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Nanosized magnetite modified with poly(ethylene glycol) for efficient sorption of L-lysine-α-oxidase from the culture fluid

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ABSTRACT

Fe_3O_4@PEG have been proposed for sorption of L-lysine-α-oxidase (LO) from the culture fluid of *Trichoderma harzianum Rifai F-180* for the first time. To synthesize the Fe_3O_4@PEG nanoparticles, an original method based on aqueous biphasic systems has been developed. The PEG-modified magnetite provide a high sorption ability towards LO in contrast to the non-modified Fe_3O_4 synthesized by the traditional precipitation method. The morphology and structure of the prepared nanoparticles were characterized by TEM, FTIR and XRD. The data on magnetic properties and stability in physiological media are presented. The synthesized nanoparticles ensure quantitative sorption and desorption of LO during at least 3 cycles.

1 Introduction

In recent years the search for new antitumor drugs based on enzymes has been underway. Significant progress has been made in preclinical and clinical trials of enzyme–based drugs. Bacteriostatic, antiprotozoal, antifungal, antiviral, antitumor properties of oxidoreductases enzymes have been revealed. L-lysine-α-oxidase (LO) is one of the enzymes promising in therapy of tumors based on different sensitivity of normal and tumor cells to deficiency of growth factors [1]. The creation of a dosage form of LO is hindered by a lack of effective technology for enzyme obtaining. Fungi of *Trichoderma harzianum Rifai F-180* are known as producers of the culture of extracellular L-amino acid oxidase [2]. The common method of preparation of LO is precipitation with ammonium sulfate solution and subsequent chromatographic or membrane isolation [3].

Enzyme immobilization on the surface of a nanoparticle is a decision to the problem of its purification and bioseparation. The solid carrier used to immobilize the enzyme must preserve the activity of the biomolecule. The review [4] describes the features of enzyme immobilization by magnetic nanoparticles (MNPs), including Fe_3O_4, γ-Fe_2O_3. The MNPs are characterized by superparamagnetic properties, a large surface area, the ability to immobilize enzymes through physical adsorption, covalent binding or cross-linking. The MNPs can be stored for a long time without inhibition of enzymatic reactions and be used as biosensors, contrasting agents for MRI, markers of biomolecules, carriers for targeted therapy and controlled local hyperthermia of tumors [5,6]. The recent works describe the adsorption of proteins, amino acids, cells, bacteria on MNPs [7,8].

In this work, a new method of LO immobilization on the MNPs, including Fe_3O_4 modified with poly(ethylene glycol) (PEG), from the culture fluid of *Trichoderma harzianum Rifai F-180* has been developed. The comparative study of physicochemical characteristics and sorption properties of Fe_3O_4 and Fe_4O_4@PEG MNPs has been carried out.

2 Experimental part

2.1 Preparation of the MNPs

Fe_3O_4 MNPs were synthesized by the traditional co-precipitation method. The weighed portions of Fe(II, III) chlorides in a molar ratio of Fe^{3+}:Fe^{2+} 2:1 were dissolved with 5 mL of 2 M HCl. Ammonium hydroxide was added drop wise to the metal solution.

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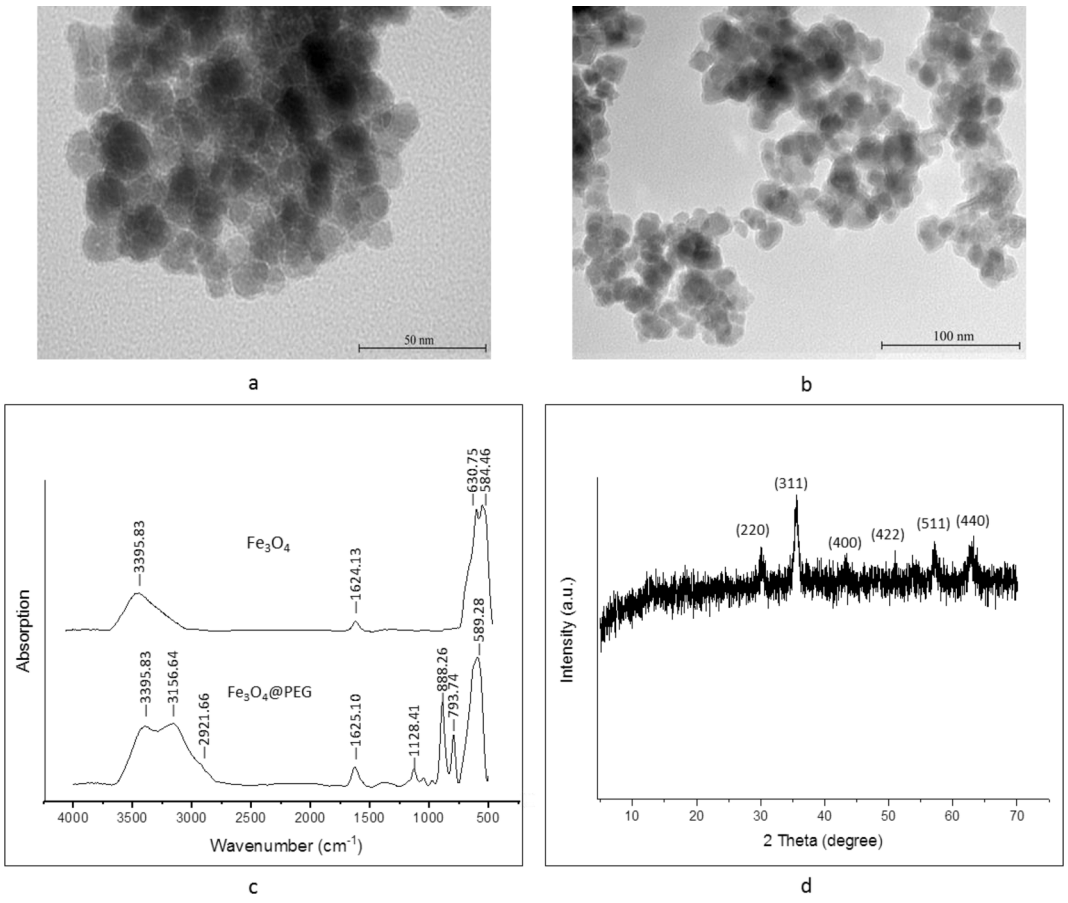

Fig. 1. TEM images for Fe_3O_4 (a) and Fe_3O_4@PEG (b), FT–IR spectra for Fe_3O_4 and Fe_3O_4@PEG (c) and XRD pattern for Fe_3O_4@PEG (d).

Fe_3O_4@PEG MNPs were prepared at the interface of aqueous biphasic system based on PEG and phase-forming salt. PEG-4000 and ammonium sulfate with a concentration of 50 wt% (for both components) were mixed in equal parts. After separation of phases, PEG was enriched with precursors of metal oxides (Fe(II, III) chlorides in a molar ratio of Fe^{3+}:Fe^{2+} 2:1.32), while a precipitating agent (NaOH) was injected into the salt phase. The polymer phase was carefully layered onto the salt phase, after that, the MNPs were precipitated at the interface of the biphasic system.

The obtained Fe_3O_4 and Fe_3O_4@PEG MNPs were rinsed with water up to pH 6–7, dried at 80 °C. The morphology of MNPs was characterized by transmission electron microscope HT7700 (Hitachi, Japan); the chemical structure was studied using an FT–IR spectrometer IRPrestige-21 (Shimadzu, Japan) and X-ray diffractometer D8 Advance (Bruker, USA); magnetic measurements were carried out with a Lake Shore 7410 vibration sample magnetometer (VSM) (Lake Shore Cryotronics, USA) at room temperature; zeta potential was measured by a Zetasizer Nano ZS (Malvern Instruments, UK).

2.2 Sorption procedure

The cultivation of the fungus was carried out by the submerged method on a medium supplemented with wheat bran [3]. Sorption of LO was carried out at room temperature for 10 min with 20 mg of both types of MNPs and 1 mL of the culture fluid diluted 5 times with distilled water. Then the loaded MNPs was washed by distilled water and contacted with 1 mL of phosphate buffer (pH 7.4) for 10 min for LO desorption. The aqueous phases were analyzed by determining the hydrogen peroxide formed during the reaction of oxidative deamination of L-lysine catalyzed by LO [2].

3 Results and discussion

3.1 Synthesis of the Fe_3O_4@PEG nanoparticles

The proposed method of MNP synthesis in aqueous biphasic system is a new environmentally friendly method that provides the preparation and modification of nanoparticles in one stage. The PEG-salt system is versatile due to low interfacial tension between the phases, low viscosity, higher phase separation rate, and low material cost. PEG used as polymer phase and modifying agent is one of the biocompatible polymers most commonly used to stabilize nanoparticles.

3.2 Characterization of MNPs

As it is shown at TEM images (Fig. 1a), the prepared Fe_3O_4 MNPs have a spherical shape with an average particle size of 16 nm. The average diameter of Fe_3O_4@PEG MNPs is 11–13 nm with a shell thickness of 1–2 nm (Fig. 1b). Both samples are characterized by a high surface area to volume ratio and thereby increased the sorption capacity. The specific surface area was calculated based on the density and size of the nanoparticles [9]; for Fe_3O_4@PEG MNPs the obtained value was 82 m^2/g.

FT–IR spectroscopy and X-ray diffraction were used to determine the crystal structure and to confirm the presence of PEG functional groups on the surface of Fe_3O_4@PEG nanoparticles. The absorption peak at 584.64 cm^{-1} (Fig. 1c) in Fe_3O_4 sample indicates the formation of Fe-O bond and confirms the crystal structure of magnetite. A similar peak at 589.28 cm^{-1} is observed for Fe_3O_4@PEG nanoparticles. The appearance of vibration peak at 3395.83 cm^{-1} associated with –OH group indicates the absorption of a water molecule on the surface of both samples. The peaks of C–O–H 888.26 cm^{-1}, C–O 1128.41 cm^{-1}, C–H

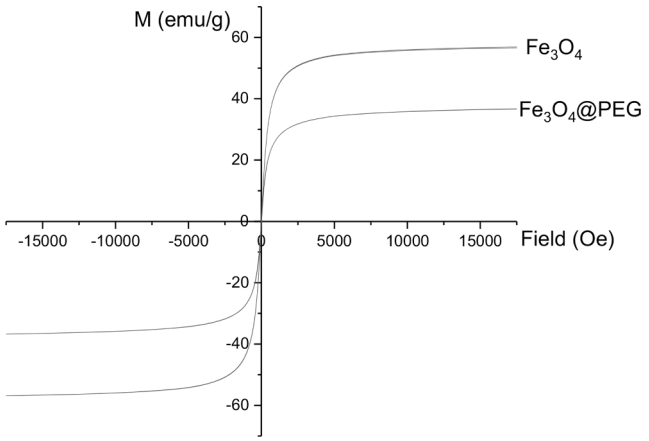

Fig. 2. Magnetization curves for Fe_3O_4 and Fe_3O_4@PEG samples.

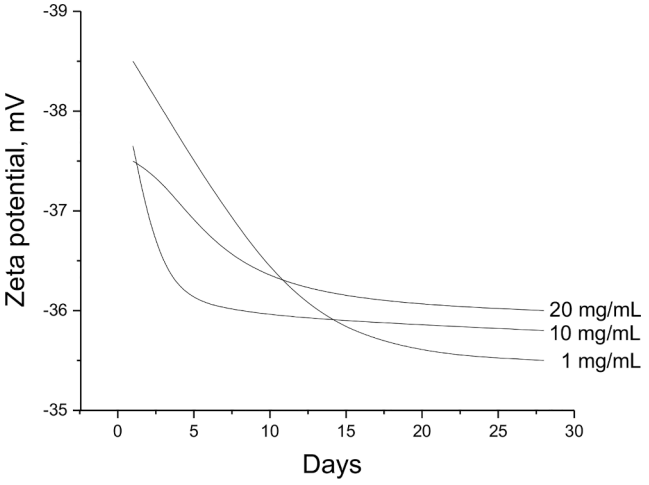

Fig. 3. Change of zeta-potential for Fe_3O_4@PEG MNPs over time.

2921 cm^{-1} and 3156 cm^{-1} are observed in Fe_3O_4@PEG sample, confirming the coating of MNPs with PEG. The diffraction peaks with 2θ angles of 30.3°; 35.5°; 43.1°; 57.1°, and 63.1° were observed for Fe_3O_4@PEG indicating the cubic spinel structure of magnetite (Fig. 1d). The functionalization did not alter the crystal structure of the material.

The magnetization curves of both samples demonstrate the superparamagnetic behavior, which typical for MNPs with a size up to 20 nm (Fig. 2). Practically zero values of coercivity and remanence magnetization was detected. The saturation magnetization was equal to 56.7 emu·g^{-1} and decreased by 35% to 36.9 emu·g^{-1} for the PEG-coated MNPs. The expressed amount of non-magnetic polymer molecules on MNP surface leads to the decrease of specific value of magnetization. This result is typical for surface-modified nanoparticles and confirms the successful modification.

Studies on the stability of the obtained particles in a physiological environment were carried out using a solution of phosphate buffered saline (pH 5.9). The zeta potential of unmodified Fe_3O_4 is 18 mV. Such a value of the electrokinetic potential does not contribute to the repulsion of suspended particles from each other, and thus their aggregation is possible. The surface charge of the modified Fe_3O_4@PEG particles was −38 mV, which is sufficient for the electrostatic stabilization of nanoparticles (Fig. 3). The zeta potential has not changed significantly during a month for various MNP concentrations.

3.3 Application of the MNPs for sorption of LO

The extracellular accumulation of the enzyme by the fungus *Trichoderma harzianum Rifai F-180* makes it possible to create a scheme for the isolation of LO. Comparative experiments were carried out using the synthesized magnetic sorbents Fe_3O_4 and Fe_3O_4@PEG (Fig. 4).

Experimental data presented at Fig. 4a show that the degree of LO sorption by Fe_3O_4 MNPs does not exceed 30%. However, the modification of the magnetite surface with PEG allows reaching the quantitative sorption of LO; sorption equilibrium is established within 10 min. Most probably, the enzyme immobilization is carried out by means of covalent interactions: amino acid residues of LO covalently bind to hydroxyl groups of PEG on the surface of the MNPs [10].

A quantitative desorption of LO was achieved using a phosphate buffer solution (pH 7.4). It was found that the efficiency of sorption and desorption is maintained for at least three cycles (Fig. 4b).

4 Conclusions

A new sorption nanomaterial Fe_3O_4@PEG has been synthesized in aqueous biphasic system and applied for LO immobilizing. The synthesized magnetic sorbent is stable in physiological media during at least a month and ensures fast and efficient sorption of LO from cultural fluid without changing its enzymatic activity. The developed procedure could be applied for LO purification and the delivery of its dosage form for enzyme therapy.

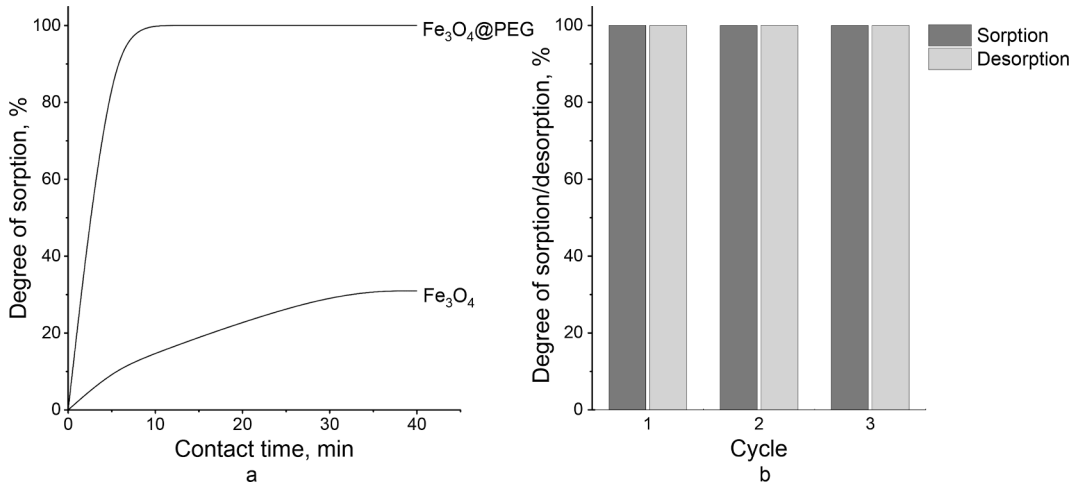

Fig. 4. Degree (%) of LO sorption by Fe_3O_4 and Fe_3O_4@PEG MNPs (a); degree (%) of LO sorption/desorption by Fe_3O_4@PEG MNPs during 3 cycles (b).

CRediT authorship contribution statement

V. Shkinev: Conceptualization, Methodology, Supervision. **V. Maksimova:** Investigation, Validation, Visualization, Writing – original draft. **O. Mokhodoeva:** Visualization, Writing – review & editing. **V. Larichev:** Investigation, Validation. **B. Spivakov:** Supervision. **O. Osmolovskaya:** Investigation, Formal analysis. **A. Egorova:** Investigation. **I. Smirnova:** Resources. **R. Dzhenloda:** Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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