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Research Article

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Purification of Ovalbumin From Egg White Using Molecular Imprinted Cryogels

Moleküler Baskılanmış Kriyojeller Kullanılarak Yumurta Akından Ovalbumin Saflaştırılması

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ABSTRACT

In recent years, food allergy is one of the most important problems worldwide. Ovalbumin is one of the main allergens found in egg whites. In this study, ovalbumin imprinted and non-imprinted poly(hydroxyethyl methacrylate-methacrylic acid (poly(HEMA-MAA)) cryogels were synthesized using molecular imprinting method. After characterization of ovalbumin imprinted cryogels, the effect of flow rate, pH buffer, einitial ovalbumin concentration, ionic strength, and temperature parameters are examined. The maximum ovalbumin adsorption amount was determined to be 37.78 mg/g of ovalbumin imprinted cryogels at pH 5.5, 25°C and a flow rate of 0.5 mL/min. The selectivity studies of ovalbumin imprinted and non-imprinted cryogels were performed using lysozyme and transferrin as competitor molecules. In addition, reusability experiments of ovalbumin imprinted cryogels were performed. The purity of ovalbumin was determined from egg white using sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The purity of ovalbumin was found to be approximately 85.4%.

Key Words

Ovalbumin, cryogel, molecular imprinting method, purification.

ÖZ

Son yıllarda, dünya çapında gıda alerjisi en önemli sorunlardan biridir. Ovalbumin yumurta akında bulunan ana alerjanlardan biridir. Bu çalışmada, ovalbumin baskılanmış ve baskılanmamış poli(hidroksietil metakrilat-metakrilik asit) poli(HEMA-MAA) kriyojeller moleküler baskılama yöntemi kullanılarak sentezlenmiştir. Ovalbumin baskılanmış kriyojellerin karakterizasyonundan sonra, akış hızı, pH tamponu, ilk ovalbümin konsantrasyonu, iyonik kuvvet ve sıcaklık parametrelerinin etkisi incelenir. Maksimum ovalbumin adsorpsiyon miktarı, ovalbumin baskılanmış kriyojel için pH 5.5, 25°C ve 0.5 mL/dk akış hızında 37.78 mg/g olarak belirlenmiştir. Ovalbumin baskılanmış ve baskılanmamış kriyojellerin seçicilik çalışmaları, yarışmacı moleküller olarak lizozim ve transferrin kullanılarak gerçekleştirilmiştir. Ayrıca ovalbumin baskılanmış kriyojellerin yeniden kullanılabilirlik deneyleri gerçekleştirilmiştir. Ovalbuminin saflığı, sodyum-dodesil sülfat poliakrilamid jel elektroforezi (SDS-PAGE) kullanılarak yumurta akından belirlenmiştir. Ovalbuminin saflığı yaklaşık% 85.4 bulunmuştur.

Anahtar Kelimeler

Ovalbumin, kriyogel, moleküler baskılama yöntemi, saflaştırma.

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INTRODUCTION

Food allergy is a major health problem worldwide. Ovalbumin, the most abundant protein in egg white, is known as the main allergen [1-4]. Ovalbumin constitutes 60-65% of the total protein in egg white. Ovalbumin is a complex glycoprotein consisting of 386 amino acids with a molecular weight of 44 kDa. Ovalbumin contains six cysteines with a single disulfide bond between Cys74 and Cys121 [5-10]. It causes food allergies with the most common immunoglobulin E, especially in infants and young children [11-18]. Today, typical methods such as ovalbumin electrophoresis, ion exchange chromatography, size exclusion liquid chromatography, ultrafiltration obtained from egg white are used [19-21]. However, some of these methods are multi-stage and costly. Also, extracting and purifying proteins from a complex matrix such as egg whites is a difficult task.

Cryogels are gel matrices prepared by freezing solutions containing monomeric or polymeric precursors, in part under freezing conditions. Wide interconnected porous mesh structure is the main characteristic feature of cryogels [22,23]. Cryogels show very low flow resistance due to their spongy morphology and macroporous structure, and practically allow diffusion of soluble matter of any size without hindering. As macroporous substances, cryogels are used as a matrix, as they allow not only diffusion of small molecular weight solutes, but also macromolecules and cells without hindering diffusion [24,25]. This is due to the interconnected structure of the macropores, and, the spongy and elastic morphology of the cryogels as a whole. The most common application areas of cryogels are separation, catalysis, and affinity chromatography [26-29]. Molecularly imprinted polymers are often used as recognition elements in food, life, medicine, and environmental science [30-33]. One of the application areas of molecularly imprinted polymers are cryogels as affinity support materials [33,35]. In recent years, molecularly imprinted cryogels are utilized in determining the biologically active molecules such as drugs, proteins, peptides, amino acids thanks to their higher selectivities [36-39].

In this study, we prepared ovalbumin imprinted and non-imprinted poly(hydroxyethyl methacrylate-methacrylic acid poly(HEMA-MAA) cryogels for purification of ovalbumin from egg white. Characterization studies of ovalbumin imprinted and non-imprinted cryogels were carried out with fourier transform infrared spectroscopy (FTIR), swelling degree, surface area and scanning electron microscopy (SEM) measurements. In the adsorption studies of ovalbumin imprinted cryogels, the effects of pH, initial ovalbumin concentration, temperature, ionic strength, flow rate, and reusability were investigated with continuous system.

MATERIALS and METHODS

Chemicals

Ammonium persulfate (APS), ovalbumin (OVA), methacrylic acid (MAA), 2-hydroxyethyl methacrylate (HEMA), N,N'-tetramethylethylenediamine (TEMED) and N,N'-methylene bisacrylamide (MBAAm) were purchased from Merck (Switzerland). In the selectivity studies, lysozyme (Lyz) and transferrin (Tf) were obtained from Sigma (St. Louis, USA). During the experimental studies, deionized water was used.

Preparation of Ovalbumin imprinted and non-imprinted cryogels

Complexes with 1:1, 1:2 and 1:3 M different molar ratios of monomer:template (MAA:OVA) were prepared for the preparation of ovalbumin imprinted (MIP) and non-imprinted (NIP) cryogels. Three different OVA imprinted cryogels were synthesized with MAA:OVA complexes at each different molar ratio. MAA:OVA complex in a ratio of 1:1 M for cryogel named MIP-1, MAA:OVA complex in molar ratio of 1:2 M for cryogel named MIP-2 and MAA:OVA complex in molar ratio of 1:3 M for cryogel named MIP-3 were prepared for the synthesis of 3 different OVA imprinted cryogels. HEMA and MBAAm were dissolved in 10 mL of deionized water and was prepared separately for each 3 different OVA imprinted cryogels. Three different molar ratio of MAA:OVA complexes were added to separately this monomer solution. TEMED (1%) and APS, 1% were used as the initiator/activator pairs. These mixture for each OVA imprinted poly(HEMA-MAA) cryogels were placed in separately 5 mL plastic bottom-sealed syringes and frozen for 24 h at -12°C. Non-imprinted cryogel was synthesized by the same polymerization procedure without using OVA.

Characterization Studies

The characterization studies of OVA imprinted and non-imprinted poly(HEMA-MAA) cryogels were carried out with scanning electron microscopy (SEM) measurements, fourier-transform infrared spectroscopy (FTIR), surface area, macroporosity and swelling degree (SD). FTIR spectra of poly(hydroxyethyl methacr-

ylate-methacrylic acid) cryogels were examined in the range 600-4000 cm^{-1} using a FTIR spectrometer. The specific surface areas of the OVA imprinted and non-imprinted cryogels were determined by multipoint Brunauer–Emmett–Teller (BET) method (Quantachrome, Nova 2200E, USA). SEM measurements were made to examine the surface morphology of OVA imprinted poly(hydroxyethyl methacrylate-methacrylic acid) cryogels. Firstly, the cryogels were dried (m_{dried}) and weighed for the swelling properties of OVA imprinted and non-imprinted cryogels. Deionized water was placed in a 20 mL beaker and placed in cryogel. The wet cryogel was removed from beaker, wiped and weighed (m_{wet}). Macroporosity is calculated using the weight of the cryogel in its pores when the water is swollen and after being squeezed. The swelling degree and macroporosity were determined using Eq (1,2).

$$\text{Swelling degree} = \frac{W_0 - W_{sw}}{W_0} \quad (1)$$

$$\text{Macroporosity } \% = \frac{W_{sw} - W_{sq}}{W} \quad (2)$$

Adsorption Studies

The effects of temperature, ionic strength, pH, flow rate and initial concentration on the ovalbumin adsorption capacity were studied for OVA imprinted cryogels. The effect of pH on the adsorption capacity was studied in acetate and phosphate buffers pH 3.0-8.0 at ovalbumin concentration of 0.5 mg/mL. The adsorption capacity of OVA imprinted cryogels was determined by performing along 2 h analyzes by performing adsorption studies in the range of OVA concentration of 0.1-2.5 mg/mL. The effect of temperature (4-45°C), flow rate (0.5-2.5 mL/min) and ionic strength (0-1.0 M, NaCl) on the adsorption capacities were also investigated by using OVA imprinting cryogels. The amount of the adsorbed ovalbumin was determined spectrophotometrically at 280 nm wavelength. Desorption studies of OVA imprinted cryogel were carried out using 1.0 M NaCl solution. Finally, OVA imprinted cryogels were repeated 10 times using the same cryogel in adsorption-desorption studies.

For purification of ovalbumin from egg white, the egg white was first separated from the egg yolk. After, the globulins were precipitated by adding 2 mL of 50% (v/v) saturated ammonium sulfate, and the precipitate was centrifuged at 3000xg for 30 min. The pH of the supernatant was adjusted to pH 4.6 by adding 2.0 M acetic acid and centrifuged again at 3000xg for 30 min. After centrifugation, the precipitate containing ovalbumin was suspended in pH 7.0 phosphate buffer. SDS-PAGE analysis was used for ovalbumin purity from egg white. For SDS-PAGE analysis, 12% separation gel (9 × 7.5 cm) was used and electrophoresis was operated at 110 V for 2 h. After analysis, stacking gels (%6) were prepared and stained with coomassie brillant prepared in water-acetic acid-methanol mixture [4]. The purity of ovalbumin from egg white was analysed by SDS-PAGE using 12% separating gel [22,40].

Selectivity experiments

One of the most important features of molecular imprinting method is that cavities of the imprinted molecule are formed on the polymer surface. Ovalbumin, lysozyme, and transferrin are an important glycoproteins found in egg white. Lysozyme (Lyz, MW: 14.3 kDa) and transferrin (TF, MW: 55.8 kDa) molecules were chosen as competitive agents due to their presence with ovalbumin (OVA, MW: 44 kDa) in egg white as well as their molecular structure and weight [41]. Lyz and TF solutions were prepared at a concentration of 0.5 mg/mL and adsorption studies were carried out on ovalbumin imprinted and non-imprinted cryogels. K_d (distribution coefficients), k (selectivity) and k' (relative selectivity coefficient) for Lyz and TF molecules relative to OVA molecules were calculated by following Eq (3,4,5).

$$Kd = \left(\frac{C_i - C_f}{W_0} \right) * V / m \quad (3)$$

$$k = \frac{K_{d(\text{template protein})}}{K_{d(\text{competing protein})}} \quad (4)$$

$$k' = \frac{K_{MIP}}{K_{NIP}} \quad (5)$$

The distribution coefficient (mL/g) is K_d, volume of the solution (mL) is V, OVA concentrations (mg/mL) is C_i (initial) and C_f (final), and weight of polymer (g) is m.

RESULTS and DISCUSSION

Characterization Studies

OVA imprinted poly(HEMA-MAA) cryogels with code name (MIP-3) were observed aliphatic C-H stretch band at 2917 cm^{-1}, carbonyl band at 1728 cm^{-1} and amide band 1541 cm^{-1}. N-H stretching, C=O of amide I and C-N-H of amide II vibration of the OVA was observed at 3201 cm^{-1}, 1652 cm^{-1} and 1538 cm^{-1}, respectively [42]. As can be seen from FTIR-ATR spectra, MAA monomer and ovalbumin molecule were found to enter the cryogel structure (Figure 1A). The surface morphology of OVA imprinted (MIP-3) cryogel was investigated by SEM. As clearly shown in Figure 1B, OVA imprinted cryogel has large continuous interconnected and homogeneous porous surface structure.

The swelling degree, macroporosity and specific area of ovalbumin imprinted and non-imprinted cryogels were characterized and given in Table 1. When comparing OVA imprinted cryogels (code MIP-1, MIP-2 and MIP-3 cryogels) with non-imprinted (NIP) cryogels, it was observed that as the amount of ovalbumin increased, the degree of swelling and other variables increased. In addition, an increase in the specific surface area was observed with the increase in the imprinting ratio and caused an increase in water intake ratios

Adsorption Experiments

When the adsorption capacity of OVA imprinted and non-imprinted cryogels were compared, it was observed that the adsorption capacity of OVA imprinted cryogels was higher. This shows us that ovalbumin specific binding sites have been formed. The maximum imprinting factor value was observed at a 1:3 M ratio of MAA:OVA and all adsorption experiments were performed with OVA imprinted cryogel (MIP-3) synthesized at this molar ratio. In all experiments, ovalbumin concentration in the solution was determined spectrophotometrically at 280 nm.

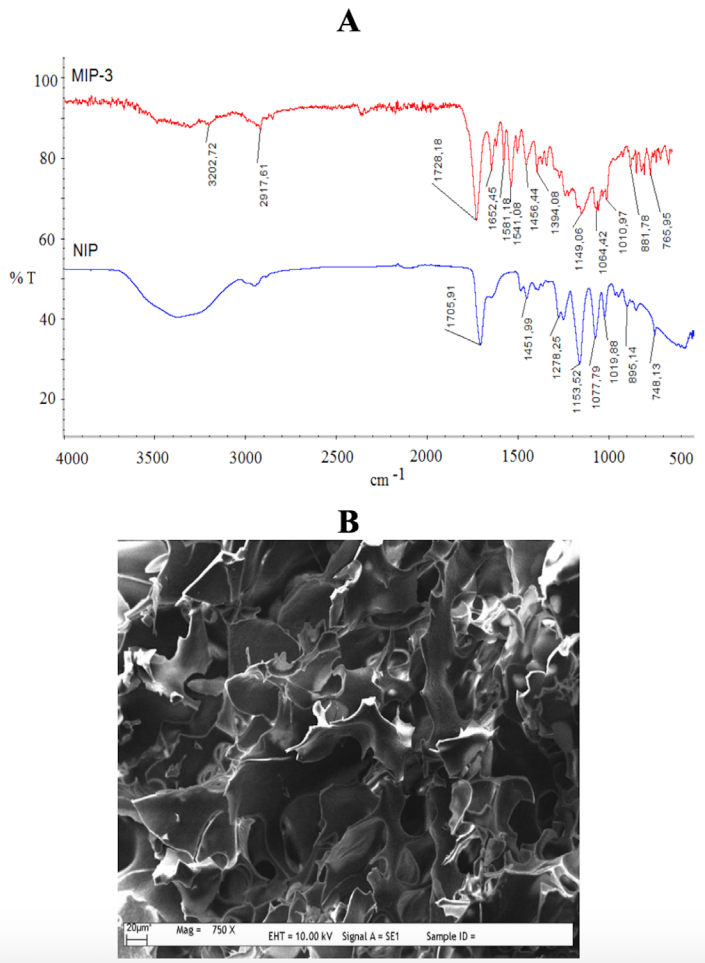

Figure 1. FTIR spectra of OVA imprinted (MIP-3) and non-imprinted (NIP) cryogels (A) and SEM image of OVA imprinted cryogel (B).

Table 1. Physical properties of OVA imprinted (MIP) and non-imprinted (NIP) cryogels.

Polymer Code	Swelling Degree	Macroporosity (%)	Surface Area
MIP-1	7.23 g H_2O/g polymer	71.15	25.7
MIP-2	9.02 g H_2O/g polymer	76.39	27.4
MIP-3	12.74 g H_2O/g polymer	81.84	32.5
NIP	5.63 g H_2O/g polymer	56.26	21.6

The effect of pH buffer on the adsorption capacity of OVA imprinted cryogel were performed by preparing different acetate (pH 3.0, 4.0, 5.0, 5.5) and phosphate buffers (pH 6.0, 7.0 and 8.0) at a concentration of 0.5 mg/mL OVA. Figure 2 shows the effect of different buffer types on ovalbumin adsorption capacity with OVA imprinted cryogel. The maximum ovalbumin adsorption capacity was observed as 11.55 mg/g in pH 5.5 phosphate buffer [43]. The isoelectric point of ovalbumin molecule is in the range of 4.43-4.9. It has been observed that the solution pH has the highest adsorption capacity at pH where it is close to the isoelectric point.

The effect of flow rate on the ovalbumin adsorption capacity of OVA imprinted cryogels (MIP-3) was investigated at a concentration of 0.75 mg/mL ovalbumin. While the flow rate increased from 0.5 mL/min to 2.5 mL/min, the adsorption capacity of OVA imprinted cryogels decreased from 17.38 to 5.44 mg/g polymer (Figure 3). It has been observed that the reason for the decrease in the adsorption capacity with the increase of the flow rate is due to the decrease in the retention time of ovalbumin [44]. The maximum ovalbumin adsorption capacity is determined as the lowest flow rate of 0.5 mL/min.

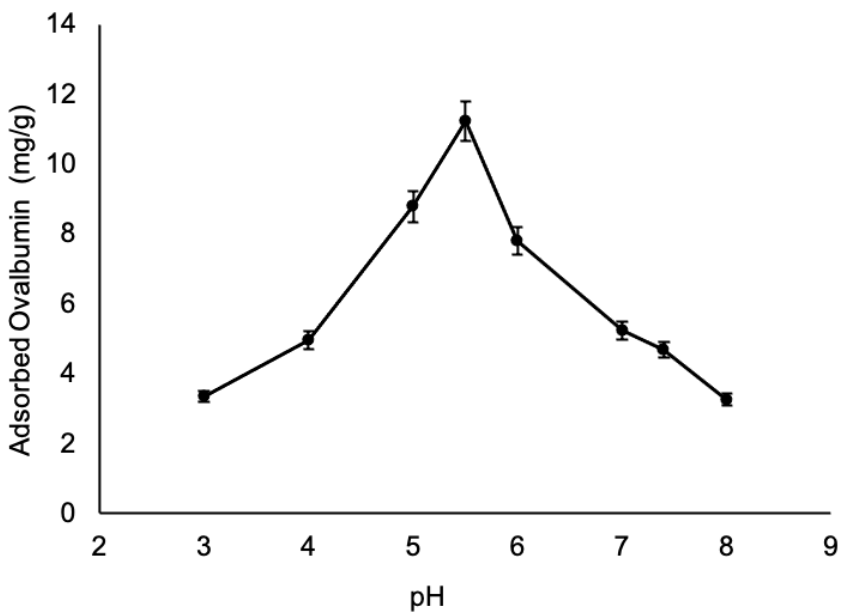

Figure 2. Effect of pH buffer (Flow rate: 0.5 mL/min, T: 25°C).

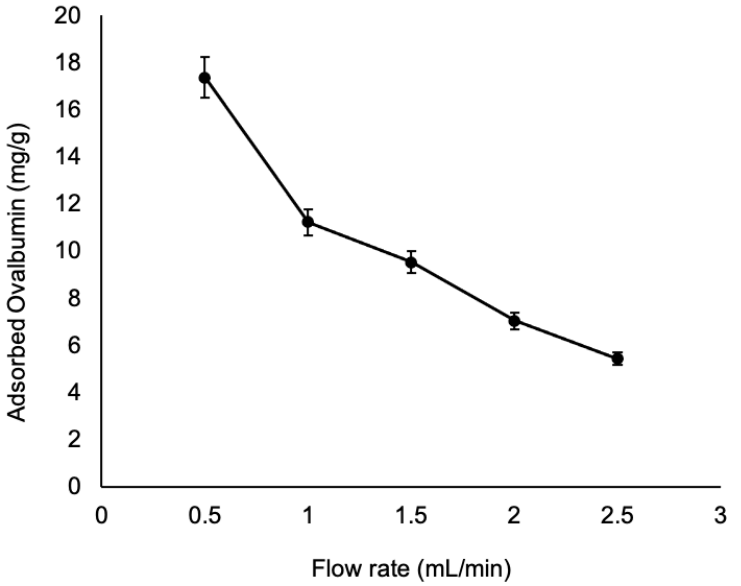

Figure 3. Effect of flow rate (pH 5.5 acetate buffer, T: 25°C).

Ovalbumin aqueous solutions were prepared at different concentrations (0.1-2.5 mg/mL) and the effect of the initial concentration of ovalbumin was investigated for OVA imprinted and non-imprinted cryogels. The adsorption capacity of ovalbumin molecules to MIP-1, MIP-2, MIP-3 and NIP cryogels increased with increasing concentrations of ovalbumin. With the increase in the ovalbumin concentration, the adsorption value increased too. But, at 1.5 mg/mL of ovalbumin concentration, a saturation value was reached. This is due to the saturation of the binding cavities on the ovalbumin imprinted cryogels. The maximum ovalbumin adsorption capacities of NIP and MIP-1, MIP-2 and MIP-3 cryogels were found as 3.23, 18.99, 28.99 and 37.78 mg/g, respectively. (Figure 4). It was found that the maximum ovalbumin adsorption capacity of MIP-3 was the highest due to the higher imprinted amount. After the ovalbumin rebinding, desorption studies were carried out using 1.0 M NaCl solution and the amount of ovalbumin removed was determined spectrophotometrically at 280 nm.

When the effect of ionic strength on the adsorption capacity of OVA imprinted cryogel was examined, the adsorption capacity of ovalbumin decreased from 11.23 to 3.03 mg/g OVA imprinted cryogel with the increasing concentration of NaCl (Figure 5). This decrease in adsorption capacity with increasing ionic strength occurs in affinity interactions due to electrostatic interactions between ovalbumin molecules and the monomer and ovalbumin in the MIP-3 cryogel [45].

The effect of temperature on the adsorption capacity of OVA imprinted cryogel was studied at different temperature (4-45°C). As seen in Figure 6, a decrease in the adsorption capacity of OVA imprinted cryogel was observed with increasing temperature. While the kinetic energy of ovalbumin molecules increases with the increase in temperature, the hydrogen bonds and ionic interaction between the monomer to ovalbumin resulting in decreasing of adsorption capacity [46]. The adsorption capacity of OVA imprinted cryogel (MIP-3) decreased from 15.50 to 6.77 mg/g with increasing temperature.

One of the most important features of molecularly imprinted polymers is that they can be used over and over. This depends on the interaction between the analyte molecule and the adsorbent and the desorption efficiency of the adsorbed molecule [46]. The reusability of ovalbumin imprinted cryogel with the MIP-3 code number was achieved by performing an adsorption-desorption cycle from the ovalbumin aqueous solution 10 times. After each adsorption-desorption cycle, ovalbumin imprinted cryogel was desorbed with 1.0 M NaCl solution. As seen in figure 7, it was observed that there was no significant decrease in the adsorption capacity of MIP-3 cryogel.

One of the most experiments for determining the selectivity of OVA imprinted cryogel is the selectivity experiments. The selectivity parameters describe the

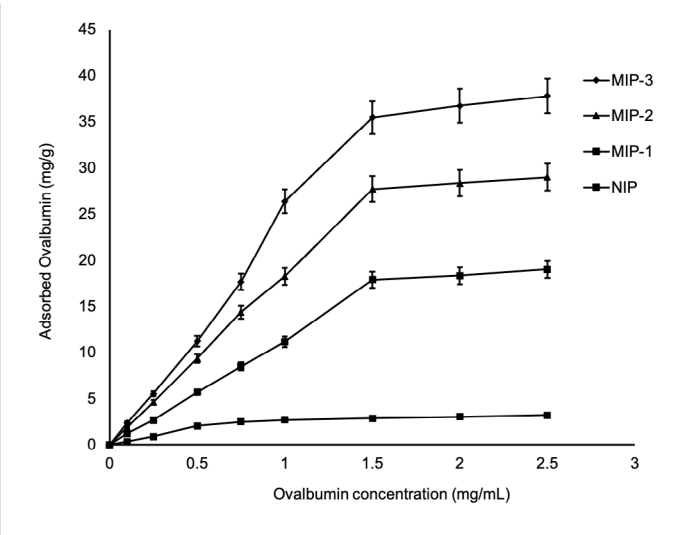

Figure 4. Effect of ovalbumin adsorption capacities (flow rate: 0.5 mL/min, pH 5.5 acetate buffer, T: 25°C).

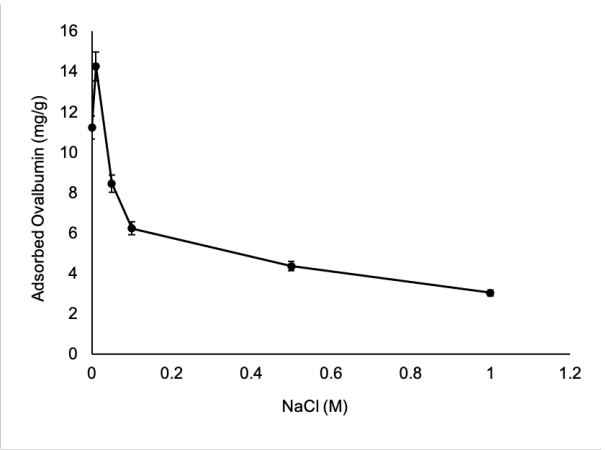

Figure 5. Effect of ionic strength (flow rate: 0.5 mL/min, pH 5.5 acetate buffer, T: 25°C).

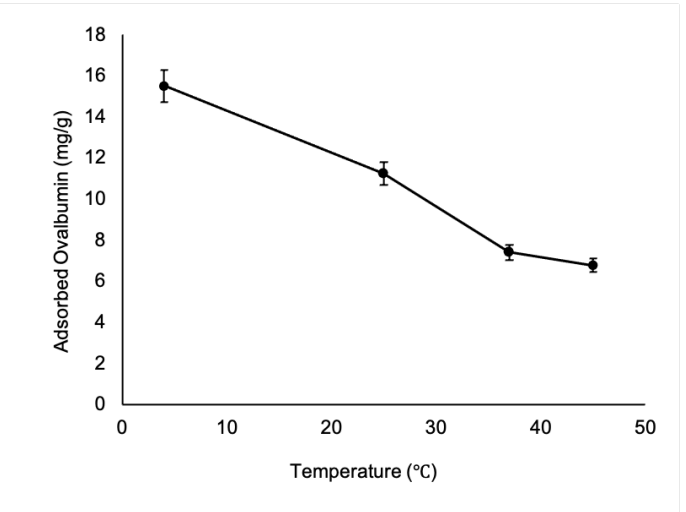

Figure 6. Effect of temperature (flow rate: 0.5 mL/min, pH 5.5 acetate buffer).

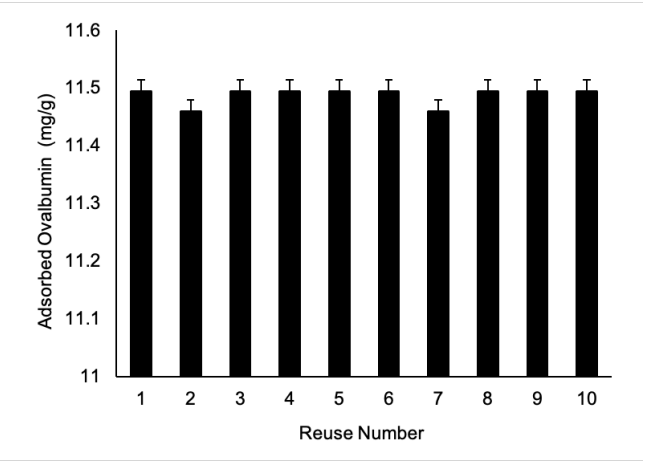

Figure 7. Effect of reusability (flow rate: 0.5 mL/min, pH 5.5 acetate buffer, T: 25°C).

imprinting efficiency of the adsorbents and the interactions of template molecules with the imprinted cavities [47]. TF and Lyz were selected as competing molecules for the selectivity of OVA imprinted and non-imprinted cryogels. The selectivity studies were carried out at ovalbumin concentration of 0.5 mg/mL. The highest adsorption capacity of OVA imprinted cryogels for OVA, TF and Lyz is 11.227, 0.834 and 0.347 mg/g, respectively (Figure 8). The adsorption capacity of ovalbumin non-imprinted cryogels for OVA, TF and Lyz is 1.586, 0.959 and 0.862 mg/g, respectively (Figure 8). As seen in the Table 2, it was clearly seen that the adsorption capacity of MIP-3 cryogel for OVA was higher than that of NIP cryogel.

After purification of ovalbumin from egg white, its molecular weight and purity were checked by SDS-PAGE analysis (Figure 9). The lanes 1, 2, and 3 are the final (egg white after adsorption), eluted (sample desorbed after ovalbumin adsorption) and initial (egg white prior to adsorption) samples, respectively. The ovalbumin amount was clearly decreased in lane 1 (final) compared to lane 3 (initial) after binding onto ovalbumin imprinted cryogel. The desorption process of the OVA imprinted cryogel after adsorption and the purity of ovalbumin was proved by the presence of the band in lane 2. It has been observed that ovalbumin suppressed cryogel provides an effective method with high adsorption capacity and high selectivity for ovalbumin purified from egg white. The separation gel was scanned using an ImageQuant 300 (Amersham, USA) and then analyzed using ImageQuant Software (Molecular Dynamics). Data analysis was performed to calculate the purity of protein based on the percentage of ovalbumin to total protein staining [22,46]. The purity of the desorbed ovalbumin was about 85.4% and the recovery was about 77.6%.

The recovery was calculated using Eq. (6) [48].

$$\%\ \text{Recovery} = \left(\frac{C_{\text{adsorbed}} - C_{\text{recovered}}}{C_{\text{adsorbed}}} \right) * 100 \qquad (6)$$

Table 2. Selectivity parameter values of MIP-3 and NIP cryogels.

	MIP-3 cryogel		NIP cryogel		
	K_d	k	K_d	k	k´
OVA	5.132	-	0.719	-	-
TF	3.559	1.442	2.145	0.335	4.304
Lyz	1.117	4.594	1.458	0.493	9.318

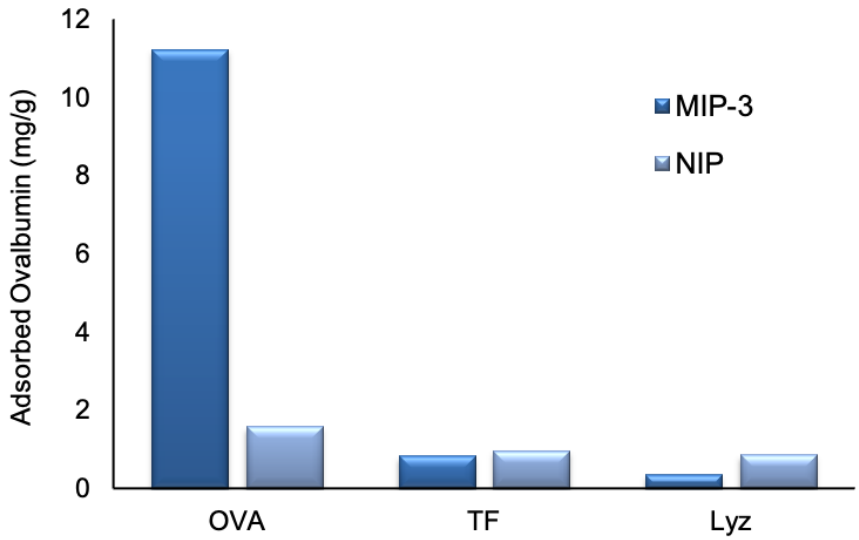

Figure 8. Competitive adsorption of ovalbumin (flow rate: 0.5 mL/min, phosphate buffer pH 5.5, T: 25°C).

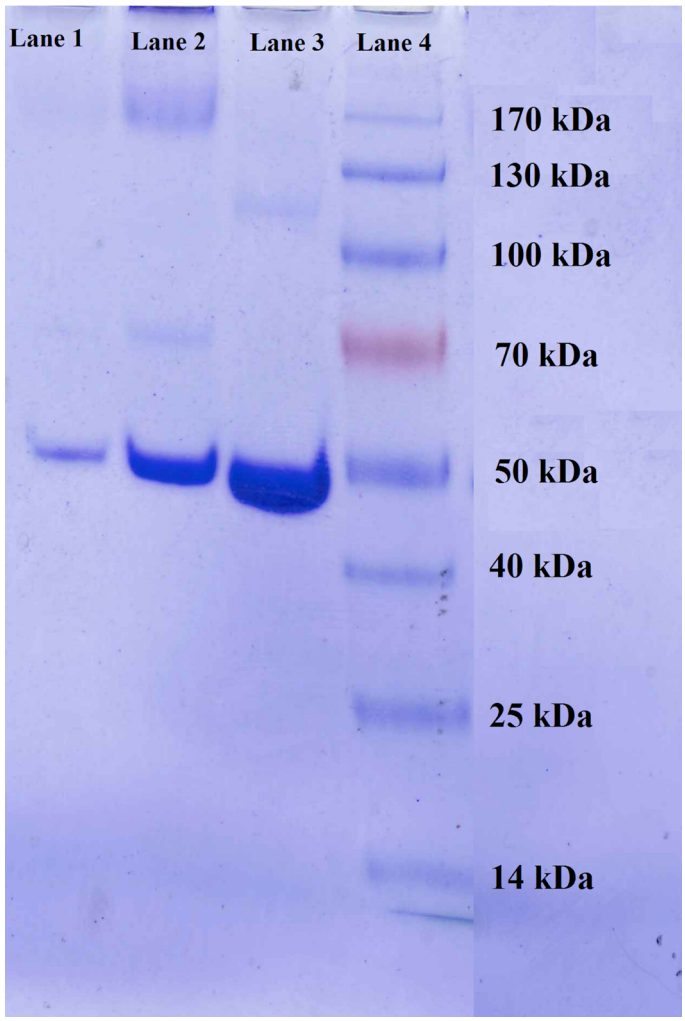

Figure 9. SDS-PAGE image (Lane 1: Egg white after adsorption (final), Lane 2: Sample desorbed after ovalbumin adsorption (eluted), Lane 3: Egg white prior to adsorption (initial), Lane: 4 Biomarker (Fermentas)).

CONCLUSIONS

Ovalbumin is an important glycoprotein that causes food allergy, especially in infants. Therefore, it is important to purify ovalbumin from natural sources [49]. In this study, OVA imprinted cryogels were prepared for purification of ovalbumin. Cryogels are produced at low temperatures, polymerization, which forms the macroporous polymer structure with large interconnected pores. Cryogels have spongy morphology. The most important features that distinguish cryogels from other conventional matrices are their large porous structure, short diffusion path, and retention time. Recently, cryogels are accepted as the new generation fixed phase in separation technique [50]. Some studies on the purification of ovalbumin in the literature are summarized as follows. Geng et al. [51] used olyethylene glycol (PEG) precipitation and isoelectric precipitation methods for ovalbumin purification. Initially, ovalbumin was separated from ovomucin, ovotransferrin and ovomucoid from chicken egg white under experimental conditions (PEG concentration 15%, pH 6.5, salt concentration 100 mmol/L, and temperature 10°C). The purity of ovalbumin in chicken egg white were analyzed by HPLC with a retention time of 14.5 min. Ovalbumin was obtained with a yield of 46.4 and a purity of 95.1% from chicken egg white. Zheng et al. [52] prepared tungstotellurate(VI)-coated magnetic nanoparticles for separation and purification of ovalbumin in egg white. The prepared magnetic nanoparticles were characterized with with infrared spectroscopy, energy dispersive spectroscopy and scanning electron microscopy. The maximum adsorption efficiency is 91.6% for 100 µg/mL ovalbumin solution. Pereira et al. [53] investigated the selectivity of ovalbumin from egg white from polyethylene glycols (PEG) with different molecular weights and potassium citrate/citric acid solutions prepared in different pH buffers. pH buffers, PEG molecular weight and the amount of phase forming components were optimized for the selective extraction of ovalbumin in the aqueous biphasic systems. Ovalbumin purity and yield were determined using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and size exclusion high performance liquid chromatography (SE-HPLC) methods. According to the experimental results, it was confirmed that ovalbumin purification from egg white was achieved completely in one step with a recovery yield of 65%. Jiang et al. [54] was purified ovalbumin from salted egg white by the aqueous two phase flotation method. n this study, an aqueous two-phase flotation consisting of ammonium sulfate ($(NH_4)_2SO_4$) and polyethylene glycols (PEG) was applied in order to reduce the contamination of salted egg white treated as waste and by-products in the production of salted egg yolk. The effects of PEG and $(NH_4)_2SO_4$ concentration, flow rate and flotation time on flotation efficiency and purity of ovalbumin were investigated. Under optimal experimental conditions, the flotation efficiency (Y) and purity (P) of ovalbumin was calculated to be 82.15 ± 0.24% and 92.98 ± 0.68%, respectively. Croguennec et al. [55] developed a simple and fast procedure based on the frontal chromatography principle for the purification of ovalbumin from egg white using an anion exchanger. Two hundred and fifty milliliters of Q-sepharose FF gel were isocratically separated with 9.55 g of ovalbumin extracted with 0.5 M NaCl at 83% purity. In addition, 94% ovalbumin purity was recovered by an isocratic elution using 0.14 M NaCl. In this study, the parameters effecting the capacity of ovalbumin adsorption were investigated with ovalbumin aqueous solutions and egg white. OVA imprinted and non-imprinted cryogels were prepared for ovalbumin purification from egg white. The maximum ovalbumin adsorption capacity of OVA imprinted cryogel (MIP-3) was found to be 37.78 mg/g from aqueous solutions. The reusability of OVA imprinted cryogels was tested by performing 10 times adsorption-desorption cycles. Additionally, the purified ovalbumin from egg white was performed by SDS-PAGE using a 12% separation gel and the recovery was about 77.6%. When the experimental results are examined, it is seen that the study is a useful and promising method that can be applied in ovalbumin purification from egg white.

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