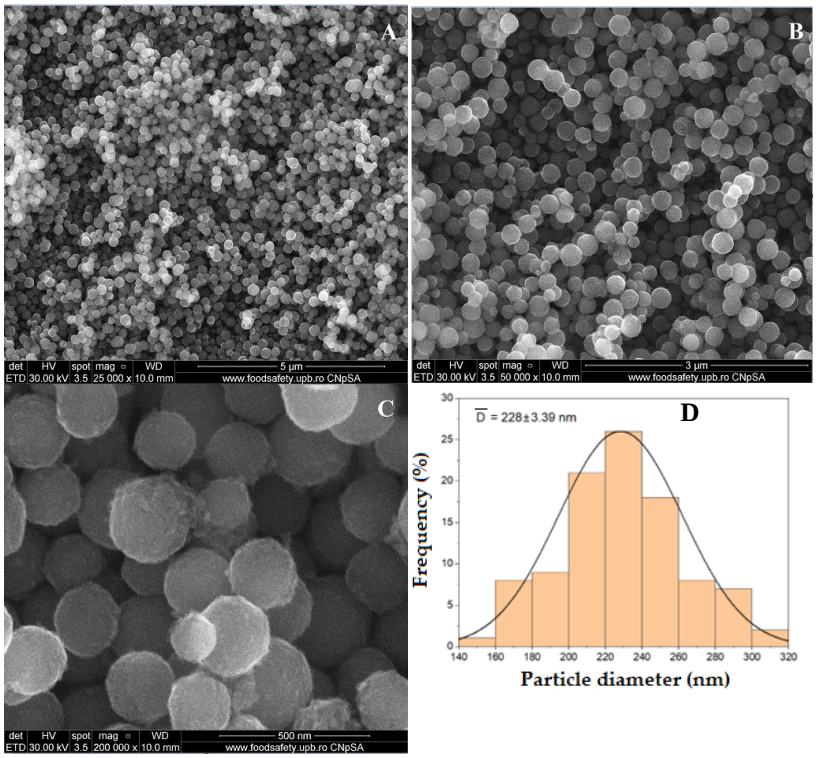

Fig. 3 Micrographs of Fe_3O_4 obtained at different magnifications (A, B, and C) and the associated size distribution of the particles (D).

3.2. Drug loading analysis

Furthermore, the UV-Vis spectroscopy analysis allowed for assessing the amount of drug loaded within magnetite particles by determining the concentration of the drug in the supernatant. Results showed that 5-fluorouracil does not attach to the surface of the particles, while the concentration of loaded irinotecan increases with the time allowed for contact between the drug molecules and magnetite particles. In this context, Fig. 4 depicts the loading capacity measured for the Fe_3O_4@Iri sample.

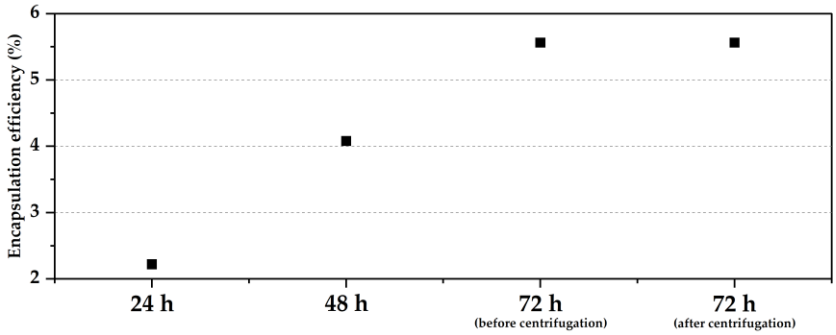

Fig. 4 Encapsulation efficiency determined at different time intervals for the Fe_3O_4@Iri sample.

The reason behind the lack of 5-fluorouracil attachment to the magnetite particles could be attributed to its molecular structure that causes steric repulsion and low binding energies [16]. Additionally, the lower encapsulation efficiency as compared to previous studies could be associated to an increased particle size that leads to lower surface areas [17]. Therefore, only the Fe_3O_4@Iri sample was selected for the subsequent characterization steps.

The characteristic bonds and functional groups of the pristine and irinotecan-loaded particles were assessed through FT-IR analysis (Fig. 5).

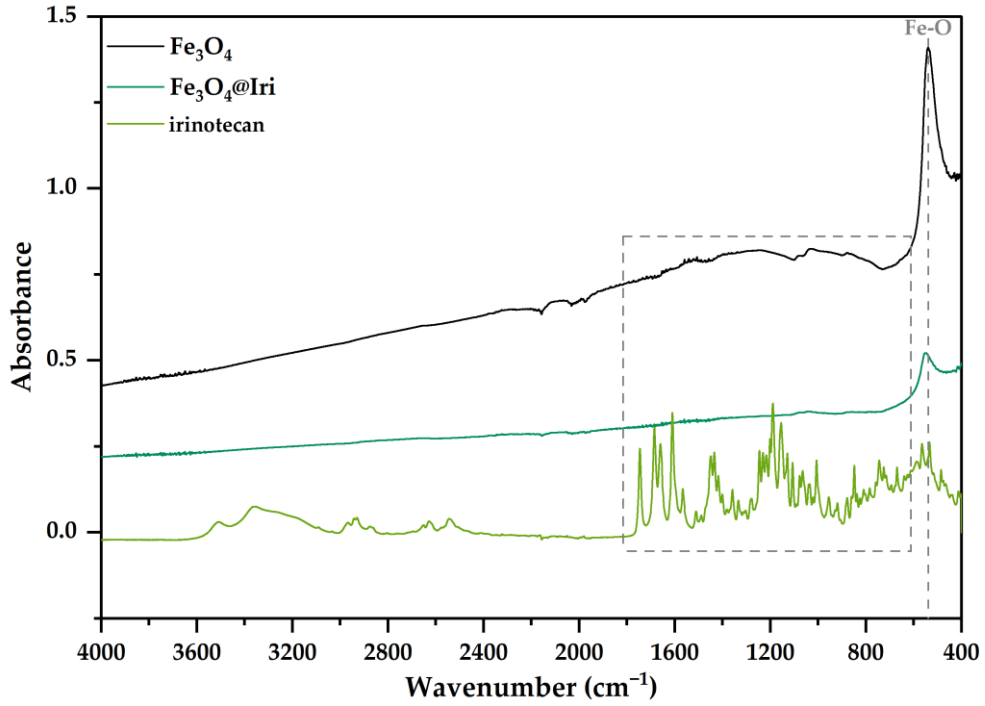

Fig. 5 FT-IR spectra of Fe_3O_4, Fe_3O_4@Iri, and irinotecan.

At the 540 cm^{-1} wavenumber, the representative Fe-O bond can be observed [18], thus confirming the magnetite formation. Nevertheless, the irinotecan loading leads to a shift of the absorption band to higher wavenumbers, i.e., from 540 to 548 cm^{-1}, and a decrease in absorbance, which are typically caused by the physical adsorption of the molecules onto the substrate and a decrease in the amount of the functional group, respectively [11]. Furthermore, as both PEG and irinotecan are characterized by fingerprints in the same region, the vibration bands specific for irinotecan are difficult to distinguish within the Fe_3O_4@Iri sample.

Fig. 6 depicts the TG-DSC curves registered for the Fe_3O_4 and Fe_3O_4@Iri samples. As it can be seen, they are losing 0.09% and 0.29%, respectively, of their initial mass up to 160 °C, caused by the elimination of water molecules. After 160 °C, there is a recorded mass gain of 3.42% and 3.19%, respectively, associated to the magnetite-maghemite transformation, i.e., Fe^{2+} to Fe^{3+}, through oxidation processes [19]. Nevertheless, the DSC curve shows two complementing exothermic peaks, which further indicates that the process does not occur in a single step, being controlled by the oxygen diffusion to the core of the particles,

which is oxidized at the highest temperature of ~400 ℃. The transformation of maghemite to hematite is evidenced by the small exothermic effect from 507.7 and 512.6 ℃ [20, 21]. The reddish residual mass is 94.10 and 93.16%. The amount of irinotecan loaded within the magnetite particles is approximately 1%, which differs from the value of approximately 5% estimated from UV-Vis due to differences in dry and wet sample measurements.

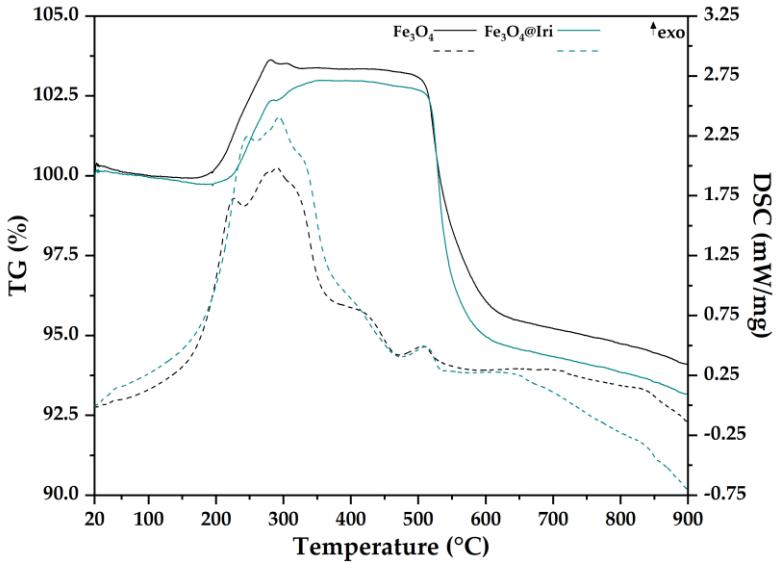

Fig. 6 TG-DSC curves of Fe_3O_4 and Fe_3O_4@Iri samples.

3.3. Drug release analysis

The release profile of irinotecan from the Fe_3O_4@Iri sample is depicted in Fig 7:

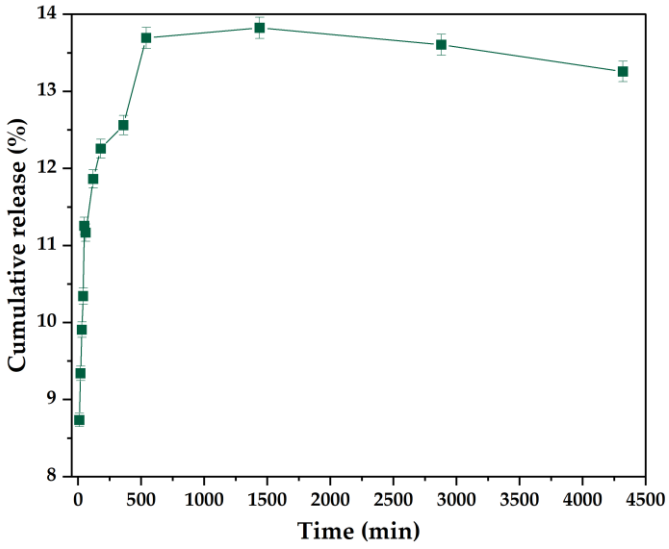

Fig. 7 Cumulative release profile for the irinotecan-loaded magnetite particles.

As it can be observed, the release profile reaches a plateau in the first 8 hours of release, with a maximum cumulative release of approximately 14%. The irinotecan release is sustained for almost 72 hours, thus confirming the potential of using magnetite particles as carriers for the controlled release of cytostatic agents. Nevertheless, an improved release profile in terms of prolonging the release time and ensuring higher drug encapsulation could be achieved by obtaining magnetite-based core-shell systems [22-24]. Silica, especially mesoporous silica, is one of the most widely investigated types of carriers that ensures high loading capacities and encapsulation efficiencies and controlled and prolonged drug release profiles [25-27].

5. Conclusions

The present work aimed to obtain Fe_3O_4 particles through the solvothermal reduction method that would be used for the loading and controlled release of chemotherapeutic agents (5-fluorouracil and irinotecan). The obtained systems were analyzed through XRD, SEM, FTIR, TG-DSC, and UV-Vis spectroscopy. XRD analysis confirmed that magnetite is the unique mineralogical phase, and it revealed a large crystallite size of approximately 115 nm. SEM provided information on the morphology and size of the microspheres, demonstrating the formation of spherical and uniform structures with an average particle diameter of 228 nm. UV-Vis spectroscopy showed that only irinotecan could be loaded onto the particles, with an encapsulation efficiency of about 6%. Furthermore, FT-IR spectroscopy and TG-DSC analysis proved the drug presence within the systems. Moreover, the release profile evaluated through UV-Vis reached a plateau after 8 h, thus proving the possibility to use magnetite particles as carriers for the controlled release of irinotecan. These results represent a step forward towards developing innovative applications in the field of medicine and targeted therapy.

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