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Adsorption and desorption studies of lysozyme by Fe_3O_4–polymer nanocomposite via fluorescence spectroscopy

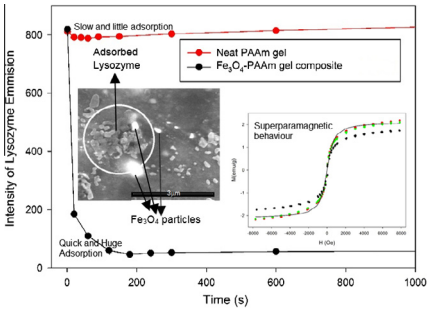
Kenan Koc [a,*], Esra Alveroglu [b]

[a] Yildiz Technical University, Faculty of Science and Letters, Department of Physics, 34210 Esenler, Istanbul, Turkey
[b] Istanbul Technical University, Faculty of Science and Letters, Department of Physics Engineering, 34469 Maslak, Istanbul, Turkey

HIGHLIGHTS

- Fe_3O_4 nanoparticles were synthesized by in situ in polyacrylamide hydrogels.
- Fe_3O_4–polymer composites show super-paramagnetic features.
- Higher monomer concentration composites present lower saturation magnetization.
- Adsorption/desorption kinetics of lysozyme were investigated via PL spectroscopy.
- Compact structures caused increasing adsorption and desorption rates of lysozyme.

GRAPHICAL ABSTRACT



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ABSTRACT

The work have been undertaken in this study is to synthesis and characterize Fe_3O_4–polymer nanocomposites which are having different morphological properties. Also, investigation of the adsorption and desorption behaviour of lysozyme onto Fe_3O_4–polymer nanocomposites have been studied. Fe_3O_4 nanoparticles, synthesized by in situ in polyacrylamide hydrogels, show super-paramagnetic behaviour and saturation magnetization of composite material have been tuned by changing the hydrogel conformation. Adsorption and desorption studies of lysozyme were followed by using pure water at room temperature via fluorescence measurements. Fluorescence measurements showed that, the composite materials adsorbed lysozyme molecules less than 20 s and higher monomer concentration of composite materials cause faster adsorption. Besides, structure of lysozyme molecules were not changed during the adsorption and desorption. As a result Fe_3O_4–polymer nanocomposites could be used for drug delivery, protein separation and PAAm gels could be used for synthesis of magnetic composites with varying magnetic properties.

Introduction

Magnetic nanoparticles have been extensively investigated due to their size-dependent intrinsic magnetic properties and also usage in the area of medical engineering and other applications [1–6]. There are several methods to synthesize and use them in such applications and generally they have been used by introducing them into a matrix. Similarly several methods to synthesize them, there are also different ways to put them into a matrix by using in-situ and ex-situ methods. The most applied method is mixing the nanoparticles into the polymer matrix after synthesizing the magnetic nanoparticles, this method known as ex-situ [2]. However, ex-situ methods generally cause the high agglomeration

* Corresponding author. Tel.: +90 0212 3834283.
E-mail address: kockenan@gmail.com (K. Koc).

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tendency of the nanoparticles in the polymer [2]. Formations of magnetic nanoparticles in the hydrogels by in-situ method have been developed to overcome this problem. Additionally this method available to develop composite material which have gained some advantageous for many biomedical applications due to their similarity with natural living tissue and inherent biocompatibility [2,3]. Hydrogels have three-dimensional, hydrophilic, polymeric networks and they can allow swelling upon interaction with aqueous solutions due to their cross-linking structures [4]. Magnetic nanoparticles-hydrogels composites are promising candidates for medical applications because of the combination of good biocompatibility, stability and excellent magnetic properties [2].

Ferrite particles have been studied intensively in literature and they have wide range applications in various fields [2–6]. Especially addition of organic molecules to the surface of these particles make them very important for magnetic separation or immobilization in biological applications [2,5]. Ferrites generally show super-paramagnetic behaviour when the particle size decrease under 30 nm [7]. The small size, high stability, high capacity and strong magnetic responsiveness of super-paramagnetic ferrite nanoparticles can have significant influence on adsorption and desorption of the proteins. Because of these reasons, lots of study about immobilization or adsorption of enzymes with ferrite nanoparticle-polymer composites has been under consideration in literature [8–13].

Lysozyme (N-acetylmuramide glycanhydrolase) is a commercially valuable enzyme for food industry and pharmaceutical applications [14]. The potential for its usage as an anticancer drug and cancer chemotherapy have been studied by animal and in vitro cell culture experiments [14]. There are also other studies in literature about adsorption or immobilization of lysozyme by using ferrite particles and polymers. Previously, Fuertes et al. [15] reported on the immobilization of lysozyme and they showed that lysozyme could be immobilized in core/shell composite containing iron oxide ferrite nanoparticles. Shamim et al. [16] reported on the adsorption of lysozyme, on thermosensitive poly(*N*-isopropylacrylamide) coated nanomagnetic Fe_3O_4 particles. They observed that a maximum amount of lysozyme was adsorbed at a temperature above the lower critical solution temperature of the polymer and at the isoelectric point of lysozyme. Haynes and Norde [17] reported that adsorbed lysozyme maintains a relatively high internal cohesion on the adsorption of lysozyme and α-lactalbumin on polystyrene and hematite.

Polyacrylamide (PAAm) hydrogel is one of the ideal matrix for Fe_3O_4 nanoparticle, for this reason many of magnetic–polymeric composite materials were produced by introducing Fe_3O_4 nanoparticles into PAAm hydrogel [18–21]. In this study, we have investigated adsorption and desorption kinetics of lysozyme by using Fe_3O_4–PAAm composite materials via fluorescence technique. Although there are various studies about adsorption and desorption, we have not seen any publication about the effect of gel morphology on the synthesis of nanomagnetic particles and adsorption–desorption kinetics of lysozyme by using Fe_3O_4–polymer nanocomposites.

Experimental

PAAm hydrogels were synthesized in varying concentration by free radical cross-linking polymerization at 60 °C. Acrylamide (AAm, used as monomer), N,N-methylenebis acrylamide (BIS, used as cross linker) and ammonium persulfate (APS, used as initiator) were dissolved in pure water and placed into the 60 °C heat bath. All the chemicals were supplied from Sigma–Aldrich and used as received, without further purification. After gelation performed, the samples left for 24 h to be sure the reaction was complete. All of the samples codes and compositions are given Table 1.

The gels were washed with distilled water to get rid of any unreacted compound and purifications during two days. Then Iron (II) and Iron (III) chloride tetrahydrate (molar ratio 1:2) were dissolved in a beaker, and gels left into this solution during two days. The colour of gels were turned from transparent to yellowish. After then 2 M NaOH solution was prepared and the gels were taken from the previous solution and put into it. Fe_3O_4 nanoparticles were prepared in this aqueous medium which chemical reaction of formation can be written as

$$Fe^{2+} + 2Fe^{3+} + 8OH \rightarrow Fe_3O_4 + 4H_2O$$

The schematic representation of preparation of samples and photographs of gels at each stage are seen in Fig. 1. As seen from the photograph in Fig. 3c, the gel seems very dark when Fe_3O_4 particles are formed in it.

Samples were characterized by X-ray diffractometry (XRD, RIGAKU), Secondary Electron Microscope (SEM, FEI Nova NanoSEM 450), Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) (ATR-FTIR, Bruker Tensor 27) and Vibrating Sample Magnetometer (VSM).

Adsorption and desorption kinetics of lysozyme by Fe_3O_4 containing gels were studied by Varian Cary Eclipse model spectrometer equipped with a temperature controller and quartz vessel was used during the measurements. First, 10^{-3} M lysozyme solution was prepared in pure water, then the gel which is cut in certain weight placed in the lysosome solution and fluorescence spectra were taken from the solution excited with 300 nm wavelength at certain time intervals.

Results and discussion

Structural characterization

XRD analysis

After synthesizing of Fe_3O_4 particles in PAAm gel, composite material were dried and powdered and then XRD pattern shown in Fig. 2 has been taken. This diffractogram (peaks at 30.18°, 35.54°, 43.16°, 53.10°, 57.0° and 62.88°) shows Fe_3O_4 particles crystalized in face-centred cubic structure and diffraction peaks are indexed of Fe_3O_4 JCPDS No. 19-629. As shown in Fig. 2, peaks are very sharp because of the big Fe_3O_4 clusters in the gel.

ATR-FTIR analysis

The ATR-FTIR spectra of PAAm hydrogel and Fe_3O_4–PAAm magnetic composite are shown in Fig. 3. ATR spectrum of Fe_3O_4–PAAm magnetic hydrogel shows the strong absorption at 500–600 cm^{-1} corresponding to characteristic vibration of Fe—O in the Fe_3O_4

Table 1
The composition of the pre-gel solution of PAAm gels.

Sample code	AAm molarity (Mol/l)	BIS molarity (Mol/l)	APS molarity (Mol/l)
A1-H	1	0.008	0.007
A1-N	1	0.015	0.007
A1-L	1	0.030	0.007
A2-H	2	0.015	0.014
A2-N	2	0.030	0.014
A2-L	2	0.060	0.014
A3-H	3	0.022	0.022
A3-N	3	0.045	0.022
A3-L	3	0.090	0.022
A4-H	4	0.030	0.029
A4-N	4	0.060	0.029
A4-L	4	0.120	0.029