Adsorption Behaviours of Lysozyme onto Poly-Hydroxyethyl Methacrylate Cryogels Containing Methacryloyl Antipyrine-Ce(III)

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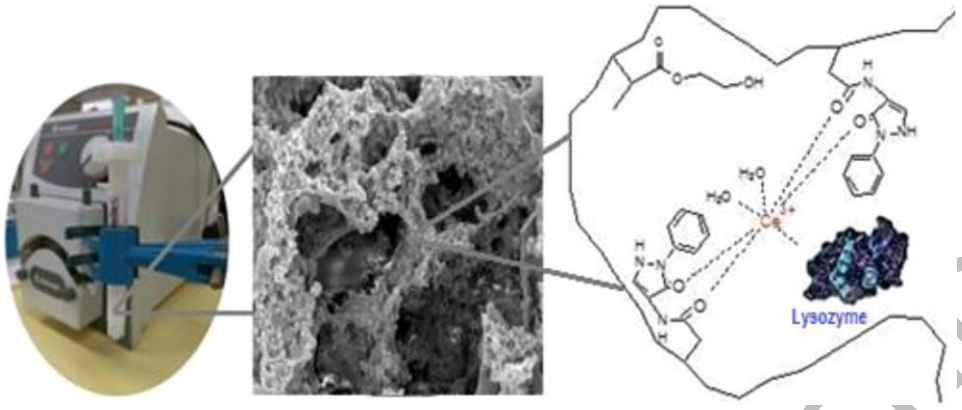
Abstract

In this study, we prepared Ce^{3+} based cryogel with methacryloyl antipyrine (MAAP) and 2-hydroxyethyl methacrylic acid (HEMA) [p(HEMA-MAAP-Ce^{3+}]. MAAP-Ce^{3+} complex was characterized by UV-near infrared and energy dispersive X-ray spectroscopy. Cryogel was characterized by Fourier transform infrared spectroscopy, scanning electron microscopy, and swelling test. Pore size of the cryogel was found to be about 30-50 μm. The effects of flow rate, pH, temperature and initial enzyme concentration have been investigated. Maximum adsorption capacity was found to be 57.84 mg/g cryogel at pH 6.0. After 7 times adsorption-desorption cycles of same cryogel, it was observed that there is negligible decrease in the adsorption capacity.

Graphical Abstract



KEYWORDS: Cryogel, Cerium^{3+}, Lanthanide, Adsorption, Lysozyme

1. Introduction

Ovalbumin, ovotransferrin, ovomucoid, ovomucin and lysozyme are egg white proteins. But most important of them is lysozyme [1]. Lysozyme (EC. 3.2.1.17; muramidase or *N*-acetyl muramic hydrolase) is one of the bacteriolytic enzyme. It exits in all secreted body fluids and tissues of both humans and animals [2]. Lysozyme hydrolyzes β- linkages between the *N*-acetyl muramic acid and *N*-acetylglucosamine of the mucopolysaccharides in the bacterial cell wall. It has 14.400 Da molecular weight and 11.2 isoelectric point [3,4]. Lysozyme is one of the commercial valuable enzymes. It is used in different areas, for example, pharmaceutical and food industries. Its common applications are cell disrupting agent for extraction of bacterial intracellular products, food additive in milk products and drug for treatment of ulcers and infections [3,5-7]. Therefore, lysozyme isolation is important and its applications need efficient and cost effective techniques. It occurs naturally in chicken egg white even if other natural lysozyme sources are known. Content of chicken egg white lysozyme is given in Table 1 [1].

Hydrogels, which are polymeric materials, have large water content. If hydrogels are synthesized at subzero temperatures, they may be named as cryogels which are macroporous polymeric materials. Cryogels studies started in 1970s, being of continuous interest since then [8]. They have been used in many areas of chemistry, biology, bioengineering and biotechnology. When polymerization takes place, most of solvent (commonly water) is frozen and monomers are concentrated in non-frozen solvent. Frozen water crystals form pores, thus interconnected porous (~10-100 μm) polymeric structure is constructed. In addition, cryogels have many advantages compared with the conventional adsorbents, such as, high porosity, high mechanical strength, low diffusion path, and they are very strong materials under serious deformation [9-11]. This feature of these polymers renders them different from other macroporous materials. They are also easy to prepare and cheap materials. The advantages of cryogel have seen great interest from biotechnological and another applications [6,12-15]. The preparation of different cryogels with several metal ions and their lysozyme adsorption efficiency have been reported by some research groups [16,17].

Recently, immobilized metal affinity chromatography (IMAC) has become a more useful separation method for the purification and recovery of a wide range of biological molecules, such as, peptides, proteins, hormons and enzymes [18-20]. In this technique, metal ions form complexes with various ligands for specific separation purposes. Some coordinate sites of the metal ions remain free for the binding to solvent or protein [21]. Cryogels can also be easily functionalized with different type of ligands and are

applicable to different affinity systems in this technique [22]. Some research groups have used metal affinity ligands and cryogel with different metal ions and biological molecules [23-26].

In the present study, we aimed to prepare cryogel which contains a lanthanide complex for pseudo-specific protein adsorption. For this aim, firstly, methacryloyl chloride was reacted with 4-aminoantipyrine and Ce^{3+} to prepare methacryloyl antipyrine (MAAP-Ce^{3+}) complex. MAAP was used as ligand for efficient coordination of Ce^{3+} ions. In addition, chemical activation of the cryogel was eliminated. Complex has been characterized by ultraviolet-visible-near infrared (UV-NIR) and energy dispervive X-ray (EDX). The cryogel which contains 2-hydroxyethyl methacrylic acid-methacryloyl antipyrine-Ce^{3+} [p(HEMA-MAAP-Ce^{3+})] was prepared by free radical polymerization and characterized by Fourier Transform Infrared Spectroscopy (FTIR), scanning electron microscope (SEM), and swelling test. Then, the cryogel was used for model enzyme lysozyme adsorption from aqueous solution. Effects of different parameters, such as, flow rate, pH, temperature and initial enzyme concentration on lysozyme adsorption capacity were studied. The reusability of the cryogel and desorption studies were also investigated.

2. EXPERIMENTAL

2.1. Materials

Lysozyme (from chicken egg white, catalog number: Sigma 62970), 2-hydroxyethyl methacrylic acid (HEMA), *N,N'*-methylene bisacrylamide (MBAAm), *N,N,N',N'*-tetramethylethylene diamine (TEMED) and ammonium persulfate (APS) were supplied

by Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used were of analytical grade and purchased from Merck A.G. (Darmstadt, Germany).

2.2. Preparation Of Methacryloyl Antipyrine-Ce^{3+}

MAAP was synthesized according to Keçili et al. [27]. 0.5 g 4-aminoantipyrine and 0.2 mL of pyridine were dissolved in 100 mL of dry $CHCl_3$ and the solution was cooled to 0 ^oC. Then, 0.26 mL of methacryloyl chloride was slowly poured into the solution and stirred for 2 h. At the end of the reaction, the solution was washed with 50 mL of diluted HCl and 50 mL of diluted NaOH, respectively. The organic phase was evaporated in a rotary evaporator and residue was crystallized in petroleum benzene-ethyl acetate solution. Ce^{3+} chelated MAAP was prepared in 100 mg/50 mL of $Ce(NO_3)_3$ solution and 130 mg MAAP. The solution was then stirred at 100 rpm for 24 h at room temperature. Ce^{3+} ion concentration in the resulting solution was determined by an atomic absorption spectrometer. The amount of adsorbed Ce^{3+} was calculated by using the Ce^{3+} concentration of the initial solution and the equilibrium.

2.3. Preparation Of P(HEMA-MAAP-Ce^{3+}) Cryogel

P(HEMA-MAAP-Ce^{3+}) cryogel was prepared as follows: 1.3 mL of HEMA and 100 µL of MAAP-Ce^{3+} (20 mg/mL) were mixed in 5 mL of deionized water. On the other part, 0.283 g MBAAm was separately dissolved in 10 mL of deionized water. Then, these two solutions were mixed. The cryogel was obtained by free radical polymerization by adding 20 mg APS and 50 µL of TEMED with stirring for 2-3 min, which was then poured into a plastic syringe (5 mL). The solution was frozen at -18 ^oC for 24 h for polymerization. The

cryogel was thawed at room temperature and washed with 50 mL of deionized water to discard unreacted monomers, then stored in 0.02% sodium azide at +4 ºC.

2.4. Characterization Studies

MAAP-Ce^{3+} was analysed by using ultraviolet visible-near infrared (UV-NIR) and energy dispersive X-ray (EDX) spectroscopy techniques. The surface morphology of p(HEMA-MAAP-Ce^{3+}) cryogel was determined by using SEM-EDX (FEI Quanta FE6-250 model, USA). Cryogel (20 mg) was taken from the sample and the pellet was sprinkled on the platinum surface. Then, polymer was coated with gold nano-scale with a thickness of 20 nm, to increase the conductivity. The SEM images were obtained at different magnifications. The structure of p(HEMA-MAAP-Ce^{3+}) cryogel was confirmed by FTIR spectroscopy (FTIR 800 Series, Shimadzu, Japan).

In order to calculate the swelling ratio of p(HEMA-MAAP-Ce^{3+}) cryogel, the cryogel was dried and weighed until constant weight (m_{dried}). Then, it placed in a 50 mL vial containing distilled water and kept at 25 ºC for 2 h. The cryogel was removed from water, wiped by a filter paper and weighed again (m_{wet}). The swelling ratio was calculated according to following Eqn (1);

$$S = m_{wet} - m_{dried} / m_{dried} \qquad (1)$$

For the measurement of macroporosity percentage (*M%*) of cryogels, the mass of water-saturated cryogels (m_{wet}) was weighed. The cryogel was squeezed to remove free water which is found in the pores ($m_{squeezed}$), and the mass of the cryogel without water was weighted. *M%* was calculated according to following Eqn (2);

$$M\% = m_{wet} - m_{squeezed} / m_{wet} \times 100 \quad (2)$$

2.5. Lysozyme Adsorption From Aqueous Solutions

The cryogel was equilibrated with 10 mL of 20 mM phosphate buffer solution (pH 6.0) after washed with 50 mL of water. The prepared lysozyme solution (10 mL) was passed through the cryogel column by a peristaltic pump for 2 h. Lysozyme adsorption was followed by determining the protein amount of samples before and after adsorption according to Bradford protein assay method at 595 nm [28]. All adsorption studies were performed at 25 ºC.

The amount of adsorbed lysozyme was determined as follows (Eqn 3):

$$Q = C_o - C \quad V / m \quad (3)$$

where Q is the amount of adsorbed lysozyme (mg/g), C_o and C are lysozyme concentration before and after adsorption, respectively (mg/mL), V is the volume of lysozyme solution and m is the mass of the cryogel (g)

Effects of pH, flow rate, initial lysozyme concentration and temperature on the adsorption capacity were studied. To investigate the effect of pH, the solution pH was adjusted with 20 mM acetate buffer for pH 5.0 and 20 mM phosphate buffer for pH 6.0, 7.0 and 8.0. The flow rate was altered in the range of 0.5-3.0 mL/min. The effect of lysozyme concentration on the adsorption was investigated in the range of 0.25-1.5 mg/mL and the temperature was changed in the range of 4-45 ºC.

Experiments were carried out in three replicates. For each set of data, standard statistical methods were used to determine the mean values and standard deviations.

2.6. *Desorption And Reusability Studies*

The adsorbed lysozyme was desorbed from the p(HEMA-MAAP-Ce^{3+}) cryogel using 1 M NaCl in 20 mM phosphate buffer (pH 6.0). For this aim, 30 mL of desorption solution was pumped through the cryogel at a flow rate of 0.5 mL/min for 120 min. Amount of desorbed enzyme was determined spectrophotometrically at 595 nm. Desorption percentage was calculated as follows (Eqn 4);

$$\text{Desorption} \quad \% \quad = \text{Desorbed lysozyme} \times 100 / \text{Adsorbed lysozyme} \qquad (4)$$

After the desorption process, p(HEMA-MAAP-Ce^{3+}) cryogel was washed with 30 mL of distilled water and equilibrated with a 20 mM phosphate buffer solution (pH 6.0) for the next adsorption steps and lysozyme adsorption-desorption cycle was repeated for 7 times using the same cryogel column.

3. RESULTS AND DISCUSSION

3.1. Characterization Studies

P(HEMA-MAAP-Ce^{3+}) cryogel was produced by cryopolymerization of HEMA and MAAP-Ce^{3+}. MBAAm, APS and TEMED were used as a cross-linker, initiator and activator, respectively. Functional metal-chelate monomer, MAAP-Ce^{3+}, was selected to interact with lysozyme and to make metal complexing polymeric receptors for selective binding of lysozyme. MAAP-Ce^{3+} complex was detected by UV-NIR. As shown in Figure 1, MAAP has no peak, but five different peaks at 916, 1037, 1081, 1109 and 1141 nm were observed for MAAP-Ce^{3+} complex.

EDX spectrum of MAAP-Ce^{3+} showed that 15.12% N and 2.89% Ce^{3+} were found in the MAAP-Ce^{3+} complex. As seen in Figure 2, the presence of nitrogen and Ce^{3+} showed the incorporation of the MAAP-Ce^{3+}.

The FTIR spectrum of p(HEMA) and p(HEMA-MAAP-Ce^{3+}) cryogel are given in Figure 3. The p(HEMA-MAAP-Ce^{3+}) cryogel has the characteristic vibration bands of –OH bond around 3412 cm^{-1} and –NH stretching at 3104 cm^{-1} and C=O bonds of ester, amid and cyclic carbonyl groups at 1768-1712 cm^{-1}.

Surface morphology of the p(HEMA-MAAP-Ce^{3+}) cryogel is given in Figure 4. As shown in the figure, the cryogel has interconnected pores and porous structure. Pore size was found about 30-50 μm. The equilibrium swelling degree and macroporosity of the p(HEMA-MAAP-Ce^{3+}) cryogel were 7.39 g H_2O/g cryogel and 80.8%, respectively.

3.2. Effect Of Flow Rate

The amount of adsorbed lysozyme at different flow rates is shown in Figure 5A. The adsorption capacity of lysozyme decreased slightly from 57.84 mg/g to 47.35 mg/g with the increase of the flow rate. It can be concluded that lysozyme adsorption capacity was independent of flow rate. The lysozyme molecules could not directly pass through the cryogel without interaction with the macropores. The mobile phase was forced to flow through transporting the lysozyme through the active binding sites by convection. The flow independency of adsorption is due to the connective mass transfer property of p(HEMA-MAAP-Ce^{3+}) [29].

3.3. Effect Of Ph

pH is one of the parameters that affects the adsorption capacity of lysozyme. Various pH values using 0.5 mg/mL lysozyme solution in 20 mM acetate (pH 5.0) and phosphate (pH 6.0, 7.0 and 8.0) buffer systems were studied. The maximum adsorption of lysozyme was observed at pH 6.0 (57.84 mg/g cryogel). At the same time, some previous studies indicated that lysozyme adsorption capacity had been found to be 1.0 mg/g by using poly(vinyl acetate) particles at its isoelectric points [30] and 17.3, 26.8 and 36.4 mg/g with Ni^{2+}, Zn^{2+} and Cu^{2+} ions chelated poly-(L)-histidine immobilised poly(glycidyl methacrylate) cryogel discs, respectively [31]. As shown in Figure 5B, enzyme adsorption capacity decreased above or below pH 6.0. The decrease in the lysozyme adsorption capacity can be attributed to electrostatic interaction between charged groups. Protein adsorption in IMAC is mainly through chelation between some metal ions and amino acids, such as, histidine (His) and cysteine (Cys). Lysozyme has one His residue on its surface and pKa value of His in most proteins is in the range of 5.5 to 8.5 [32]. Over this value, imidazole group of His is deprotonated and suitable for interaction with positive charged metal ions [25].

3.4. *Effect Of Temperature*

Different forces, such as, hydrophobic, ionic, electrostatic or van der Waals interactions or hydrogen bonding affect adsorption capacities of proteins. In addition, adsorption type affects the temperature on the adsorption of protein onto polymer. Lysozyme adsorption analyses were carried out at 4, 25, 35 and 45 ºC and results were given in Figure 5C. Lysozyme adsorption capacity increased considerably (31%) when the temperature

increased from 4 to 25 ºC, which may be due to increasing of van der Waals interaction which increases with temperature [14,33]. Lysozyme adsorption capacity slightly decreased (12%) from 25 to 45 ºC. MAAP has hydrogen bonding and hydrophobic interaction capacity. This slight decrease may be explained with this way.

3.5. Effect Of Lysozyme Concentration

Figure 5D shows the effect of initial lysozyme concentration on the lysozyme adsorption. As seen here, adsorption capacity increased with increasing amount of lysozyme and reached a saturation value of 78.6 mg/g at 1 mg/mL of lysozyme concentration indicating that all ligands bound to the lysozyme.

Adsorption equilibria are the important physicochemical aspect for biomolecules with the adsorbent in the adsorption process. For this aim, Langmuir and Freundlich isotherms are commonly used as equilibrium models. Equilibrium data have been applied by using these two models [34]. The Langmuir and Freundlich isotherms are given in Equations 4 and 5, respectively.

$$1/qe = \quad 1/Qmax \quad + \quad 1/Qmax.b \quad 1/Ce \qquad (4)$$

$$lnqe = \; 1/n \quad lnCe \quad + \; lnK_F \qquad (5)$$

where b is the Langmuir isotherm constant, K_F is the constant, n is the Freundlich exponent. $1/n$ is a measure of the surface heterogeneity ranging between 0 and 1, becoming more heterogenous as its value gets closer to zero. The ratio qe gives the theoretical monolayer saturation capacity of the polymer. Table 2 shows the kinetic constants of Langmuir and Freundlich adsorption isotherms. The obtained R^2 values of

the isotherms suggest that Langmuir isotherm (R^2 = 0.994) is applicable for this adsorption system.

3.6. Desorption And Reusability Studies

The desorption results show that adsorbed lysozyme was desorbed within 40 min and 95.7% of the lysozyme was desorbed (Figure 6). The lysozyme adsorption capacity decreased about 12% at the end of the seven adsorption-desorption cycles using same cryogel, and there was not any noticeably changes during the repeated adsorption-desorption cycles. With these results we can say that p(HEMA-MAAP-Ce^{3+}) cryogel can be repeatedly used in lysozyme adsorption-desorption without significant losses in its adsorption capacity and shows a good reusability in lysozyme adsorption.

4. CONCLUSION

In this study, methacryloyl antipyrine-Ce^{3+} (MAAP-Ce^{3+}) containing p(HEMA-MAAP-Ce^{3+}) cryogel for lysozyme adsorption was prepared. In this procedure, co-monomer (MAAP-Ce^{3+}) was used as the pseudo-affinity ligand therefore there is no need to activate the matrix. Combination of these techniques is going to be powerful tool for protein purification technology. In the present study, lysozyme adsorption capacity was found as 57.84 mg/g cryogel at pH 6.0. Adsorption-desorption studies showed no significant decrease in the adsorption capacity. The obtained results indicated that this newly synthesized adsorbent with MAAP-Ce^{3+} can be considered as a potential and suitable for purification and separation technology. In addition, in this way the NIR

property of Ce^{3+}, near-IR featured luminescence imaging materials can be prepared for lysozyme or other biomolecules.

CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

REFERENCES

[1] Mine, Y. *Trends Food Sci. Tech.* **1995,** *6(7)*, 225.

[2] Türkmen, D.; Denizli, A. *Colloid Surface A.*. **2014,** *123*, 859.

[3] Kalburcu, T.; Tabak, A.; Öztürk, N.; Tüzmen, N.; Akgöl, S.; Çağlar, B.; Denizli, A. *J. Mol. Struct.* **2015,** *1083*, 156.

[4] Zhu, X.; Zhang, L.; Fu, A.; Yuan, H. *Mater. Sci. Eng.* **2016,** *59*, 213.

[5] Derazshamshir, A.; Ergün, B.; Peşint, G.; Odabaşı, M. *J. Appl. Polym. Sci.* **2008,** *109*, 2905.

[6] Perçin, I.; Şener, G.; Demirçelik, A. H.; Bereli, N.; Denizli, A. *Appl. Biochem. Biotech.* **2015,** *175*, 2795.

[7] Ghosh, R.; Cui, Z. F. *J. Membrane Sci.* **2000,** *167*, 47.

[8] Carvalho, M. A.; Da Silva, S. L.; Da Silva, L. H. M.; Minim, V. P. R.; Da Silva, M. C. H.; Carvalho, L. M.; Minim, L. A. *Sep. Purif. Rev.* **2014,** *43*, 241.

[9] Lozinsky, V. I.; Galaev, I. Y.; Plieava, F. M.; Savina, I. N.; Jungvid, H.; Mattiasson, B. *Trends Biotechnol.* **2003,** *21*, 445.

[10] Plieava, F. M.; Galaev, I. Y.; Mattiasson, B. *J. Sep. Sci.* **2007,** *30*, 1657.

[11] Ertürk, G.; Mattiasson, B. *J. Chromatogr. A.* **2014,** *1357*, 24.

[12] Perçin, I.; Yavuz, H.; Aksöz, E.; Denizli, A. *Biotechnol. Progr.* **2012,** *28*, 756.

[13] Yavuz, M.; Baysal, Z. *J. Food Sci.* **2013,** *78*, E238.

[14] Yavuz, M.; Cakir, O.; Baysal, Z. *Turk. J. Chem.* **2016,** *40*, 720.

[15] Andrabi, S. M.; Tiwari, J.; Singh, S.; Sankar, J.; Verma, N.; Kumar, A. *Int. J. Polym. Mater.* **2016,** *65(12)*, 636.

[16] Savina, I. N.; Mattiasson, B.; Galaev, I. Y. *Polymer* **2005,** *46*, 9596.

[17] Xu, P.; Yao, Y.; Shen, S.; Yun, J.; Yao, K. *Chinese J. Chem. Eng.* **2010,** *18*, 667.

[18] Da Silva Junior, W. F.; Cano, R.; Totola, A. H.; Milena de Carvalho, L.; Cerri, M. O.; dos Reis Coimbra, J. S.; Pinto De Carvalho, G. G.; Aparecida de Carvalho, B. M. *J. Chromatogr. A.* **2015,** *1395*, 16.

[19] Mooney, J. T.; Dale, P.; Fredericks, P.; Zhang, C.; Christensen, T.; Jespergaard, C.; Schniodt, C. B.; Hearn, M. T. W. *Protein Expres. Purif.* **2014,** *94*, 85.

[20] Mirza, M. R.; Rainer, M.; Messner, C. B.; Güzel, Y.; Schemeth, D.; Stasyk, T.; Choudhary, M. I.; Huber, L. A.; Rode, B. M.; Bonn, G. K. *Analyst*, **2013,** *138*, 2995-3004.

[21] Kagedal, L. (1998), Immobilized metal ion affinity chromatography. In: Janson J.C. Ryden L (eds). Protein purification: principles, high-resolution methods and applications. 3 rd edn. (Wiley, New York), pp. 183-201.

[22] Uygun, M. *Methods Mol. Biol.* **2015,** *1286*, 213.

[23] Wang, L.; Shen, S.; He, X.; Yun, J.; Yao, K.; Yao, S. -J. *Biochem. Eng. J.* **2008,** *42*, 237.

[24] Dainiak, M. B.; Kumara, A.; Plieva, F. M.; Galaev, I. Y.; Mattiasson, B. *J. Chromatogr. A.* **2008,** *1045*, 93.

[25] Tüzmen, N.; Kalburcu, T.; Denizli, A. *Process Biochem.* **2012,** *47*, 26.

[26] Uygun, M.; Akduman, B.; Akgöl, S.; Denizli, A. *Appl. Biochem. Biotech.* **2013,** *170*, 1815.

[27] Keçili, R.; Say, R.; Yavuz, H *Int. J. Biol. Macromol.* **2006,** *39*, 250.

[28] Bradford, M. M. *Anal. Biochem.* **1976,** *72*, 248.

[29] Perçin, I.; Aksöz, E.; Denizli, A. *Appl. Biochem. Biotech.* **2013,** *171*, 352.

[30] Dos Santos, D. P.; Alves, T. L. M.; Pinto, J. C. *Polimeros* **2016,** *26*, 282.

[31] Cimen, D.; Turkmen, D.; Denizli, A. *Adsorpt. Sci. Technol.* **2016,** *34*, 469.

[32] Lu, A. X.; Liao, X. P.; Zhou, R. Q.; Shi, B. *Colloids Surface A.* **2007,** *301*, 85.

[33] Baysal, Z.; Bulut, Y.; Yavuz, M.; Aytekin, C. *Starch-Starke* **2014**, *66*, 484.

[34] Akduman, B.; Uygun, M.; Aktaş Uygun, D.; Antalik, M. *J. Nanopart. Res.* **2013,** *15*, 1564.

Table 1. Major proteins of chicken egg white [1].

	Molecular weight (Da)	Isoelectric point (p*I*)	Total protein (%)
Ovalbumin	44.500	4.5	54
Ovotransferrin	77.700	6.1	12
Ovomucoid	28.000	4.1	11
Ovomucin	5.500-8.800	4.5-5.0	3.5
Lysozyme	14.300	10.73	4

Table 2. Adsorption constants for Langmuir and Freundlich isotherms.

Langmuir adsorption isotherm	Freundlich adsorption isotherm
Q_{max} = 81.97 mg/g	K_F = 180.34 mg/g
b = 406.66 mL/mg	$1/n$ = 0.243
R^2 = 0.994	R^2 = 0.517

Figure 1. UV-NIR spectrum of MAAP and MAAP-Ce^{3+} complex.

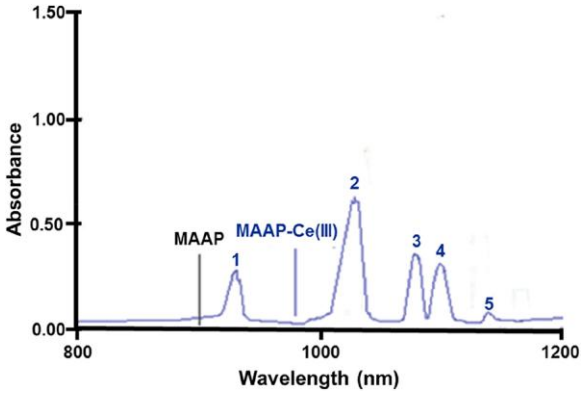


Figure 2. EDX spectrum of MAAP-Ce^{3+} complex.

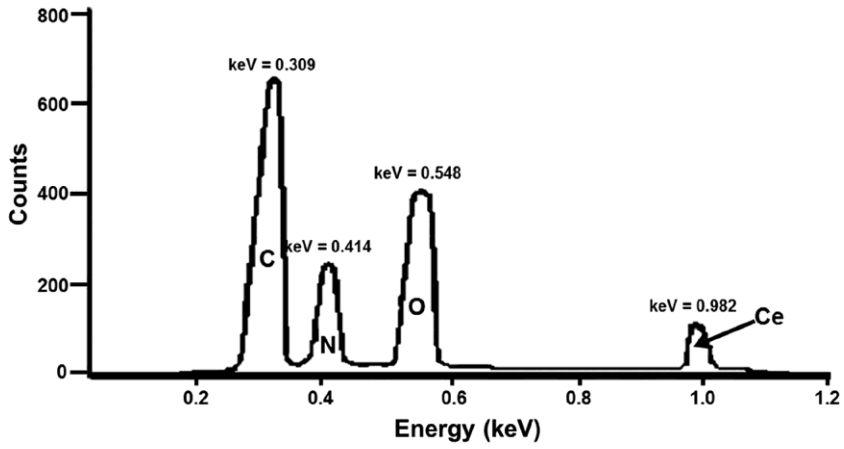


Figure 3. FTIR spectra of HEMA and p(HEMA-MAAP-Ce^{3+}) cryogels.

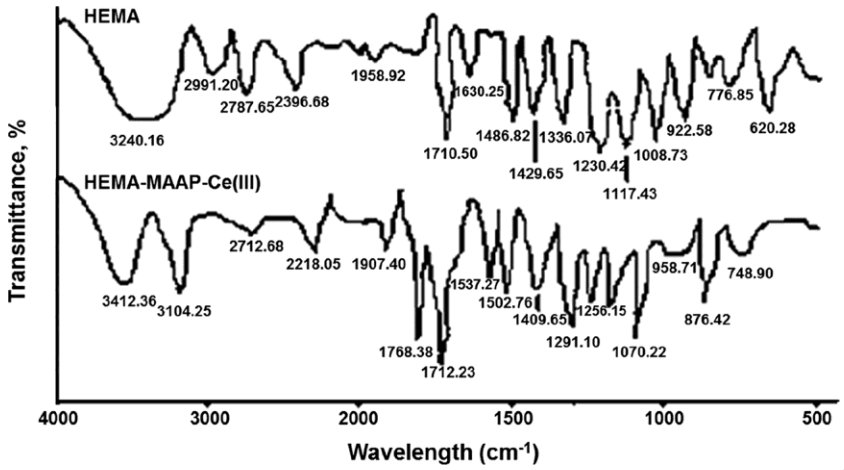


Figure 4. SEM photograph of p(HEMA-MAAP-Ce^{3+}) cryogel at **A)** 750× and **B)** 1500× magnifications.

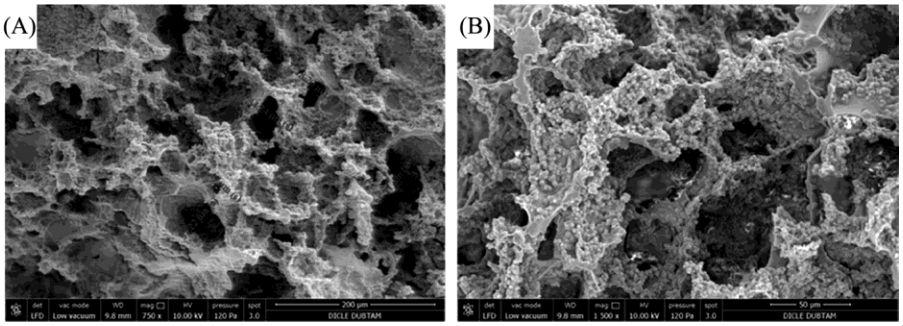


Figure 5. A) The effect of flow rate on lysozyme adsorption; 0.5 mg/mL initial lysozyme concetration, T: 25 ºC, pH: 6.0, **B)** The effect of pH on lysozyme adsorption; 0.5 mg/mL initial lysozyme concetration, T: 25 ºC at 0.5 mL/min flow rate, **C)** The effect of temperature on lysozyme adsorption; 0.5 mg/mL initial lysozyme concetration, pH: 6.0 at 0.5 mL/min flow rate, **D)** The effect of initial lysozyme concentration; pH: 6.0, T: 25 ºC at 0.5 mL/min flow rate.

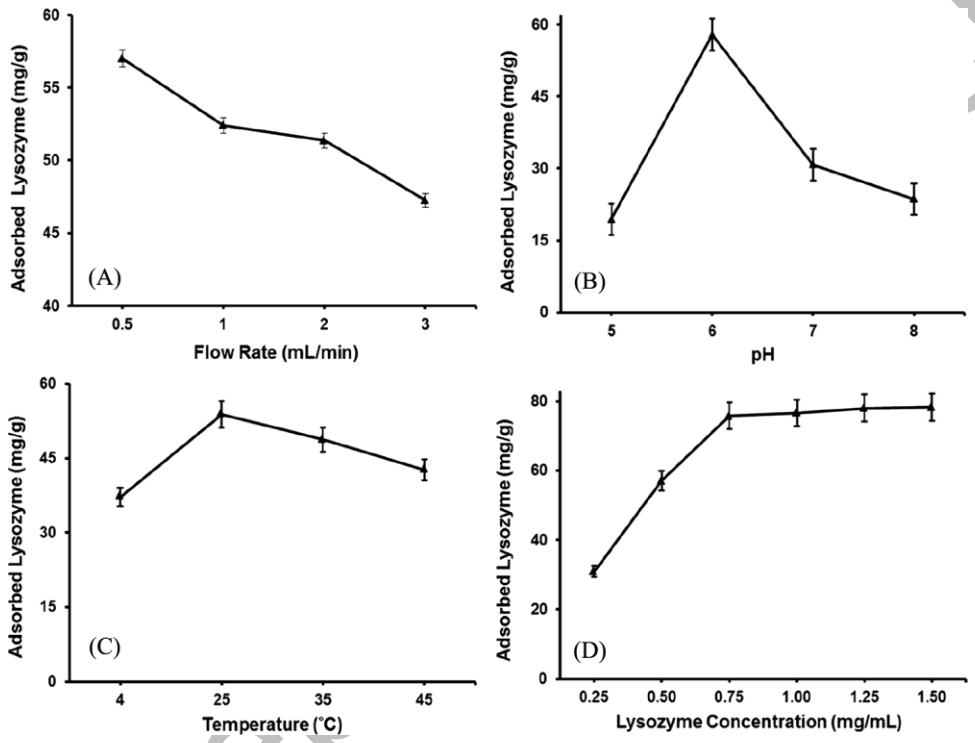


Figure 6. Desorption of lysozyme at different times; pH: 6.0, T: 25 ºC at 0.5 mL/min flow rate.