SYNTHESIS AND CHARACTERIZATION OF NEW MAGNETITE NANOPARTICLES BY USING THE DIFFERENT AMINO ACIDS SUCH AS STABILIZING AGENTS

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This paper presents the obtaining of magnetite mesoporous nanoparticles by using L-proline, L-aspartic acid and L-asparagine as stabilizing agent, by using co-precipitation. In order to analyse the synthesized nanoparticles, Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction (XRD), Brunauer–Emmett–Teller (BET) and scanning electron microscopy (SEM) have been used. According to the obtained data, the as obtained nanoparticles were characterized from the point of view of structure and morphology. Moreover, the biological assays highlight that the as obtained magnetite@aminoacids core@shell structures (aminoacids = L-proline, L-aspartic acid and L-asparagine) does not affect cell morphology and viability of HCT8 cells. The highest impact on these cells was found for the aspartic acid (a known neurotransmitter) so, their use can be further exploited in cancer treatment, in a targeted drug delivery approach.

Keywords: magnetite nanoparticles, co-precipitation methods, specific surface area

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1. Introduction

In recent years, the researches achieved on magnetic nanoparticles are of particular interest due to the applicability in various fields. Of the areas of applicability of these nanoparticles those in biomedical field should be mentioned: magnetic resonance imagining, catalysis, biomedicine, drug delivery system, hyperthermia, sensor, etc. as well as in the environmental field, especially for waste water remediation, etc. [1-8].

According to the literature data, mesoporous magnetite nanoparticles can be synthesized by different methods such as: solvothermal, co-precipitation, sol-gel, hydrolysis, thermal decomposition [1, 2, 9]. According to Ha *et al*. [1] mesoporous magnetic nanoparticles were obtained through solvothermal method, by using a gas for the shape induction of mesoporous. The as obtained nanoparticles exhibit large specific surface area but their use in specific applications should involve the functionalization of their surface [1]. One of the most efficient methods of obtaining magnetite nanoparticles is co-precipitation method. Wei *et al.* [10] have obtained the magnetic nanoparticles starting from magnetite precursors, oleic acid and sodium citrate by co-precipitation method.

In this paper the magnetite mesoporous nanoparticles have been synthesized by co-precipitation method using various stabilizing agents such as: L-aspartic acid, L-proline and L-asparagine. Therefore, the structure and the morphology of the nanoparticles were investigated by using Fourier Transform InfraRed Spectroscopy (FTIR), powder X-ray diffraction (XRD), Brunauer–Emmett–Teller (BET) and scanning electron microscopy (SEM).

2. Experimental data

2.1. Materials

For preparation of mesoporous magnetite, the following precursors have been used: sodium hydroxide – NaOH (Silal Trading), iron (III) chloride – $FeCl_3$ (Sigma-Aldrich), ammonium iron (II) sulphate hexahydrate – $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$ (Silal Trading), L-proline $C_5H_9NO_2$ (Riedel-De Haën Ag Seelze-Hannover), L-aspartic acid $C_4H_7NO_4$ (Merck), L-asparagine $C_4H_8N_2O_3$ (Sigma). The precursors were used without purification.

2.2. Methods

The mesoporous magnetite nanoparticles were prepared by co-precipitation. In a 400 mL beaker 2.5 g stabilizing agent (L-aspartic acid, L-proline and L-asparagine) and 10 g NaOH were dissolved in 250 mL distilled water under vigorous stirring at room temperature. Over the resulted solutions, 100 mL of a mixture of Fe^{3+} and Fe^{2+} in the molar ratio 2:1 (6.9920 g $FeCl_3$ and

8.4520 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$) was sprayed), while black precipitates of Fe_3O_4@aminoacids are obtained. The spraying of the mixture of Fe^{2+} and Fe^{3+} is carried out under continuous mechanical stirring, the pH being set at 12. After the completion of spraying of the mixture, the suspensions obtained are placed in the ultrasonic bath for 30 minutes. Then the obtained precipitates were filtered and washed with distilled water until pH = 7 is obtained. Finally, the obtained magnetic powders are dried under vacuum, at 60 °C. Based on the above presented procedure, magnetite (Fe_3O_4), magnetite with L-asparagine (Fe_3O_4@L-asparagine), magnetite with L-proline (Fe_3O_4@L-proline), and magnetite with L-aspartic acid (Fe_3O_4@L-aspartic acid) have been obtained.

2.3. Equipment

The mesoporous magnetite powder has been characterized by FTIR, XRD, SEM and BET.

The FTIR spectroscopic analysis was made on a Nicolet iS 50 FT-IR spectrometer; the measurements were carried out in the range of 400 – 4000 cm^{-1}, using the resolution 4 cm^{-1} and co-adding 32 scans per each spectrum.

The X-ray difraction has been performed on a Panalytical X'Pert Pro MPD equipment, whose radiation is CuK_a, in the scan range of $2\theta = 10^o - 80^o$.

Scanning electron microscopy (SEM) analysis was made on QUANTA INSPECT F SEM device equipped with a field emission gun (FEG) and an EDS (X-ray energy dispersive spectrometry) detector.

The Brunauer–Emmett–Teller (BET) analysis was achieved for measuring specific surface area and the pore size by using Micrometrics Gemini V equipment.

2.4. Biological evaluation

For biological evaluation, 1.105 HCT-8 human colon cancer cells (**ATCC**® CCL-244™) were seeded in each well of 24 well plates and maintained in Dulbecco's Modified Eagle Medium (DMEM):F12 (Thermo Scientific, Hyclone Laboratory Inc, SUA), supplemented with 10% foetal bovine serum (Biochrom, Germany), at 37°C in a 5% CO_2 humidifies atmosphere. After 24h, the cells were treated with 100 mg/mL coated magnetic nanoparticles. For morphological evaluation, the cellular monolayers were fixed in 70% ethanol for 10 minutes, stained with 10 µg/mL propidium iodide and observed at inversed microscope. Cross-sectional images were obtained using the Zeiss Observer D microscope equipped with module for fluorescence. For cell cycle analysis, the magnetite mesoporous nanoparticles treated cells were harvested and fixed in cold ethanol for 30 minutes at -20 ^oC. Thereafter, the cells were centrifuged and stained with propidium iodide containing RNAse A solution (Cell Signalling Technology,

Netherland) and quantified using Beckman Coulter TM flowcytometer and analysed using FlowJo software, and Dean-Jett-Fox calculation method.

3. Results and discussion

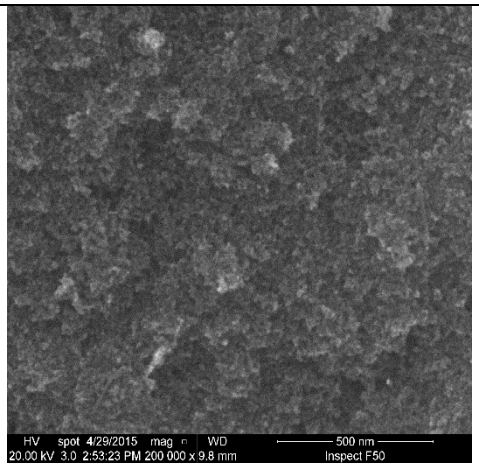
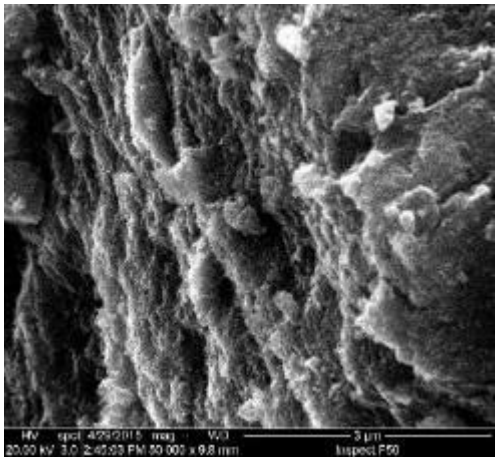
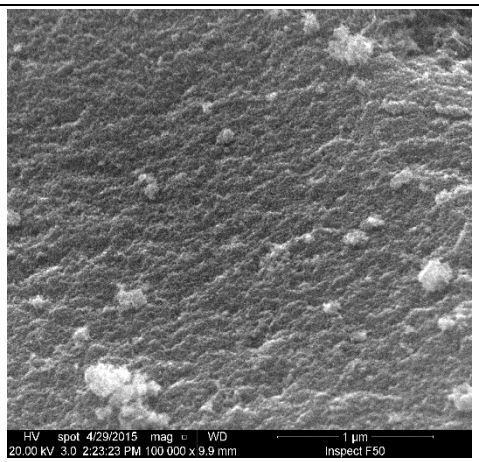
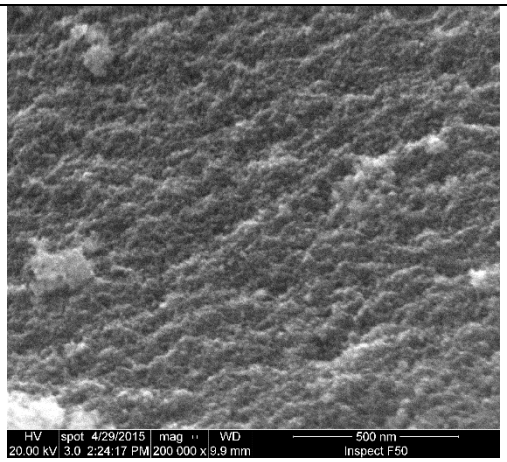
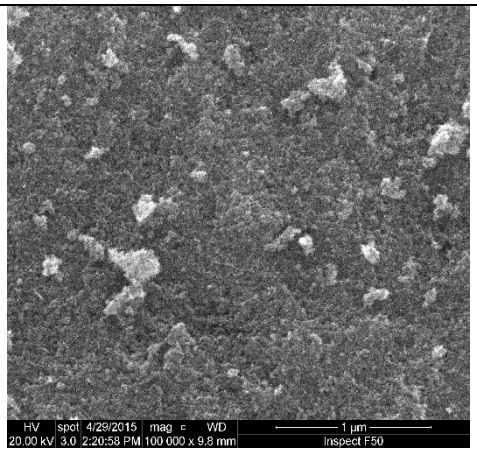
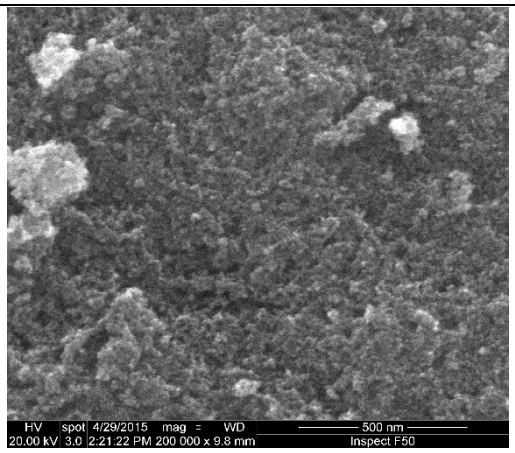
The magnetic nanoparticles have been characterized from point of view of their average pore size and specific surface area. According to the BET data, these characteristics are dependent on the used stabilizing. The bare magnetite sample shows an average pore size of 5.7 nm and a specific surface area of 156 m²/g; while in the presence of L-asparagine, the average pore size decreases to 5.4 nm and the surface area increases to 159 m²/g. In the case of magnetite nanoparticles stabilized with L-aspartic acid, the pore size became 6.96 nm, a significant increase of the pore size compared to the magnetite sample, while the specific surface area remains practically unchanged. In the case of magnetite stabilized with L-proline, a slightly pore size increase is observed from 5.75, to 6.2 nm, and a significant decrease in specific surface area, becoming 126 m²/g. These differences in the specific surface area and average pore size are due the used stabilizing agent. The resulted data of the BET analysis are presented into Table 1.

Table 1.

BET data of the magnetic nanopowders

Samples name	Specific surface area S_{BET} (m^2/g)	Average pore size D_{BET} (nm)
Fe_3O_4	156.0278	5.75
Fe_3O_4@L-asparagine	158.9864	5.41
Fe_3O_4@L-aspartic acid	155.7192	6.96
Fe_3O_4@L-proline	126.4056	6.20

The SEM images recorded for the mesoporous magnetic nanoparticles are presented in Fig. 1. Depending on the nature of the stabilizing agent, modification of the morphology of the mesoporous magnetic nanoparticles can be observed. In the case of bare Fe_3O_4, the particles have a nearly spherical shape and with rough particle surface which grow by aggregation of the small particles. By coating magnetite with the selected aminoacids, the as obtained magnetic nanoparticles show good dispersion.

Fe_3O_4	 
Fe_3O_4@L-asparagine	 
Fe_3O_4@L-aspartic acid	 

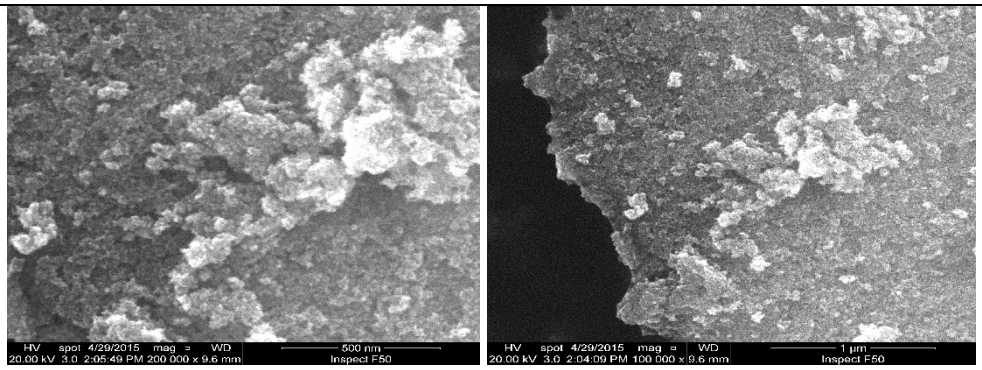
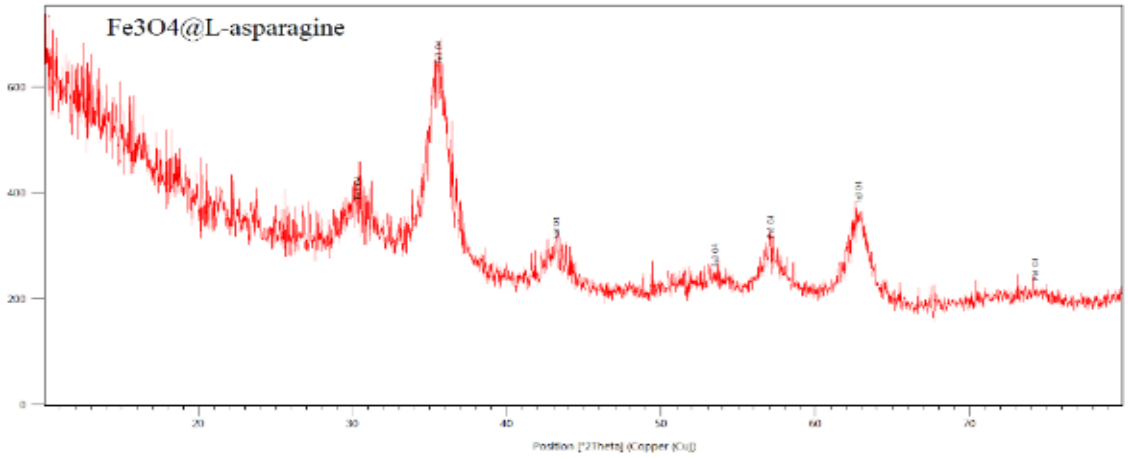
Fe_3O_4@L-proline	

Fig. 1. SEM images of the stabilized and non-stabilized magnetic nanoparticles obtained by co-precipitation

The X-ray diffraction patterns are shown information about crystallographic structure of the magnetic powders (crystallinity of the sample and the crystallite size of the obtained mesoporous nanoparticles) as well as phase composition and purity of the synthesized nanomaterials. The X-ray diffraction patterns are shown in Fig. 2. In all cases the peaks have similar characteristics denoting that these nanopowders exhibit similar crystallinity and crystallite size. All the XRD diffraction patterns reveal the peaks corresponding to (220), (311), (400), (422), (511) and (540) crystallographic planes, corresponding to the typical peaks of spinel structure, specific to magnetite. Based on the XRD patterns, the crystal structure is not altered because of the used stabilizing agent. Also, it should mention that no significant shielding due to the organic shell can be observed.



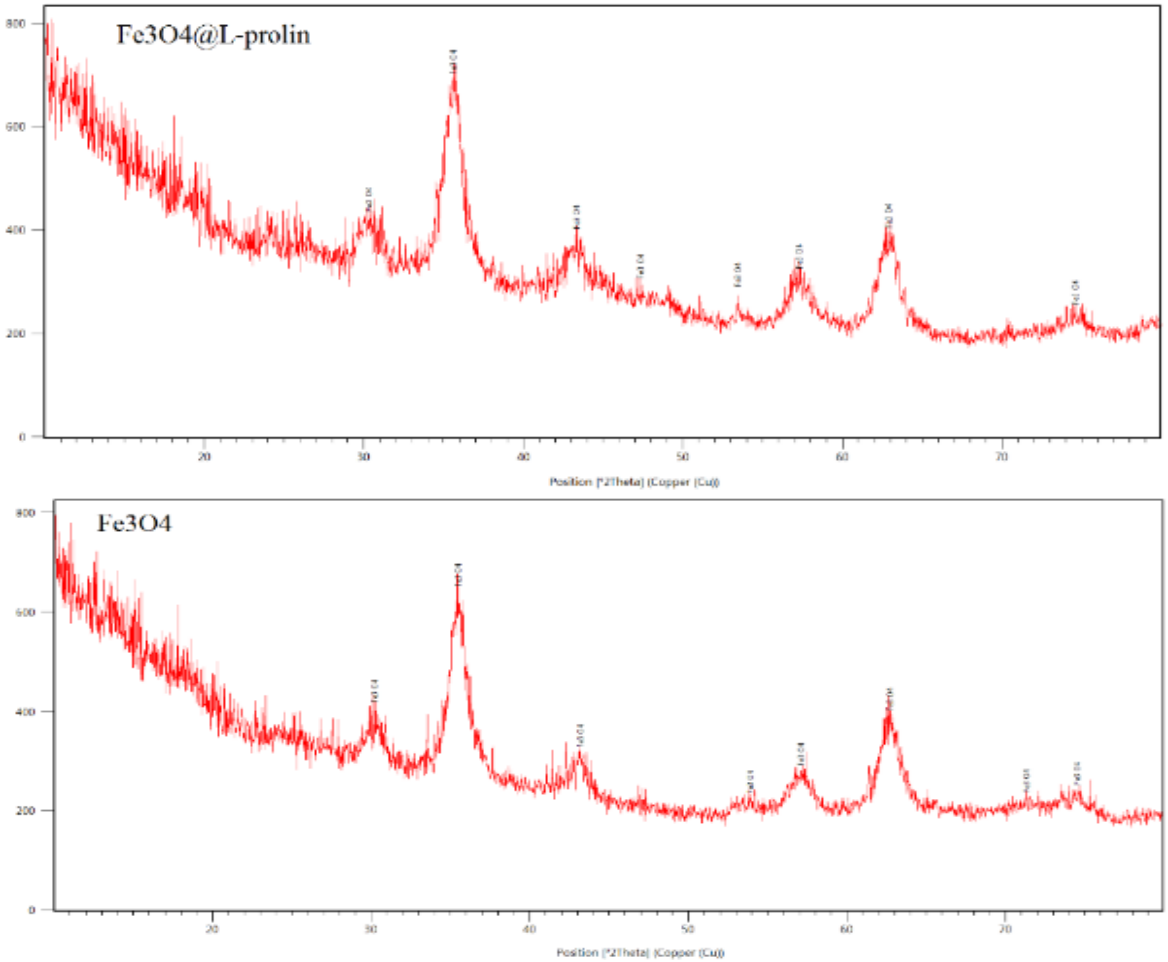


Fig. 2. X-ray diffraction patterns of coated and uncoated Fe_3O_4 nanoparticles

FTIR spectra of mesoporous magnetic nanoparticles are shown in Fig. 3. In IR spectra, the characteristic bands of Fe_3O_4 and stabilization agent can be seen. The characteristic band at 579 cm^{-1} is characteristic to Fe-O bond characteristic to Fe_3O_4 [11, 12]. This band is shifted to 530 cm^{-1} for acid coated materials indicating successful functionalization on the surface of the magnetite nanoparticles. The band at ~1635 cm^{-1} is assigned to the OH stretching vibration of Fe_3O_4. The appearance of band at 1600 –1630 cm^{-1} is characteristic to carboxyl group (-COOH) and corresponds to stretching vibration of C=O characteristic for the stabilized nanoparticles. The bands at 1337cm^{-1} and 1530 – 1552 cm^{-1} correspond to the asymmetric and symmetric stretching vibrations of carboxyl group (COO^-) of coating agents. Here, two binding modes have been suggested. In one mode, carboxylate is connected to the surface through one oxygen atom giving rise to symmetric and asymmetric vibrations, while in the other mode it is bound symmetrically to the surface giving rise to only stretching vibrations. Due

to the stabilization agents the magnetic nanoparticles exhibit a band at 1630 – 1635 cm^{-1} corresponding to NH bending and a broad band at 3333–3346 cm^{-1} indicating the presence of NH_2 and OH groups attached to the surface of the nanoparticles [11, 13].

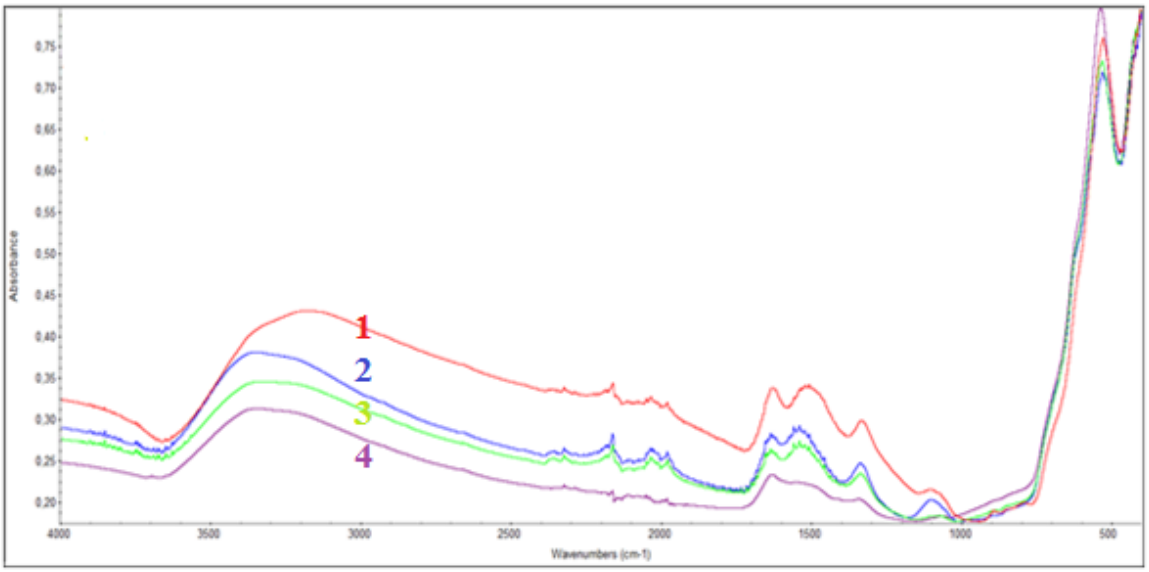


Fig. 3. FTIR spectra of coated and uncoated magnetic samples (1- Fe_3O_4, 2-Fe_3O_4@L-proline, 3-Fe_3O_4@L-asparagine, 4Fe_3O_4@L-aspartic acid)

The TEM images of obtained mesoporous coated nanoparticles with different magnification show that the magnetic nanoparticles are spherical (Fig. 4). Also, the TEM images revealed that the particle size for Fe_3O_4 is in the range of 4.5 – 5 nm while that obtained in the presence of L-asparagine and L-aspartic acid measure ~5.5 nm. This decrease in size may be due to the coating agent, which prevents agglomeration and hence reduces the size of the nanoparticles. However, small particles have larger surface area-to-volume ratio and represent a higher energy state comparing to that of larger particles. The crystallization of L-arginine coated magnetite nanoparticles showed no reduction in magnetic property and the growth was prevented because of the steric hindrance of coating agent.

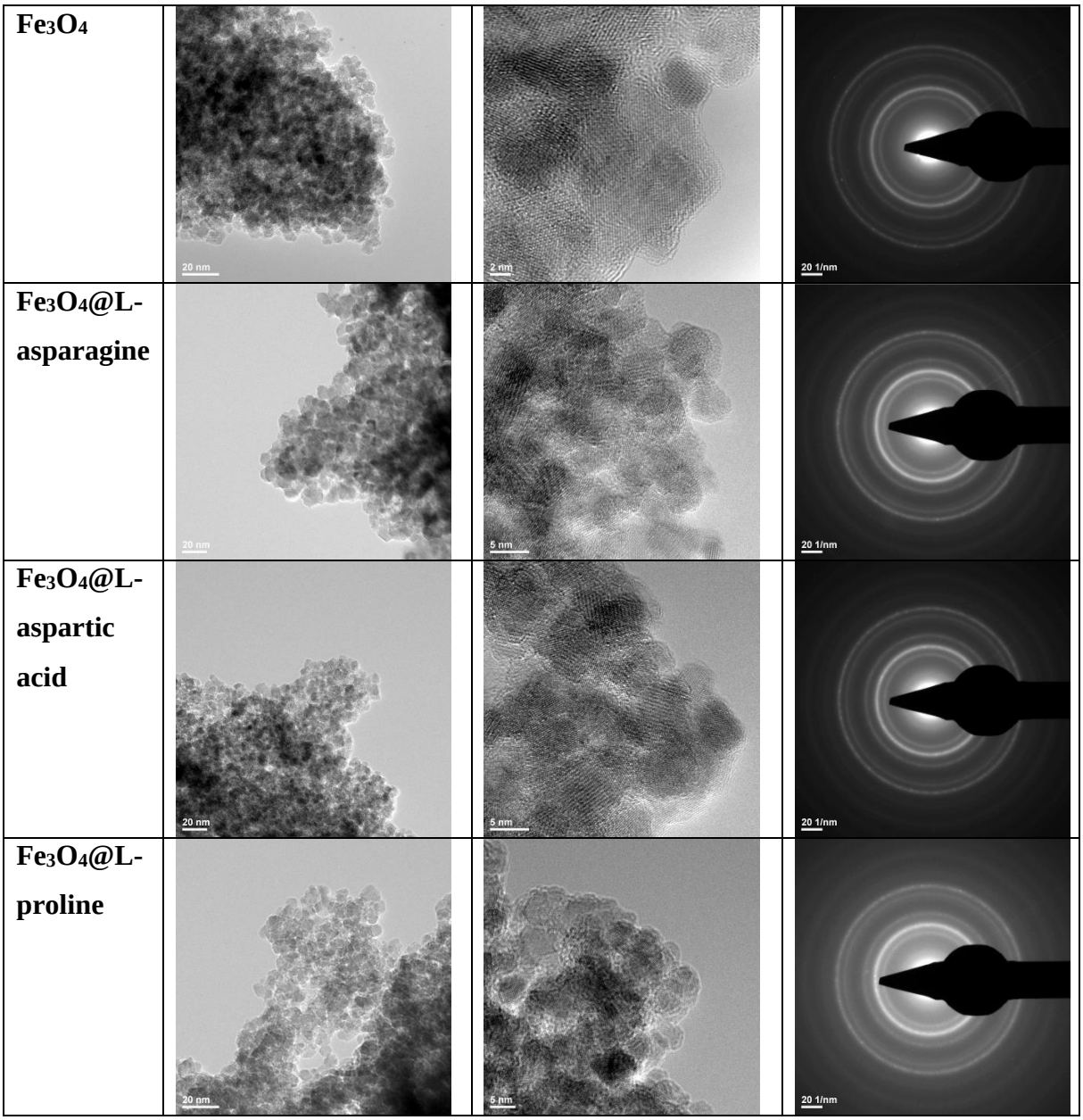


Fig. 4. TEM, HRTEM and SAED patterns of the coated and uncoated magnetic samples

The treatment with 100 μg/mL coated magnetite mesoporous nanoparticles does not affect cell morphology and viability of HCT8 cells (Fig. 5).

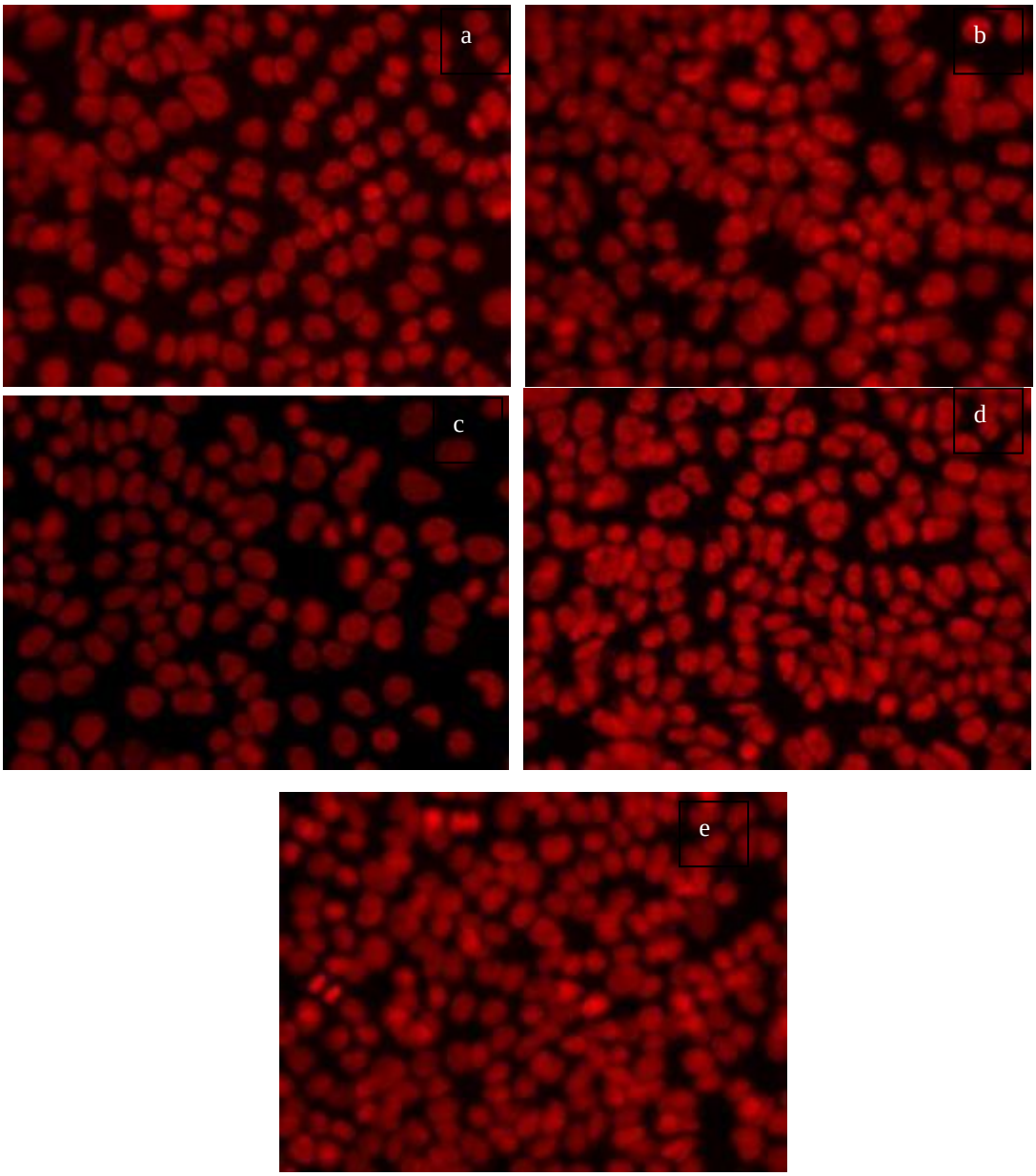


Fig. 5. The effects of the coated and uncoated magnetic samples on the HCT8 cells morphology (a) Fe_3O_4, b) Fe_3O_4@L-asparagine; c) Fe_3O_4@aspartic acid, d) Fe_3O_4@L-proline, e) untreated HCT8 cells (fluorescence images; 630x magnification)

Specific DNA staining with propidium iodide (PI), a fluorescent dye which intercalates between DNA bases, was used for a DNA content determinant in cell cycle analyses by flow cytometry (Fig. 6).

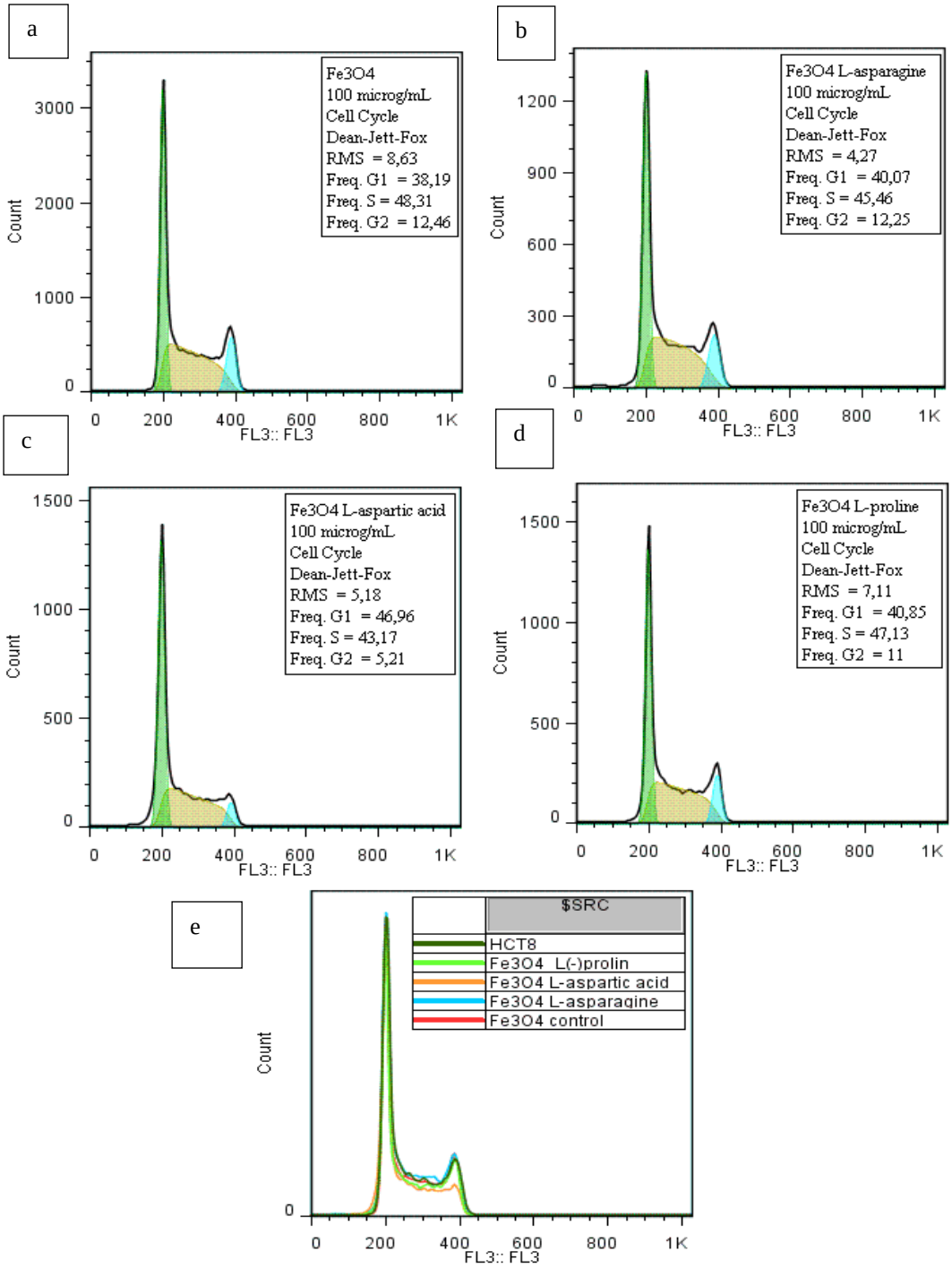


Fig. 6. The effects of the coated and uncoated magnetic samples on the HCT8 cell cycle: (a) Fe_3O_4, b) Fe_3O_4 @ L-asparagine; c) Fe_3O_4 @ aspartic acid, d) Fe_3O_4@L proline, e) overlay of all histograms highlighting the differences between them

According to Fig. 6, only treatment with Fe_3O_4@aspartic acid slightly decreased G2/M phase and increased G1 phase. However, the anti-tumor activity of free amino acids was reported by some studies and the use of aspartic acid (a known neurotransmitter) has been reported to have a slight antiproliferative activity probably functioning through N-methyl-D-aspartate receptor (NMDAR) [14].

4. Conclusions

According to the obtained results the use of L-asparagine, L-aspartic acid, L-proline as stabilizing agents of the magnetite core induce important morphological changes which can be exploited in medical applications. The use of the amino-acids is not yet well studied in the literature and so, studies will be necessary to be accomplish in order to determine the influence of these functionalization agents from the point of view of the biological activity and morphological changes against specific cells, tissues and organs. In this paper, based on the *in vitro* data, it can reveal that the use of aspartic acid (a well-known neurotransmitter) as stabilizing agent, induces a slight anti-proliferative activity probably through N-methyl-D-aspartate receptor (NMDAR). Further works will be necessary to evaluate the possibility of using them as targeted carrier for drugs, contrast agents, internalization ability of these magnetic nanoparticles as well as their bioaccumulation in different tissues and organs, especially from the point of view of their toxicity and benefits.

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