Poly(2-vinylpyridine) magnetite nanoparticles for 5-fluorouracil targeted delivery: synthesis, uptake and release study

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Abstract

One of the major limitations of anti-cancer drug is their poor selectivity and high toxicity. Present study is aimed overcoming these difficulties by development targeted drug delivery systems. Drug delivery systems were synthesized based on magnetite nanoparticles with grafted poly(2-vinylpyridine) from their pre-modified surface with 3-(trimethoxysilyl)propyl methacrylate. Physical and chemical properties of synthesized samples were examined by FTIR, XRD, VSM, EDA, Mössbauer spectroscopy. 5-FU release from nanocarriers was estimated using UV-Vis spectroscopy.

Introduction

5-FU is one of the most frequently used antimetabolite chemotherapy drugs applied for treatment of different cancer types, such as brain, breasts, colorectal cancer, gastrointestinal tract, pancreas, ovary, and liver [1]. It has two mechanisms of action: anabolic and catabolic. In anabolic route its metabolites, fluorodeoxyuridine diphosphate and fluorodeoxyuridine triphosphate can misincorporate DNA or RNA, leading to the damage to molecule, or such metabolite as fluorodeoxyuridine monophosphate binds thymidine synthase, disrupting DNA synthesis and repair mechanism. The catabolic pathway is the reduction of 5-FU to inactive 5,6-dihydro-5-fluorouracil with enzyme dihydropyrimidine dehydrogenase in the liver [2]. Though, up to 5% of patients are expressing lack of this enzyme, and standard chemotherapy dose causes adverse effects or even death. The other limiting factors are multidrug resistance, unspecific cytotoxic effect to all cells, gastrointestinal side effects, short half-life in body [3–5]. Thus, the efficiency of 5-FU chemotherapy could be increased by administrating it locally in the tumor and controlling it release.

Administration of chemotherapy agents locally to cancer tissues with high accuracy can be achieved using nanocarriers [6–8]. Magnetite nanoparticles are of great interest for these purposes: delivery can be performed using external high-gradient magnetic field, nano scaled size and physical properties making conditional penetrating trough vascular architecture, cell membranes, blood brain barrier. Also, magnetite nanoparticles were already used in cancer treatment as magnetic hyperthermia agents [9]. On the other hand, 5-FU release control can be achieved by attaching it to the polymers. So, the chemotherapy drug will be released from polymer during some time, reducing the minimal necessary dose. Researchers have made significant progress in this area of research. For instance, *Bayramgil et al* [10] synthesized poly(1-vinyl 1,2,4-triazole) hydrogels by irradiation methods for 5-FU immobilization. Authors [11] obtained pH-responsive nanogel based on poly(2-vinyl pyridine)-b-poly(ethylene oxide) for delivery curcumin, 5-FU and control release. Hamid Hashemi-Moghaddam et al [12] evaluated efficiency of magnetic delivery of 5-FU by modified iron oxide nanoparticles with 5-FU-imprinted polymer in mouse breast cancer model. The obtained results, showed significantly deposition of 5-FU in the 5-FU-imprinted polymer treated group with magnetic field. Amini-Fazl and co-workers [13] developed a magnetic hydrogel chitosan/polyacrylic acid/Fe_3O_4 nanoparticles and tested them in dosing for a long time with controlled releases in the colon and rectal conditions. In addition, magnetite nanoparticles have been used to deliver carborane and gadolinium agents for neutron capture therapy [14–16], doxorubicin, paclitaxel, cisplatin and others [17].

Thus, previous studies show that the use of magnetite with various coatings leads to an improvement in the treatment of cancer. Nevertheless, the search for methods for modifying magnetite nanoparticles, expanding the range of polymers in order to increase the efficiency of drug delivery, remains relevant.

So, it is proposed in this work to use hydrogel coated magnetite nanoparticles loaded with 5-FU. 5-FU containing 2-vinylpirydine hydrogel (2-VP), crosslinked with trimethylolpropane trimethacrylate (TMPTMA) is attached to the surface of nanoparticles with via 3-(trimethoxysilyl)propyl methacrylate (Fig. 1).

Figure 1 – Scheme of modification of magnetite nanoparticles with silane shell and attaching 5-FU molecular imprinted 2-VP hydrogel

Materials and methods

2.1 Materials

$FeCl_2 \cdot 4H_2O$, $FeCl_3 \cdot 6H_2O$, Al_2O_3, 3-(trimethoxysilyl)propyl methacrylate (TMSPM), 2-vinylpyridine (2-VP), 5-fluorouracil (5-FU), trimethylolpropane trimethacrylate (TMPTMA), HCl, ammonia hydroxide aqueous solution, *o*-xylol, dimethylformamide are of chemical grade or higher, deionized water was used in all experiments.

2.2 Synthesis methods

2.2.1 Synthesis of magnetite nanoparticles

Magnetite nanoparticles were synthesized as in [16]. In brief, 0.05 mol of $FeCl_2 \cdot 4H_2O$ and 0.1 mol of $FeCl_3 \cdot 6H_2O$ were dissolved in 100 ml of deionized water with adding of 11.8 ml of HCl. Reaction was kept under argon flux with vigorous stirring. Then ammonia hydroxide aqueous solution was added dropwise until pH of the solution became 9. Then the temperature was adjusted to 80°C and kept for 2 h. Then precipitate was magnetically decantated, washed in water and dried.

2.2.2 Functionalization of surface with C = C double bonds

The surface of magnetite nanoparticles was functionalized with TMSPM as in [16]. 1 g of magnetite nanoparticles was dispersed in 100 ml of *o*-xylol by ultrasound. After adding 0.0126 mol of TMSPM, reaction was kept at 80°C under argon flux. Then, precipitate was magnetically separated, washed in *o*-xylol and dried.

2.2.3 5-FU imprinted 2-VP hydrogel on Fe_3O_4

0.185 mol of purified by filtering through column with Al_2O_3 2-vinylpyridine was added to 79.4 ml of dimethylformamide. After adding 1 g of functionalized magnetite nanoparticles, 0.0002 mol of benzoyl peroxide, 0.002 mol of TMPTMA and 3.8 μmol of 5-FU were added. Reaction was kept at 80°C under

argon flux. Then the precipitate was magnetically separated and washed twice in dimethylformamide and 2-propanol, dried on air.

2.3 Methods of characterization

FTIR spectra were performed using FTIR spectrometer InfraLUM FT-08 (range 400–4000 cm^{-1}, 25 scans, and resolution of 2 cm^{-1}). The thermogravimetric analysis (TGA) was performed on Perkin-Elmer (Pyris 1 TGA) instrument in the temperature range of 25 to 800°C with a programmed temperature increment of 10°C min–1 in a nitrogen atmosphere. EDA spectra were performed using Hitachi TM 3030 with the microanalysis system Bruker XFlash MIN SVE at an accelerating voltage of 15 kV.

X-ray diffraction analysis was carried out on D8 ADVANCE ECO diffractometer (Bruker, Germany) using CuKα source (λ = 1.54060 Å). To identify the phases and study the crystal structure, the software BrukerAXSDIFFRAC.EVAv.4.2 and the international database ICDD PDF-2 were used.

The investigation of macromagnetic properties was carried out using the vibrational magnetometer (the Liquid Helium Free High Field Measurement System (Cryogenic Ltd.). The measurements were implemented using the induction method, through a determination of the induced electromotive force of the induction in signal coils by a magnetized sample oscillating with a definite frequency at magnetic field B = ± 1 T at 300 K temperature. DLS was performed on Fritch Nanotec Analysette 22.

2.4 5-FU release assay

Release of 5-FU was analysed by UV-Vis spectroscopy method. 0.07 g of samples were incubated in tubes with 14 ml of phosphate buffer solution (PBS) with different pH at 36.6°C for 150 h. Then aliquot (1ml) was taken and examined on UV-vis spectrometer and placed back to the tube. All the measurements were made 3 times and experiment was repeated twice.

Results and discussion

Fe_3O_4 nanoparticles were modified by TMSPM with the aim to create C = C groups on their surface for subsequent graft polymerization of 2-VP as it is presented on Fig. 1. Figure 2 performs the data of FTIR spectroscopy. Spectrum of initial magnetite nanoparticles correlating well with literature data [18]. It can be characterized by ν Fe-O-Fe (540 cm^{-1} и 630

cm^{-1}), δ O-H (1640 cm^{-1}) and ν O-H (3350 $см^{-1}$) peaks. After treating the samples with silanes (TMSPM), spectrum has significantly changed: Si-O-Si characterizing bonds (1016 cm^{-1}, 1175 cm^{-1}), C-O bonds (1300 cm^{-1}), δ_s O-CH_3 bonds (1450 cm^{-1}), C=C and C=O bonds (1635 cm^{-1} и 1718 cm^{-1}), C-H groups at 2935 cm^{-1}. Also, ν(O-H) peak gradually reduced, which indicates reaction goes by these groups. Then nanoparticles were treated with 5-FU imprinted poly (2-vinylpirydine) hydrogel. After this, N-H bonds (3425 cm^{-1}), distinctive for pyridine ring C = N peak (1605 $см^{-1}$) are appearing at FTIR spectrum. C=C and C = O peaks are indicating the crosslinking of polymer to hydrogel and related to

trimethylolpropane. In favor of this the fact that Si-O-Si peaks are reduced in contrast to C = C and C = O. Peaks of 5-FU are poorly visualized at FTIR spectra and can't be observed due to the small amount.

From EDA, after the silane treating Si and C appeared in the structure (1.24 at. % and 13.92 at. % respectively). After the next step of graft polymerization, the concentration of C and N increased up to 18.28 at. % and 0.9 at. % respectively, which indicates successful grafting of P2VP. Sorption of 5-FU is indicated by the increasing amount of F (up to 0.007 at. %), but the detection of low-weight elements such as F and N is not precise due to the specificity of EDA.

Figure 2 - FTIR spectra of synthesized samples: initial Fe_3O_4 (black line), Fe_3O_4-TMSPM (red line) and Fe_3O_4-TMSPM-p(2VP)-5FU (blue line)

Figure 3 shows the results of X-ray diffraction (XRD) of the studied samples of iron-containing nanoparticles with various types of modification. Lattice data calculated from XRD are presented in Table 1. In the initial state, the resulting structures are nanoparticles with a cubic type of crystal structure characteristic of the Fe_3O_4 phase (PDF-00-065-0731). For samples Fe_3O_4-TMSPM and Fe_3O_4-TMSPM-P2VP-5FU in the region $2\theta = 18–20$, peak broadening is observed which is characteristic for the presence of amorphous structures come from the modification of nanoparticles. At the same time, the analysis of the broadening of the main diffraction reflections showed that for the modified nanoparticles, an increase in the size of crystallites is observed, which can be explained by the modification of the surface of the nanoparticles.

Table 1
Lattice data from XRD

Parameters	Sample		
	Fe_3O_4	Fe_3O_4-TMSPM	Fe_3O_4-TMSPM-P2VP-5FU
Lattice parameter, Å	a = 8.31752 Å	a = 8.2241 Å	a = 8.84362 Å
Lattice volume, $Å^3$	575.42	576.43	580.85
Crystalline size, nm	11–13	15–17	19–22
Crystalinity degree, %	63.8	65.9	70.0

Size of nanoparticles was estimated by DLS, initial Fe_3O_4 have average hydrodynamic size of 51±5 nm, Fe_3O_4-TMSPM – 62±7 nm and Fe_3O_4-TMSPM-P2VP-5FU – 89±9 nm. We observe a regular increase in particle size from the modification stage, and we also demonstrated an increase in the size of crystallites from XRD analysis. Nanoparticle sizes of Fe_3O_4-TMSPM-P2VP-5FU are acceptable for administration into the body [19].

Magnetic characteristics of composites were studied on a universal measuring system (automated vibrating magnetometer) «Liquid Helium Free High Field Measurement System» (Cryogenic LTD) in magnetic fields ± 1 T at 300 K. The Fig. 4 shows the hysteresis loops of original and modified magnetite particles. The Table 2 presents the results of calculating the magnetic characteristics of the samples. The original particles have coercivity of 14 Oe, saturation magnetization of 62.1 mu/g, and remanence of 1.21 mu/g, typical of magnetite. The characteristics of the modified particles differ from the initial ones and correspond to a change in the content of the magnetic phase in the sample (decrease in saturation magnetization), a change in the state of the magnetic core (decrease in coercivity). The thin coating formed on the surface of the particles increases the distance between the particles, and, accordingly, the residual magnetization decreases (from 1.21 him/g).

Table 2
The results of calculating the magnetic characteristics of the samples.

	H, Oe	Mr, emu/g	Ms, emu/g
Fe_3O_4	14	1.21	62.1
Fe_3O_4-TMSPM	6.5	1.15	58.9
Fe_3O_4-TMSPM-P2VP-5FU	9.2	1.17	57.8

Figure 5 shows the TGA curves and its derivatives. As seen from the presented data, weight lost while heating from 25 ^oC to 925 ^oC is 3.7% due to the loss of physically and chemically absorbed water. 3% of mass is lost for the samples covered with silanes, 12% is lost for the nanoparticles with grafted bare P2VP hydrogel and 6.6% for nanoparticles with 5-FU imprinted hydrogel. This might be related to the transfer of radical to the C = O group of 5-FU, making it to exert inhibiting properties, reducing the amount of grafted hydrogel. Samples with immobilized silane and polyvinyl pyridine hydrogel are losing weight at 200–500 ^oC, which is well correlating with literature data [20]. 5-FU presence is undetectable in this case because it decays at 289 ^oC, which is correlating with the samples above.

Figure 5 - TGA curves and its derivatives for samples Fe_3O_4 (a), Fe_3O_4-TMSPM (b), Fe_3O_4-TMSPM-p(2VP) (c), Fe_3O_4-TMSPM-p(2VP)-5FU (d)

Release of 5-FU was examined at different pH (4.5 and 7.58) and constant temperature of 36.6 °C, results are presented in Fig. 6. Experiment have shown that the desorption goes rapidly during first 4 h and then its speed significantly decreases, but it is not finished even after 150 h. Also, the speed of the desorption and the amount of desorbed 5-FU is higher in acidic mediums (3.8 mg/l for pH 4.5 and 3.2 mg/l for pH 7.58). This can be related that isoelectric point of 2-VP is around 3.2 and desorption process is easier when closer to it.

Figure 6 - Desorption of 5-FU at pH 4.5 (red line), 7.58 (black line)

Conclusion

Thus, the method of iron oxide nanoparticles modification was developed by silanization of TMSPM and graft polymerization of 2-VP (imprinting 5-FU). Properties of synthesized samples were examined by FTIR, XRD, VSM, EDA, Mössbauer spectroscopy. It was found out that hydrodynamic diameter of Fe_3O_4-TMSPM-P2VP-5FU is 89±9 nm and magnetic properties are preserved. 5-FU release from nanocarriers was estimated using UV-vis spectroscopy. It was found that the release of the substance occurs in the first 4 hours, then the release rate drops significantly. Thus, obtained nanoparticles have potential to be used as nanocarriers for targeted delivery of 5-FU.

Declarations

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Author Contribution

I.V.K - Conceptualization, writing–review and editing, supervision. K.A.I, Zh.A.B, K.L.N. and L.I. L. - investigationA.V.Z. - investigation and writing–original draft preparationA.E.Sh - Conceptualization and investigationM.V.Z - writing–review and editing, supervision, funding acquisition

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Figures

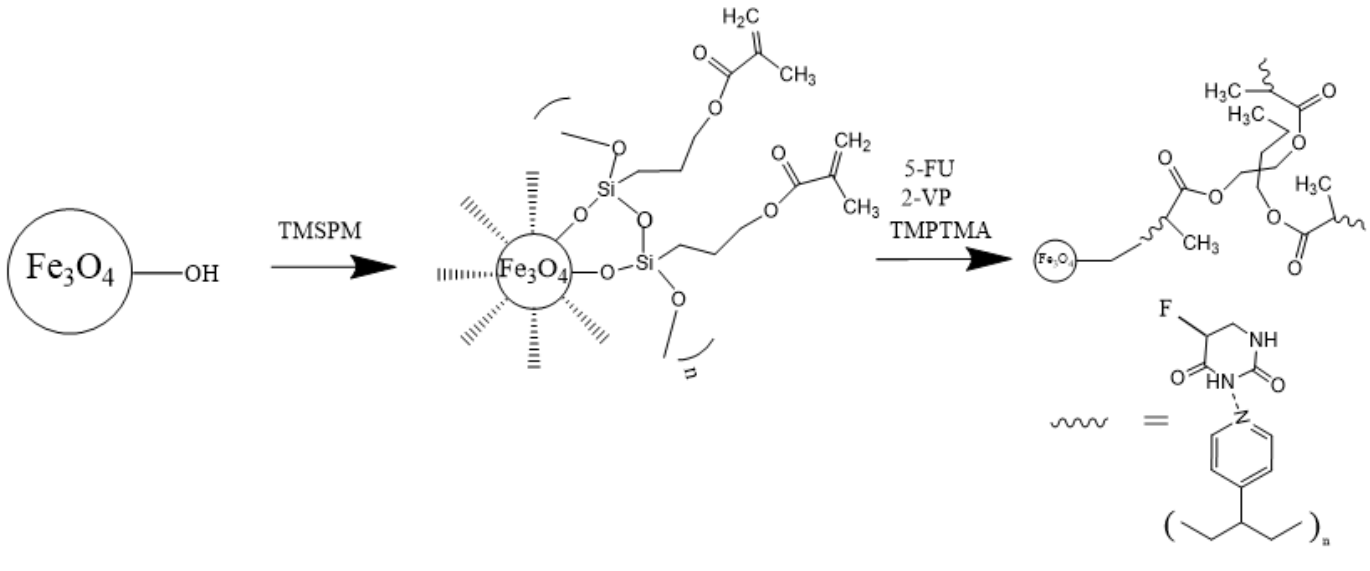


Figure 1

Scheme of modification of magnetite nanoparticles with silane shell and attaching 5-FU molecular imprinted 2-VP hydrogel

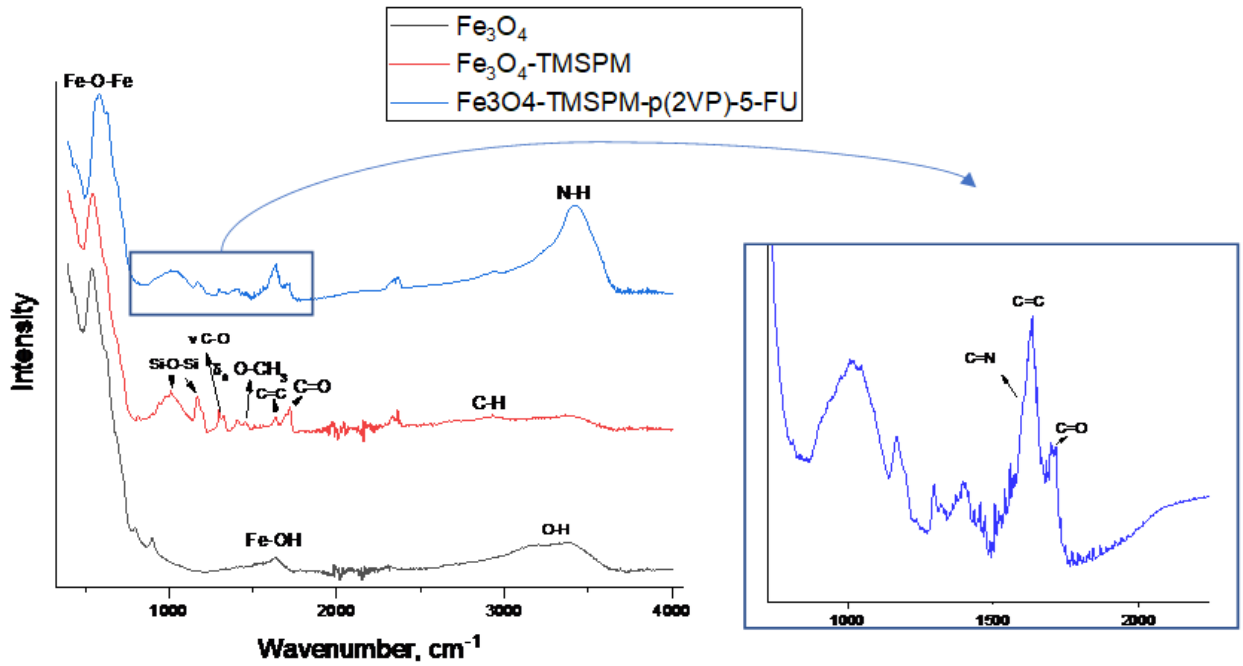


Figure 2

FTIR spectra of synthesized samples: initial Fe_3O_4 (black line), Fe_3O_4-TMSPM (red line) and Fe_3O_4-TMSPM-p(2VP)-5FU (blue line)

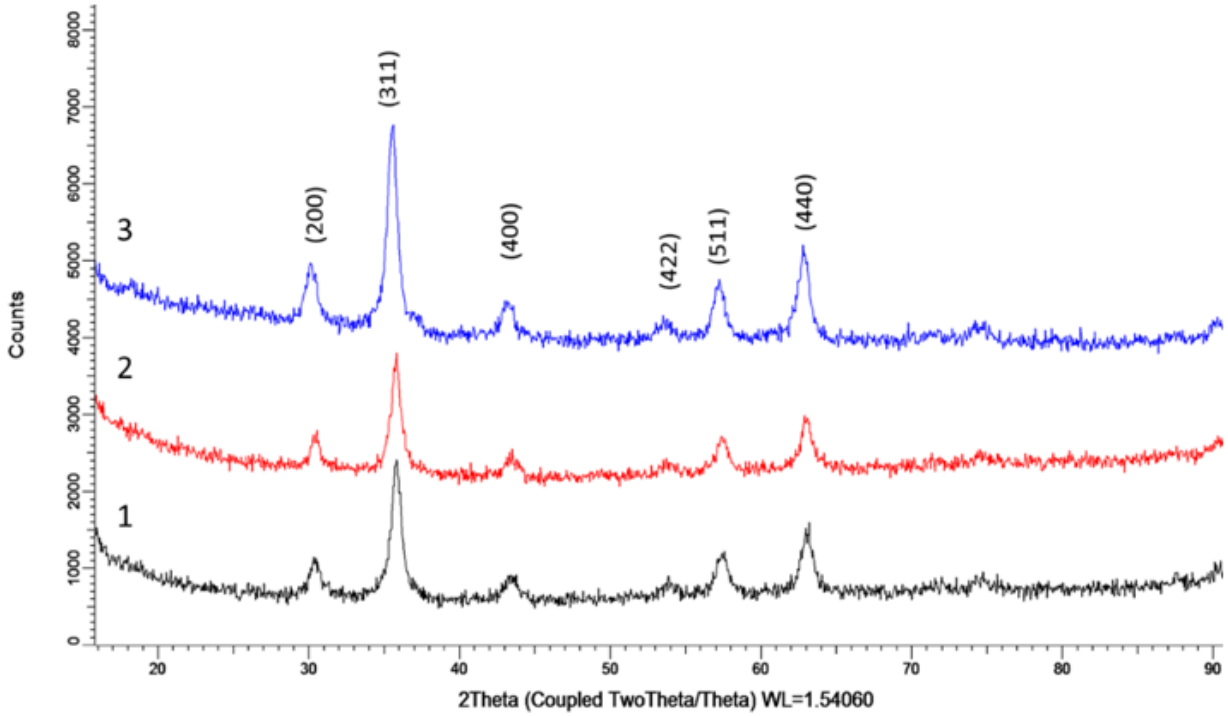


Figure 3

XRD analysis of 1- Fe_3O_4, 2-Fe_3O_4-TMSPM, 3- Fe_3O_4-TMSPM-P2VP-5FU

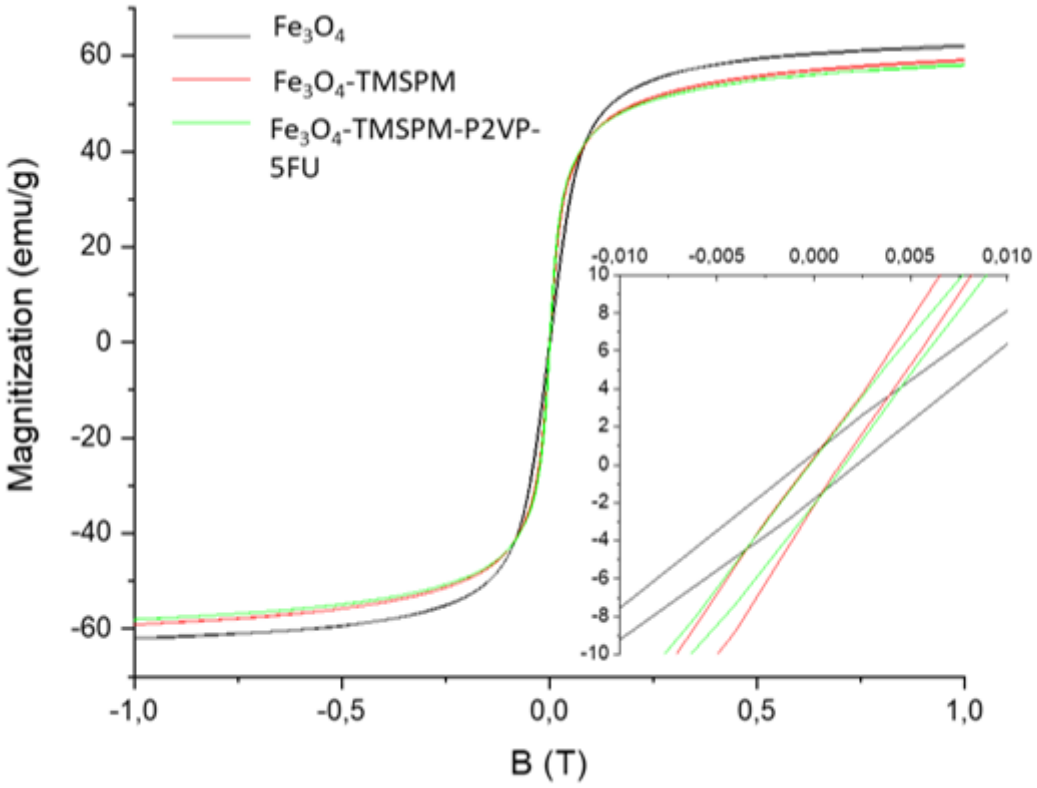
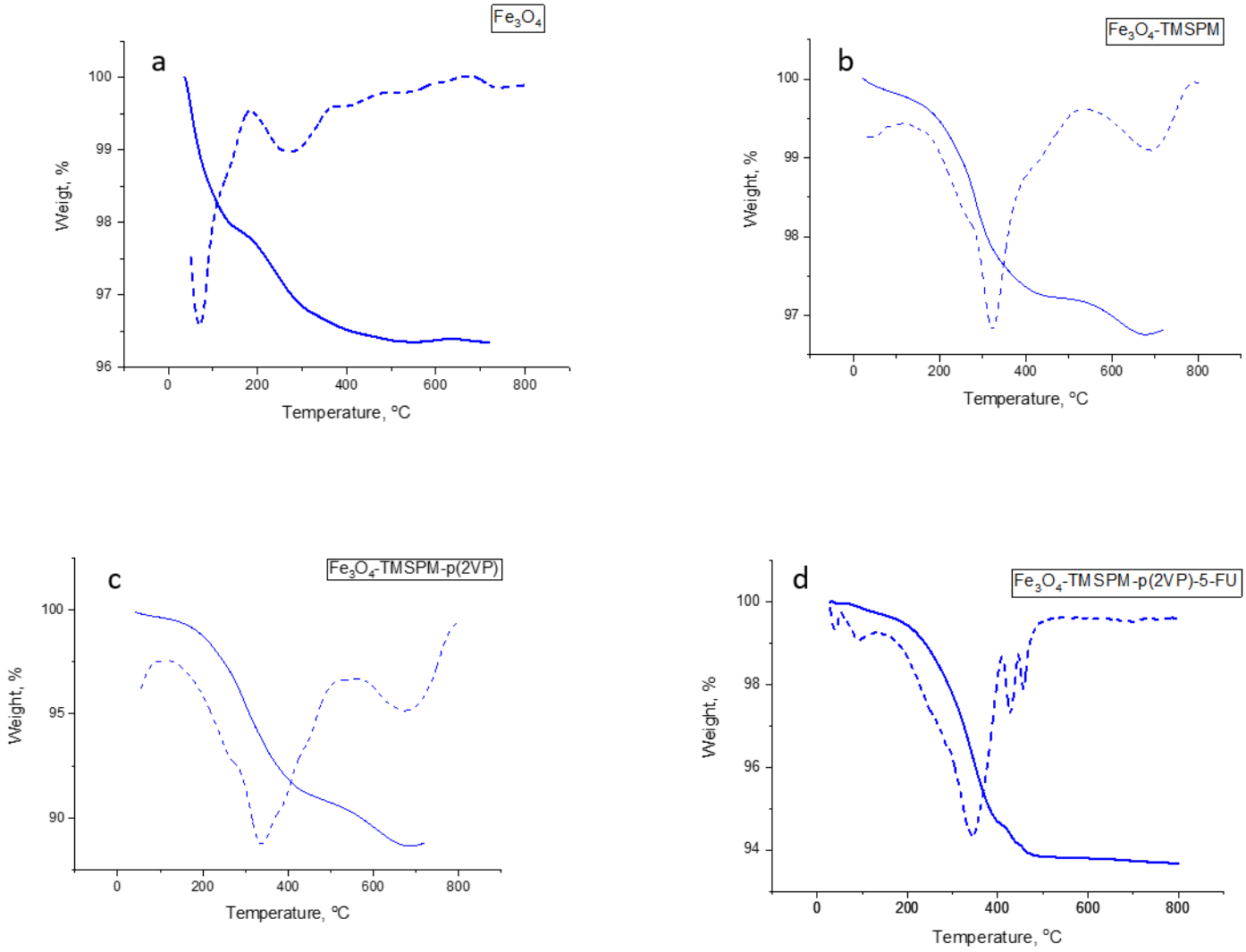


Figure 4

The hysteresis loops of original and modified magnetite nanoparticles



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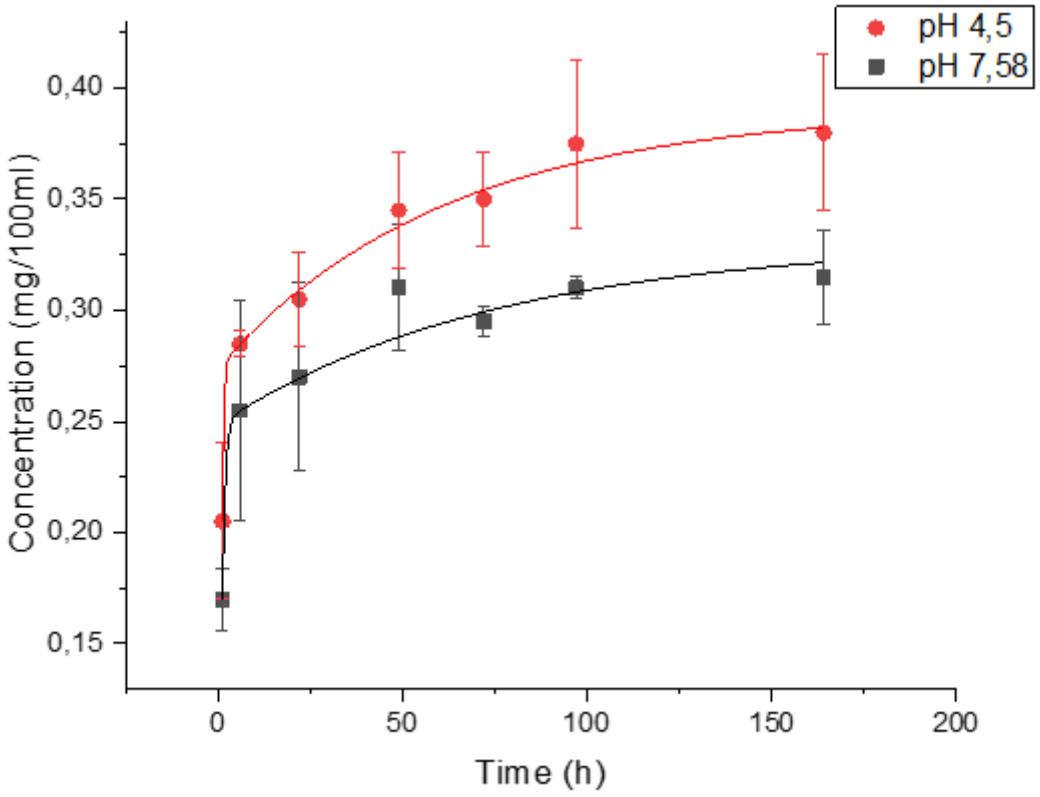

Figure 6

Desorption of 5-FU at pH 4.5 (red line), 7.58 (black line)