Study of kinetic adsorption human insulin on magnetic imprinting polymer

H. Gheybalizadeh, P. Hejazi*
Biotechnology Research Laboratory, School of Chemical, Petroleum and Gas Engineering, Iran University of Science and Technology, Zip Code: 16846-13114 Tehran, Iran
phejazi@iust.ac.ir

Abstract
In this work, Magnetic molecular imprinting polymers (MMIP) were used to separate recombinant human insulin. Different kinetic models for the adsorption of insulin on MMIPs and magnetic molecular non-imprinting polymers (MNIPs) were investigated. The results were shown that the adsorption capacity (Q, $mg.g^{-1}$) of the MMIPs, and imprinting factor (IF) are found to be 66.5 $mg.g^{-1}$, and 3, respectively. Studies demonstrate that the pseudo-second-order model has a better fit (R^2= 0.9988) for insulin adsorption kinetics on MMIPs. Moreover, the Elovich confirmed the chemisorption adsorption of insulin on MMIPs (R^2=0.998). The characterization of synthesized particles was applied by Fourier transform infrared spectrometry (FT-IR), and the vibrating sample magnetometer (VSM) and the MMIPs achieved rapid magnetic separation (19.93 emu g^{-1}).

Keywords: Fe_3O_4 magnetic nanoparticle, Insulin, kinetic adsorption model, Magnetic molecular imprinting polymer

Introduction
Molecular imprinting polymers (MIPs) is an effective technique, which can separate and recognize a specific molecule in the mixture. Because of the specific recognition, low cost, long-term storage, chemical, and physical stability in comparison with natural antibody, MIPs have attracted more attention in recent years. Molecular imprinting consists of binding sites that exactly complement template molecule either in conformational arrangement or distributions of functional monomers, which could have bound with template molecule. MIPs are synthesized with copolymerization of functional monomer(s), and cross-lineker in the presence of template molecule [1].
Compare the small molecules (<1500 Da), imprinted polymer for macromolecules have been associated with challenges due to large molecular size and conformational complexity. So, researchers have successfully developed different strategies like bulk and surface techniques in separation macromolecules [2]. However, in the bulk imprinting method due to the large size of macromolecules, diffusion limitation, and rebinding difficulties, surface imprinting technique has been introduced and was applied for separation and recognition of macromolecules such as proteins [3]. Nanoparticle materials overcome the drawbacks of conventional MIPs. Among these materials, magnetic nanoparticles (MNPs) such as Fe_3O_4 because of physical responsiveness that can be manipulated by an external magnetic field has

been received significant attention. MNPs can be used in separation, solid-phase extraction, sensor, and diagnostic application [4].
Nowadays, human insulin is synthesized by recombinant DNA technology, as the high purity and pharmaceutical quality requirements in human insulin production; therefore, it is essential to have an approach with high separation factor, fast, and acceptable selectivity [5]. Due to MIPs properties, this procedure has great potential in reducing impurities and the cost of insulin production.
Binding kinetics is an important criteria in the evaluation of molecular adsorption performance. Generally, adsorption time can change the adsorption capacity drastically. So to verify the mechanism of binding kinetic, pseudo-first-order, pseudo-second-order, and Elovich models should be used to analyze the adsorption properties. The pseudo-first-order model suggests the occupancy of the binding site in the proportion of the number of unoccupied sites, whereas the pseudo-second-order model demonstrated the chemical reaction mechanism of template molecule and MIPs [6] (listed in Table 1).
In this study, the MNPs were coated, and were functionalized with vinyl groups. MMIPs layer synthesized with Acrylamide (AAm), Methacrylic acid (MAA), 2-dimethyl aminoethyl methacrylate (DMA) as functional monomers, and methylene bisacrylamide (MBAA) as crosslinker on the surface of modified MNPs. Different kinetic adsorption models have been applied for insulin adsorption. FT-IR and VSM were used to characterize the magnetic particle.

Experimental

Materials

Human insulin (recombinant DNA) was purchased from Exir Pharmaceutical Co. (Brujerd, Iran). MAA, AAm, MBAA, DMA, iron (II) chloride tetrahydrate, iron (III) chloride hexahydrate, tetraethyl orthosilicate (TEOS), aqueous ammonium solution (25% w/w), and N,N,N′,N′-tetramethylethylenediamine (TEMED) were from Merck Co. Methacryloxypropyltrimethoxysilane (MPS), and Ammonium persulfate (APS) were obtained from Sigma Co. All the other reagents were of analytical grade without further purification, and the solutions were prepared with double-distilled water (DDW).

Methods

Preparation of the Fe_3O_4 Synthesis & coating

Fe_3O_4 magnetic nanoparticle (MNPs) were synthesized through a co-precipitation reaction. Then, silica coating on Fe_3O_4 with TEOS was applied. Subsequently, the vinyl groups were introduced to the Fe_3O_4@SiO_2 through a sol-gel method [7].

Preparation of Magnetic molecularly imprinted polymer (MMIPs)

To prepare for the MMIPs according to [8] with some modification, firstly, based on 100 ppm, 1 mol of insulin, 60 mol AAm, 15 mol MAA, and 15 mol DMA as functional monomers had being stirred in PBS (100 mM, pH=6) for 3 h at 25 ℃ in the complex mixture before the polymerization process to complete the pre-assembly via hydrogen bond, π-π stacking, and hydrophobic interactions. After 3 h stirring, MNP (20 mg), MBA (5 mol) toward 1 mol insulin, APS (10 % of total monomers), and TEMED (1:1 molar ratio with APS) was added into 10 ml vial to initiate the polymerization. After being purging with N_2, the resultant mixture was incubated at 25 ℃ and polymerized for 24 h. After polymerization, the MMIPs were collected with external magnetic and washed with PBS several times. Subsequently, in the elution procedure, the insulin embedded from the binding sites in MMIPs was washed with methanol/acetic acid 10% (v/v) for 72 h and then re-washed with distilled water for 48 h.

Molecularly non-imprinted polymer (MNIPs) were synthesized without insulin during polymerization in the same procedure for comparison.

Adsorption experiments

To study binding kinetic of MMIPs and MNIPs, 20 mg MMIPs and MNIPs were added into 10 ml vial with 100 ppm of insulin solution. After the mixtures were incubated at 25 ℃ for 3 h, the suspension was separated, and the concentration of insulin was analyzed with Bradford assay ,and the adsorption capacity (Q mg g^{-1}), and imprinting factor (IF) was calculated.

Kinetic absorption of MMIPs and MNIPs

Kinetic adsorption was carried out by dissolving MMIPs (20 mg) in the insulin solution (100 ppm). The concentration of insulin at designated intervals (0.5, 1, 1.5, 2, 2.5, and 3 h) was measured by Bradford assay. Kinetic adsorption of MNIPs was applied in the same procedure.

Characterization of the MMIPs and MNIPs

FTIR analysis was carried out before and after polymerization to justify the coating polymer' formation layer on surface MNP. The magnetic properties of Fe_3O_4, Fe_3O_4@SiO_2@MPS, MMIPs were analyzed with VSM at 25 °C.

Results and discussion

Preparation of MMIPs

The MIPs layer is formed on the surface of modified MNPs with co-precipitation polymerization. After polymerization, the results of protein analysis showed that the amount of insulin is imprinted on MMIPs is equal to Q_{imp}=69±1. By washing insulin of the binding cavities of MMIPs, more than 90 % of insulin was extracted with methanol-acetic acid elution solution. Moreover, the Q (mg/g), and IF values are obtained 68±3, and 3, respectively. The results reveal that the MMIPs has a better specific recognition ability.

Characterization of the molecularly imprinted nanoparticles

The FT-IR spectra of Fe_3O_4@SiO_2@MPS and MMIPs are shown in Fig. 1. In the IR spectra, the double peaks at 1721 and 1362 cm^{-1} showed C=C-H group on surface Fe_3O_4@SiO_2, which indicated the successful grafting MPS onto the surface of Fe_3O_4@SiO_2 [9]. The FT-IR spectrum of MMIPs clearly showed the disappearance of the vinyl group peak at 1729 cm^{-1} and also appeared new peak at 2926 cm^{-1} demonstrated the new CH_2, which corresponded to the successful synthesis of MMIPs layer [10].

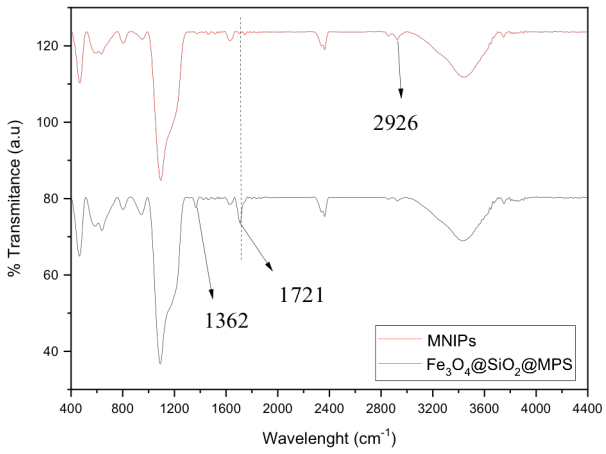

Fig. 1 FT-IR spectra of before polymerization (Fe_3O_4@SiO_2@MPS) and after polymerization (MNIPs)

The magnetization of Fe_3O_4, Fe_3O_4@SiO_2, and MMIPs are measured. The saturation magnetization values were obtained 61.3, 21.88, and 19.93 emu/g for Fe_3O_4, Fe_3O_4@SiO_2@MPS, and MMIPs, respectively. The reduction of magnetic saturation values of Fe_3O_4@SiO_2@MPS and MMIPs samples with respect to bar Fe_3O_4 confirmed coating, surface modification, and polymer layer formation [11].

Binding Kinetics

The binding kinetics of MMIPs and MNIPs for insulin was shown in Fig. 2. This kinetic was also supported by surface protein imprinted polymer reported previously [10]. The rapid initial adsorption was occurred because of the accessibility of binding sites near-surface of MMIPs, which facilitated mass transport of insulin without diffusion limitations. The adsorption of MMIPs is divided into three stages. In the first stage (t=0.5 h), because of hydrophobic and electrostatic interaction between insulin and functional monomers, insulin rapidly absorbed to MMIPs. In the second stage (t=0.5-2 h), when insulin penetrates to binding sites, face with strong steric hindrance that decreases adsorption rate. Finally, in the last stage (t=2-3 h), adsorption rate reached to equilibrium state since insulin concentration reduced and binding sites filled up. In comparison with MMIPS, adsorption kinetics of MNIPs has a lower adsorption capacity than MMIPs because of the absence of imprinting cavities and non-specific binding sites. As shown in Fig.2. MNIPs adsorption kinetics reached equilibrium after 2 h.

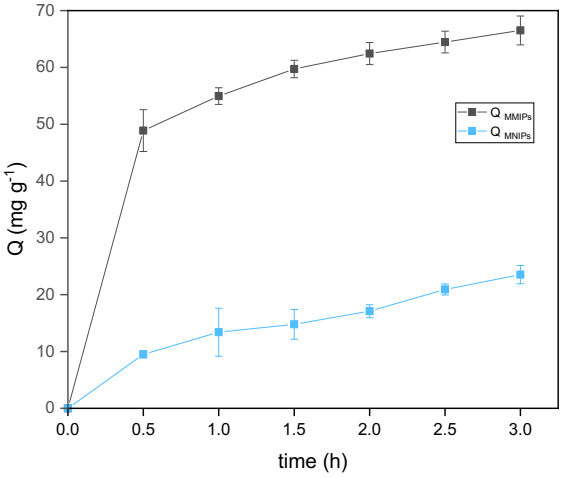

Fig. 2 Adsorption kinetics of insulin binding to MMIPs and MNIPs

Application of pseudo-first-order and pseudo-second-order models by plotting t versus Ln (Q_e-Q_t) and t versus t/Q_t are shown in Fig. 3. The corresponding constants of these models are shown in Table 1. The results showed that the adsorption behavior of insulin on MMIPs is followed on pseudo-second-order, implying that the adsorption behavior followed by chemical process. The pseudo-second-order model for MMIPs has a better fit (R^2=0.9988) than the pseudo-first-order (R^2=0.9343). Simultaneously, it was clear that Q_{exp} has a better complement to Q_{cal} in the pseudo-second-order model. The Elovich equation has a good fit for MMIPs (R^2=0.998) rather than MNIPs (R^2=0.919), which suggesting the chemical reaction involved in insulin adsorption on MMIPs [12].

The mechanism of adsorption is studied by the intraparticle Weber-Morris equation. Generally, the adsorption involves the following steps: bulk diffusion, film diffusion, and intraparticle diffusion or pore diffusion, finally, the adsorption of adsorbate on active sites. If the plot passes only one line, the intraparticle diffusion controls the adsorption mechanism. Nevertheless, other mechanisms also involve adsorption mechanisms [13].

Table 1 Constants for pseudo-first-order, pseudo-second-order, and Elovich equations for inulin adsorption

Model	Equation	Parameters*	MMIPs	MNIPs
Pseudo-first-order	$\ln(Q_e - Q_t) = \ln Q_e - k_1 t$	$Q_{e,exp}$ (mg.g^{-1})	70	25
		$Q_{e,cal}$ (mg.g^{-1})	44.66	27.77
		K_1(min^{-1})	2.03	1.09
		R^2	0.93	0.90
Pseudo-second-order	$\frac{t}{Q_t} = \frac{1}{k_2 Q_e^2} + \frac{t}{Q_e}$	$Q_{e,cal}$ (mg.g^{-1})	72	33.45
		K_2 (g mg^{-1}min^{-1})	0.49	0.02
		R^2	0.99	0.91
Elovich	$Q_t = \frac{1}{\beta}\ln(\alpha\beta) + \frac{1}{\beta} lnt$	α (mg.g^{-1} min^{-1})	44.83	71.41
		β (g mg^{-1})	0.10	0.13
		R^2	0.99	0.92

*Q_e & Q_t : insulin amount on the equilibrium & time; K_1 & K_2: rate constants; α: initial adsorption rate; β: desorption constant

The adsorption mechanism of insulin on MMIPs and MNIPs was demonstrated in Fig. 4. The results indicated that three-linearity that confirmed three steps for the insulin adsorption mechanism. Initially, the adsorption increased sharply, due to the diffusion of insulin on the surface of MMIPs and MNIPs (film diffusion phenomenon). Then, the adsorption gradually reduced, which indicated intraparticle insulin diffusion to binding sites. This step generally controls the rate of diffusion of the whole process [14]. Finally, the adsorption capacity reached to a constant amount. Further, the diffusion rate (k_i) for MMIPs (3.74) is higher than MNIPs (1.85). Similarly, the intercept of MMIPs (51.56) is bigger than MNIPs (11.38), which indicates the big thickness of the boundary layer of MMIPs.

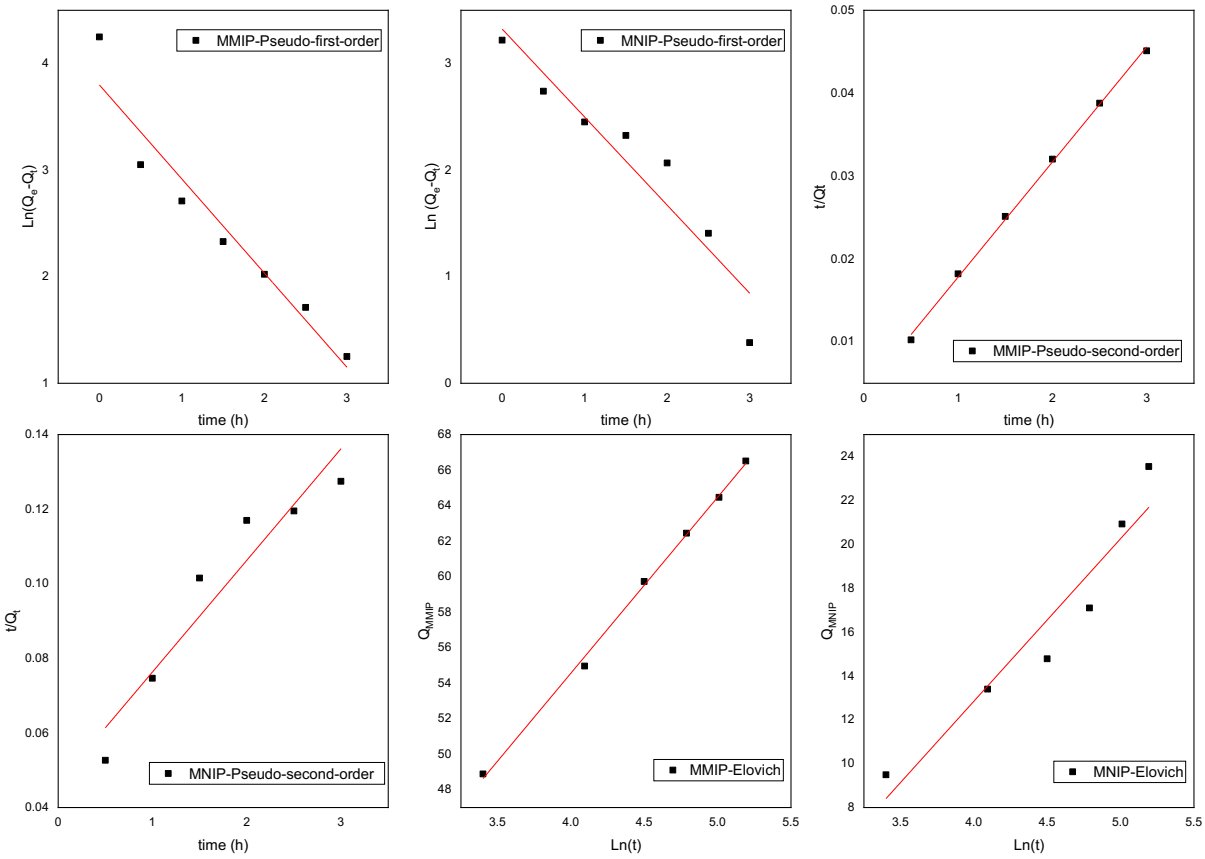

Fig. 3 Fitting of various kinetic models for insulin adsorption on MMIPs and MNIPs

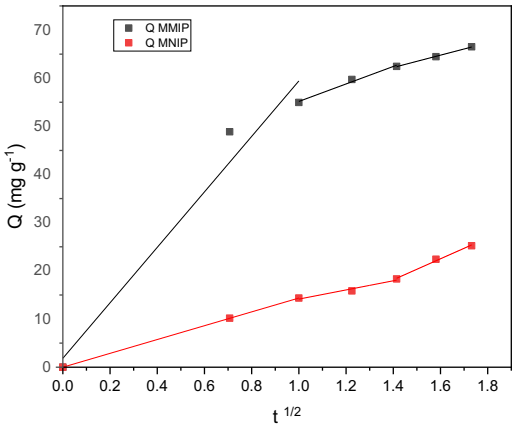

Fig. 4 Weber-Morris plot for the insulin adsorption on MMIPs and MNIPs

Conclusions

In summary, the magnetic imprinted polymers were developed for insulin adsorption, which has a rapid magnetic response for separation. The resultant MMIPs has good adsorption capacity and stability. Meanwhile, the MMIPs has fast adsorption kinetic. The results of this study showed that MMIPs could be used as biocompatible material with specific recognition properties in protein separation in aqueous media.

Reference

[1] Komiyama, M., T. Mori, and K. Ariga, "Molecular Imprinting: Materials Nanoarchitectonics with Molecular Information". Bull. Chem. Soc. Jpn., 91(7), 1075-1111 (2018).

[2] Ansari, S. and S. Masoum, "Molecularly imprinted polymers for capturing and sensing proteins: Current progress and future implications". Trac-Trend. Anal. Chem., 114, 29-47 (2019).

[3] Xu, W., et al., "Fabrication of magnetic polymers based on deep eutectic solvent for separation of bovine hemoglobin via molecular imprinting technology". Anal. Chim. Acta, 1048, 1-11 (2019).

[4] Xiaochu, D. and H.P. A., "Recent Developments in Molecularly Imprinted Nanoparticles by Surface Imprinting Techniques". Macromol. Mater. Eng., 299(3), 268-282 (2014).

[5] Zielinski, M., et al., "Expression and purification of recombinant human insulin from E. coli 20 strain". Protein Expr. Purif., 157, 63-69 (2019).

[6] Wu, Q., et al., "Well-defined nanostructured core–shell magnetic surface imprinted polymers (Fe3O4@SiO2@MIPs) for effective extraction of trace tetrabromobisphenol A from water". J. Ind. Eng. Chem., 60, 268-278 (2018).

[7] Goudarzi, F. and P. Hejazi, "Comprehensive study on the effects of total monomers' content and polymerization temperature control on the formation of the polymer-layer in preparation of insulin-imprinted magnetic nanoparticles". Eur. Polym J., 126, 109541 (2020).

[8] Goudarzi, F. and P. Hejazi, "Effect of biomolecule chemical structure on the synthesis of surface magnetic molecularly imprinted polymer in aqueous solution using various monomers for high-capacity selective recognition of human insulin". React. Funct. Polym., 143, 104322 (2019).

[9] Zhang, Z., et al., "Magnetic, core-shell structured and surface molecularly imprinted polymers for the rapid and selective recognition of salicylic acid from aqueous solutions". Appl. Surf. Sci., 435, 178-186 (2018).

[10] Xie, X., et al., "Facile preparation of photonic and magnetic dual responsive protein imprinted nanomaterial for specific recognition of bovine hemoglobin". Chem. Eng. J., 371, 130-137 (2019).

[11] Craciunescu, I., et al., "Synthesis and characterization of size-controlled magnetic clusters functionalized with polymer layer for wastewater depollution". Mater. Chem. Phys., 185, 91-97 (2017).

[12] Zhang, Y., et al., "Sorption enhancement of TBBPA from water by fly ash-supported nanostructured γ-MnO_2". J. Ind. Eng. Chem., 21, 610-619 (2015).

[13] Hosseini, S., et al., "Carbon coated monolith, a mesoporous material for the removal of methyl orange from aqueous phase: Adsorption and desorption studies". Chem. Eng. J., 171(3), 1124-1131 (2011).

[14] Arfaoui, F., et al. "Synthesis and characterization of molecularly imprinted silica for efficient adsorption of melamine". J. Tun. Chem. Soc., 19, 227-236 (2017).