Dynamic Adsorption Behaviors of Protein on Cibacron Blue-Modified PVA Nanofiber Fabrics

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Abstract

Electrospun polyvinyl alcohol (PVA) nanofiber fabrics functionalized by Cibacron Blue F3GA (CB) as an affinity ligand were prepared as efficient platforms for protein adsorption. Bovine serum albumin (BSA) was selected as a model protein to investigate their static adsorption behaviors. The protein adsorption capacities for the PVA nanofiber are 355.9 and 793.7 mg/g before and after CB modification, resulting in a 2.2 times increase. Then, dynamic experiments were conducted to determine the function of CB modification to the PVA nanofiber fabric. The effects of initial concentration and permeation rate on the dynamic adsorption behaviors for BSA of the CB-modified PVA nanofiber fabrics were also studied. The pseudo-first-order and pseudo-second-order kinetic models were used to analyze the kinetic adsorption data, and the latter was better fitted the experimental data. Furthermore, the adsorbed BSA can be easily eluted by a 0.1 M NaCl solution, and the CB-modified PVA nanofiber fabrics presented competent adsorption performance in the three-cycle reused experiment. Finally, the adsorption efficiency by the static and dynamic methods was compared. The obtained results demonstrate the potential of using the CB-modified PVA nanofiber for the affinity adsorption and isolation of proteins.

Key Words: Nanofiber fabric, Surface modification, Affinity adsorption, Protein, Kinetic model

1. Introduction

High-purity proteins, as an important group of biological products, have a broad application in our daily life [1]. Proteins are synthesized in the endoplasmic reticulum and accumulate in the apoplast in low quantities, making their recovery technically challenging. The co-existence of highly complex impurities with a tendency to bind non-specifically to adsorption media causes numerous impediments to purification [2]. In the industrial production of protein products, the protein isolation and purification processes play critical roles because of their significant influence on the purity and the production cost of downstream processing [3]. Consequently, there has been a growing demand to develop downstream purification methods that are capable of separating large quantities of products in a short period of time. Much effort has been devoted to developing effective and less expensive protein separation and purification techniques. To date, various protein isolation methods have been successfully applied, mainly consisting of precipitation, dialysis, adsorption, and chromatographic purification [4-7].

Among these techniques, adsorption is considered as an effective and simple method for protein separation applications. It also holds the advantages of flexibility of design and ease of operation. It is known that the structure of the adsorbent and the numerous adsorption sites are the keys for the efficient protein adsorption. Nanofiber fabric has been found to be superior to other adsorbents in terms of high porosity, facile preparation, large surface area to volume ratio, and ease of modification [8]. Electrospinning is a promising method applied for the development of functionalized nanofiber fabric as a scaffold for protein adsorption. Electrospun nanofiber fabric is fabricated by electrospinning, where a high voltage is applied to a polymer solvent solution causing surface repulsion which stretches the polymer into nanometer-scale fibers before deposition in a nonwoven random fashion [9]. The three-dimensional fibrous nanostructure of the electrospun nanofiber fabric enables the fabric to access the protein from the protein-containing solution easily. At the same time, there is plenty of active surface area for the binding of protein to the surface of the nanofiber fabrics [10]. Since the adsorption performance relies mainly upon the combination properties of the fabric surfaces, extensive research has been done to facilitate chemical/physical functionalization of nanofiber fabric during purification processes.
such as co-electrospinning of surface modification agents, plasma treatment, surface grafting, and wet chemical methods [11]. Chemical immobilization of surface modification agents onto the surface of nanofiber fabric is favored over physical immobilization, attributed to the uniformity, stability, and precise control over functional groups [12]. The application of nanofiber fabrics functionalized with metal ion or affinity dye has generated great interest due to the unique adsorption properties for protein. Zhu and Sun [13] modified the polyvinyl alcohol-co-ethylene nanofiber fabrics with chelating groups of iminodiacetic acid and copper ions. The resultant fabric exhibited a high lysozyme adsorption capacity of 199.0 mg/g. Cibacron Blue F3GA (CB) is a monochlorotriazine dye which contains three acidic sulfonate groups and four basic primary and secondary amino groups, which bind with considerable specificity and significant affinity to a series of other proteins [14]. For instance, Lu and Hsieh [15] prepared a highly efficient and versatile cellulose nanofiber fabric by a nucleophilic reaction of the cellulose hydroxyl with the triazinyl chloride of the CB ligand. The resulting fabric had a facile lipase loading of approximately 150.0 mg/g. Zhang et al. [16] immobilized the CB ligand on the chitosan-coated polyacrylonitrile (PAN) nanofiber fabric. The CB-attached PAN nanofiber fabric showed a capturing capacity of 161.6 mg/g towards bromelain. Zhu et al. [17] fabricated the CB functionalized poly(vinyl alcohol-co-ethylene) as affinity materials. The functionalized fabric achieved a bovine serum albumin (BSA) capture capacity of 105.5 mg/g. Although the fabrication of modified nanofiber-based mediums has certainly progressed, some bottleneck problems still exist, such as the convoluted modification processes, unsatisfactory adsorption performance, and weak mechanical properties. Therefore, it is highly urgent to exploit a terse approach for fabricating highly effective nanofiber-based adsorption media. In our previous study, we electrospun a polyvinyl alcohol (PVA) nanofiber fabric and explored the effects of buffer pH and ionic strength on its static protein adsorption performance [18].

In the present work, a simple and facile methodology is utilized to fabricate a modified PVA nanofiber fabric with high affinity power. CB and BSA were employed as a ligand-ligate model. After covalent immobilization of the CB ligand, the chemical structures of the PVA nanofiber fabrics were characterized by FTIR. The potential application properties of the resultant fabrics were evaluated by testing their static and dynamic adsorption properties. The Langmuir isotherm model was applied to elucidate the equilibrium adsorption data. The pseudo-first-order and pseudo-second-order kinetic models were also used to analyze the kinetics adsorption data. Moreover, the efficiency of the static and dynamic adsorption behaviors was examined.

2. Experimental

2.1 Materials

The electrospun PVA (Mw = 66,000 ~ 79,000) nanofiber fabrics were supplied by Japan Vilene Company, Ltd., Japan. Cibacron Blue F3GA was purchased from Polysciences, Inc., Japan. Sodium chloride (NaCl) and sodium carbonate (Na2CO3) were purchased from Wako Pure Chemical Industries, Ltd., Japan. Bovine serum albumin (MW = 67,000) was provided by Sigma-Aldrich Co. LLC., Japan.

2.2 Modification of PVA nanofiber fabrics

The CB molecules were immobilized onto the PVA nanofiber fabrics by covalent bonding between the hydroxyl group of PVA and chlorinated triazine ring of CB under the alkaline condition. Four types of PVA nanofibers with the heat treatment time of 30 minutes were supplied by Japan Vilene Company, Ltd., with varying heat treatment temperatures of 180, 150, 120, and 80 °C, respectively. And the mass per unit area of the PVA nanofiber fabrics is 40 g/m². The coupling procedure was followed by the method described previously [18, 19, 20]. Briefly, PVA nanofiber mats were immersed into a 10 mL CB solution (10 g/L). In order to stimulate the deposition of the dye on the internal surface of the fabrics, 2 g NaCl was added into the reaction mixture, and the temperature was maintained under 60 °C for 1 hour. This was followed by the addition of 0.2 g Na2CO3 in order to accelerate the reaction between the dye and the fabrics, which took place at 80 °C for 2 hours. Finally, the dyed fabrics were washed repeatedly with deionized water until the washings gave no optical absorption at 600 nm, which is CB’s maximum absorption wavelength.

2.3 Adsorption experiments

BSA (isoelectric point = 5.0) was used as a model protein. BSA solutions were prepared by dispersing a certain amount of BSA in the 0.1 M CH3COONa-CH3COOH buffer solution with pH 5.0, since BSA molecules are in most compact states and have a minimum electrostatic repulsion with CB at pH 5 [18]. The concentration of BSA was measured by a UV-1800 spectrophotometer (Shimadzu Corporation, Japan) with the UV absorption band at 280 nm. In order to obtain the optimal BSA affinity material, the adsorption performances of the four types of PVA nanofibers were compared. The four nanofibers were immersed in 8 mL solutions of various BSA concentrations and shaken at 25 °C for 6 hours to reach equilibrium. Adsorption isotherms were conducted with initial concentrations ranging from 1.0 to 6.0 g/L. The concentration of BSA was determined from the difference between the absorbance before and after adsorption. The amount of adsorbed BSA was calculated in accordance with the following
where \( q \) (mg/g) is the adsorption amount, \( C_0 \) and \( C \) (g/L) are the initial and the final concentrations of BSA, respectively, \( V \) (mL) is the volume of protein solution, and \( W \) (g) is the mass of the adsorbent.

The permeation experiments were investigated only for the CB-modified PV A nanofiber with the heat treatment temperature of 80 ℃, since the static adsorption experiments demonstrated that it was the most efficient adsorption platform of the four nanofibers. The experimental setup schematic is shown in Fig. 1. A fabric mat with a weight of 19.6 mg was packed in the Millipore filter holder (SX0002500) with an effective diameter of 2.2 cm and a permeation area of 3.8 cm². BSA solution was injected into the filter holder by the syringe pump (SRS-2, AS ONE Co., Japan). The study in details was carried out at 25 ℃ under various initial BSA concentrations (from 0.3 to 1.2 g/L), and permeation rates (from 2 to 10 mL/h). The BSA rejection \( R \) was calculated according to Eq. (2):

\[
R = 1 - \frac{C_p}{C_0}
\]

where \( C_0 \) (g/L) is the BSA concentration of feed solution, and \( C_p \) (g/L) is the BSA concentration of permeate solution.

In order to test the reusability of the CB-modified PV A nanofiber, the BSA-adsorbed nanofiber mats were eluted using buffer solutions (0.1 M NaH₂PO₄-K₂HPO₄, pH = 10.0) containing 1.0 M NaCl. The concentration of the remaining BSA in the eluent was measured at 280 nm by the UV-1800 spectrophotometer. Desorption efficiency \( \eta \) was calculated according to the following equation:

\[
\eta = \frac{q_d}{q} \times 100\%
\]

where \( q_d \) (mg/g) and \( q \) (mg/g) are the nanofiber fabric desorption and adsorption capacities, respectively.

3. Results and discussion

3.1 Static adsorption for BSA

3.1.1 Effect of initial BSA concentration

An important parameter to evaluate different adsorbents is their maximum uptake ability. The effects of initial protein concentration on the adsorption are presented in Fig. 2. For all types of the nanofibers, the adsorbed BSA amount raised with an increase in the protein equilibrium concentration. Increase in the initial concentration of BSA provides a potent driving force to overcome the mass transfer resistance between the aqueous and solid phases. The adsorption capacity of the PV A nanofiber with the heat treatment temperature of 80 ℃ is noticeably greater than the other three types of nanofibers. The four modified nanofibers with the heat treatment temperatures of 180, 150, 120 and 80 ℃ are shown in Fig. 3. The nanofiber with the lowest heat treatment temperature showed the deepest blue colour, which is due to more fixed CB molecules. Compared to the original nanofiber, the adsorption ability of the CB-modified nanofiber was improved significantly. The adsorption isotherm is the relationship between the amounts of a substance adsorbed per unit mass of adsorbent at constant temperature and its concentration in the equilibrium solutions. The experimental results fitted well with the Langmuir isothermal adsorption equation, where the model assumes adsorption to be the monolayer type and describes the adsorbent surface as homogeneous having identical surface sites [21]. The adsorption
process can be expressed by the following equation:

\[ q_e = \frac{q_s K C^*}{1 + K C^*} \]  

(4)

where \( q_e \) and \( q_s \) (mg/g) are the equilibrium adsorption capacity and the saturated adsorption capacity of BSA, respectively, \( K \) (L/g) is a constant of adsorption associated with free energy, and \( C^* \) (g/L) denotes the equilibrium concentrations of BSA in solution. The saturated adsorption capacity of \( q_s \) can be predicted by the linear form of the Langmuir equation:

\[ \frac{C}{q_e} = \frac{C}{q_s} + \frac{1}{K q_s} \]  

(5)

The BSA adsorption capacities for the PVA nanofiber with the heat treatment temperature of 80 °C are 355.9 and 793.7 mg/g before and after immobilizing CB, resulting in a 2.2 times increase. However, without CB modification, the BSA adsorption capacity of the original PVA nanofiber is much greater than that of the two nanofibers with heat treatment temperatures of 180 and 150 °C. The protein adsorption capacity of the CB modified PVA nanofibers can be considered from two aspects, one is the affinity of the matrix material, and the other is the amount of CB. After heating above 120 °C, the affinity of the matrix material was greatly impaired, and the amount of CB binding was also less. Even with the combination of the weakened affinity and the small CB binding amount, the protein adsorption capacity of the CB modified PVA nanofibers heated at the high temperatures was not as strong as the original PVA nanofiber heat-treated at 80 °C. Min et al. [22] functionalized PVA nanofiber fabrics by incorporating poly(methyl vinyl ether-alt-maleic anhydride) (PMA) and invested the effect of heat treatment on the fabric properties. The maleic anhydride on PMA has chemically crosslinked with the hydroxyl groups on PVA through an esterification reaction. It is pointed out that increased crystallinity of PVA and densely crosslinked structure due to high temperature will limit the accessibility of charged moieties to the internal binding sites of PVA. They chose the PVA fabrics with the heat treatment temperature of 120 °C in the range of 120 to 160 °C for the dye capturing study. The crosslinking agent of the PVA nanofibers in our research is also PMA. Gohil et al. [23] used maleic acid (MA) as a crosslinking agent to crosslink PVA with varying heat treatment temperatures from 120 to 160 °C. It is demonstrated that the interaction between PVA and maleic acid is inferior at a lower temperature and the heat treatment results in the elimination of water, which in turn enhances the alignment order of the polymeric chains through the formation of polyene. The hydroxyl groups are consumed by the reaction between PVA the crosslinking agent. A decrease in the hydroxyl groups reduces the affinity of PVA polymer with water.

The abundant presence of hydroxyl groups in PVA results in a hydrophilic nanofiber surface. The crosslinking reaction makes the polymer stable in water. On the other hand, the residual uncrosslinked fraction of hydroxyl groups of PVA provided the hydrophilic properties for the modified nanofiber surface [24]. Xie et al. [25] analyzed the water contact angle for the PVA hybrid membrane at different heat treatment temperatures. The water contact angle remained almost unchanged at about 45° at heating temperatures less than 140 °C, but increased significantly at higher temperatures, indicating that the hybrid membrane became less hydrophilic at the higher heating temperature. It can be attributed to the fact that the crosslinking reaction is incomplete at lower heating temperatures and more complete at higher temperatures. What is really needed is a network that provides a tight restraining without serious loss of hydroxyl groups. When the heating temperature increased higher than 120 °C, more hydrophilic groups were consumed, consequently increased the hydrophobicity of the PVA nanofibers. As displayed in Fig. 3, a lighter color of the modified nanofibers reflects less CB fixed amount, which is caused by insufficient hydroxyl groups. The better adsorption performance of the PVA nanofiber heat-treated at 80 °C is due to the excellent affinity of the matrix materials and the abundant hydroxyl groups to participate in the nucleophilic substitution reaction with CB.

### 3.1.2 Effect of contact time

To investigate the static adsorption kinetics, the CB-modified PVA nanofiber with the heat treatment temperature of 80 °C was immersed in 20 mL BSA solution (0.6 g/L) under shaking for 6 hours. As shown in Fig. 4, the BSA adsorption amount onto the nanofiber mats increased rapidly in the first 4 hours. Then, the adsorption gradually slowed down, eventually reaching equilibrium. At the initial stage of adsorption, since there were sufficient adsorption sites on the surface of the modified nanofiber, the BSA concentration was conducive to the rapid adsorption of BSA onto the nanofiber. During the adsorption process, the adsorption sites of the nanofiber surface declined, thus decreasing the adsorption rate as time elapsed. At the end of adsorption, the adsorption sites were depleted, and the adsorption reached saturation. The kinetic data was modeled using the pseudo-first-order kinetic and the pseudo-second-order kinetic. As the pseudo-first-order kinetics assumes that
the rate of change of the adsorption capacity is proportional to the concentration of available active sites per unit mass of adsorbent material [26, 27], the following formula can be expressed:

$$\frac{dq_t}{dt} = k_1(q_e - q_t)$$

where \(q_t\) (mg/g) is the adsorption amount at time \(t\) (h), \(q_e\) (mg/g) is the adsorption amount at \(t = \infty\), and \(k_1\) (h\(^{-1}\)) is the pseudo-first-order rate constant. Integrating Eq. (6), \(q_t\) can be expressed as:

$$q_t = q_e \left(1 - \exp(-k_1 t)\right)$$

and its linear form is given as:

$$\ln(q_e - q_t) = \ln(q_e) - k_1 t$$

The pseudo-second-order kinetic assumes that the rate of change of the concentration of occupied active sites per unit mass of the adsorbent material is proportional to the square of the concentration of free active sites per unit mass of sorbent [28, 29]. In terms of adsorption capacity, the pseudo-second-order rate equation can be written as:

$$\frac{dq_t}{dt} = \frac{k_2(q_e - q_t)^2}{1 + q_e k_2 t}$$

where \(q_t\) (mg/g) is the adsorption amount at time \(t\) (h), \(q_e\) (mg/g) is the adsorption amount at \(t = \infty\), and \(k_2\) (g·mg\(^{-1}\)h\(^{-1}\)) is the pseudo-second-order rate constant. Integrating Eq. (6), \(q_t\) can be expressed as:

$$q_t = \frac{q_e^2 k_2 t}{1 + q_e k_2 t}$$

and its linear form is given as:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e} + \frac{1}{q_e} t$$

Fitting was carried on plotting \(\ln(q_e - q_t)\) against \(t\) for the pseudo-first-order model, and \(t/q_t\) against \(t\) for the pseudo-second-order model. The correlation coefficients \((R^2)\) for the pseudo-first-order model and the pseudo-second-order model were 0.944 and 0.974, respectively. This implies that the pseudo-second-order model is more suitable to describe the BSA adsorption behavior. The fitting of experimental data on the pseudo-second-order model depicts that the adsorption rate of BSA onto the modified PVA nanofiber depends on the availability of the adsorption sites.

### 3.2 Characteristics of the PVA nanofiber fabrics

The PVA nanofiber with the heat treatment temperature of 80 °C had the best adsorption properties of the four PVA nanofibers, so it was selected as the adsorption platform for the dynamic experiments. Morphologies of the original PVA nanofiber were observed by scanning electron microscopy (SEM, S4300, Hitachi...
High-Technologies Corporation, Japan), operating at 15 kV. As shown in Fig. 5(a), the PVA nanofiber was bead-free and had a network shape. Diameters of the PVA nanofiber were measured by the ImageJ software. Distribution of the fiber diameter is indicated in Fig. 5(b). The fiber diameters ranged from 50 to 400 nm, and the average diameter was about 232 nm.

Direct coupling of reactive triazinyl dyes to the matrices bearing hydroxyl groups is a simple, inexpensive and safe method. Coupling is achieved at alkaline conditions by nucleophilic substitution of hydroxyl groups with the reactive chlorine on the dye molecules [30]. CB was attached onto the PVA nanofiber through the nucleophilic reaction between the chloride of the triazine ring and the hydroxyl group of PVA. The multiple aromatic part and the parts of three acidic sulfonate groups on CB influence the bindings of BSA and CB [18]. The chemical changes in the PVA nanofibers before and after CB modification were confirmed by Fourier transform infrared spectroscopy (FTIR-4100, JASCO, Japan), shown in Fig. 6. The Fourier transform infrared spectrums in the range 4000 - 400 cm\(^{-1}\) were recorded with a scan resolution of 1 cm\(^{-1}\) through an average of 16 scans. In the FTIR spectrum of CB-modified PVA nanofiber, the characteristic absorption bands at 1504 cm\(^{-1}\), 1296 cm\(^{-1}\) and 1024 cm\(^{-1}\) were observed, which were different from that of the original PVA nanofiber. The peaks at 1504 cm\(^{-1}\) characterized the benzene ring stretching vibrations. The absorption peaks at 1296 cm\(^{-1}\) and 1024 cm\(^{-1}\) were attributed to the stretching vibrations of C-N, and sulfonic acid groups, respectively [17, 31, 32]. Hence, the FTIR spectra confirms that CB molecules were successfully attached onto the PVA nanofiber.

### 3.3 Dynamic adsorption and desorption performance studies

#### 3.3.1 Mathematical models for BSA dynamic adsorption

Mathematical models were derived to analyze the dynamic adsorption results in accordance with the pseudo-first-order kinetic and the pseudo-second-order kinetic. Rejection can be defined as the ratio of BSA adsorption rate \( W_1 (dq/dt) \) to BSA inflow rate \( C_0 Q \) [27], given by:

\[
R = \frac{W_1}{C_0 Q} \frac{dq}{dt}
\]  

where \( W_1 \) is the weight of the PVA nanofiber mat (g), \( C_0 \) (g/L) is the BSA concentration of feed solution, \( Q \) is the permeation rate (mL/h), and \( q \) (mg/g) is the adsorption amount at time \( t \) (h).

Permeate time is defined as:

\[
t = \frac{V}{Q}
\]  

where \( V \) (mL) is the permeate volume of BSA solution. Substituting Eqs. (6), (7) and (13) into Eq. (12), results in the following equation:

\[
R = W_1 \frac{k_1 q_\infty}{C_0 Q} \exp\left( - \frac{k_1}{Q} V \right)
\]  

Because the value of rejection cannot be greater than 1,

\[
R = 1 \quad \text{at} \quad 0 \leq V \leq \frac{Q}{k_1} \ln \frac{W_1 k_1 q_\infty}{C_0 Q}
\]  

\[
R = \frac{W_1 k_1 q_\infty}{C_0 Q} \exp\left( - \frac{k_1}{Q} V \right) \quad \text{at} \quad V > \frac{Q}{k_1} \ln \frac{W_1 k_1 q_\infty}{C_0 Q}
\]  

Equations (15) and (16) are rejection equations obtained for the dynamic adsorption model based on the pseudo-first-order kinetic model. According to the two equations, the establishment condition of the dynamic adsorption period of \( R = 1 \) is as follows:

\[
\frac{W_1 k_1 q_\infty}{C_0 Q} > 1
\]  

where the left side of Eq. (17) is \( R \) at \( V = 0 \) in Eq. (14). Taking the logarithm on both sides of Eq. (14),

\[
- \ln R = \frac{k_1}{Q} V - \ln \frac{W_1 k_1 q_\infty}{C_0 Q}
\]  

The values of \( k_1 \) and \( q_\infty \) can be calculated by plotting a straight line with \( \ln R \) and \( V \) from the slope and the intercept.

By substituting Eqs. (9), (10) and (13) into Eq. (12), rejection equation for the dynamic adsorption model based on the pseudo-second-order kinetic model is given below:
Similarly, since the value of rejection cannot be greater than 1,

\[ R = 1 \text{ at } 0 \leq V \leq \sqrt{\frac{W_c Q}{k_2 q_c}} \]  \quad (20)

The establishment condition of the dynamic adsorption period of \( R = 1 \) is as follows:

\[ \frac{k_2 W_c q_c^2}{C_0 Q} > 1 \]  \quad (22)

where the left side of Eq. (22) is \( R \) at \( V = 0 \) in Eq. (19). The linear form of Eq. (19) is given as:

\[ \frac{1}{\sqrt{R}} = \frac{C_0 Q}{C_0 Q + 1} \frac{1}{q_c} + \frac{1}{k_2 W_f} \]  \quad (23)

The values of \( k_2 \) and \( q_c \) can be calculated by plotting a straight line with \( 1/\sqrt{R} \) and \( V \) from the slope and the intercept.

### 3.3.2 Discussion of dynamic adsorption

The PVA nanofibers have the advantages of small fiber diameter and high porosity. The small fiber diameter causes the PVA nanofibers to have an extremely large specific surface area. The large specific surface area provides abundant adsorption sites for the capturing of protein molecules. Due to the high porosity, the PVA nanofibers have an outstanding liquid permeability. The mass transfer resistance is tremendously reduced, while simultaneously allowing for elevated flow rates along with faster adsorption kinetics. It results in a rapid processing, which significantly improves the adsorption, and regeneration steps. In this study, the effects of adsorption conditions on the BSA dynamic adsorption process, such as modification of CB, concentration of BSA in permeate solution, and permeation rate, were examined in a continuous system. Adsorption results were fitted using the adsorption kinetics of the pseudo-first-order kinetic and the pseudo-second-order kinetic.

Figure 7 shows the permeate volume evolution of the BSA rejection for both of the original PVA nanofiber mat and the CB-modified PVA nanofiber mat. The BSA rejection for the original PVA nanofiber mat decreased from the initial 2 mL of the process to 0.254 after permeating 10 mL BSA solution. In comparison, a significant increase in dead volume area can be observed in the process using the CB-modified PVA nanofiber mat, and the rejection still remained 0.465 by permeating the same volume of BSA solution. The CB modification effectively improves the binding efficiency of the PVA nanofiber to the protein and avoids the rapid decline of rejection during the permeate process.

Figure 8 presents the change of BSA rejection with permeate volume under different initial concentrations. With the increase of permeate volume, the rejection revealed a similar downward trend though the margins of decline varied. As the rise of initial concentration, BSA molecules in the mobile phase have more chances to interact with the nanofiber surface, and the driving force also increases. There were the same number of adsorption sites for BSA capture for the PVA nanofiber mats of the same mass; as a result, the rejection reduced fastest for the largest permeate concentration. The effect of permeation rate on BSA rejection was investigated by permeating the 10 mL BSA solutions, with the results shown in Fig. 9. The BSA rejections showed a very similar downward trend under the permeation rates of 2 and 5 mL/h, but it was slightly higher in the former at the end of the permeate
It can be seen from Table 1 that the maximum adsorption capacity of the former is higher than that of the latter, since a slower permeation rate allows enough time for the BSA molecules to be captured. However, a noticeable decrease in rejection occurred under the permeation rate of 10 mL/h, due to insufficient residence time of BSA molecules in the pores of the PVA nanofiber mat.

All kinetic parameters and correlation coefficient ($R^2$) values obtained at different adsorption conditions are listed and compared in Table 1. The higher $R^2$ values in all these results suggest that the pseudo-second-order kinetic model fits the data better than the pseudo-first-order kinetic model. This is consistent with the conclusion obtained in the static experiment by using the two kinetic models to study the effect of time on the adsorption amount. As shown in Fig. 7, Fig. 8, and Fig. 9, the BSA rejection curves calculated by the pseudo-second-order model are in favorable harmony with the experimental results.

### 3.3.3 Desorption and reusability

The dynamic desorption experiments were performed to evaluate the reusability of the CB-modified PVA nanofibers. Phosphate buffer at pH 10.0 containing 1.0 M NaCl was used as the eluent, because the adsorption capacity of BSA on adsorbent was low at pH far from its isoelectric point, and ionic strength affects electrostatic and hydrophobic interaction between BSA and the affinity ligands [18, 32]. As presented in Fig. 10, under the eluent rate of 10 mL/h, the desorption ratios at the eluent volumes of 10, 15, and 20 mL were 47.1%, 52.6%, and 56.6%, respectively. However, under the eluent rate of 5 mL/h, the desorption ratios raised to 92.4%, 95.9%, and 97.3%. Hence, sufficient residence time and volume of eluent are necessary for the efficient desorption of the adsorbed BSA molecules.

The adsorption-desorption cycle experiments were carried out under the eluent rate of 5 mL/h by 20 mL eluent solution. The adsorption-desorption cycle was repeated three times, where the
permeation concentration of BSA was 0.6 g/L. As shown in Fig. 11, the adsorption ratio decreased gradually in the three cycles. In the three adsorptions processes, the BSA instantaneous rejection at the end of permeating 10 mL BSA solution was 0.494, 0.326, and 0.269, respectively. Total rejection can be calculated as:

\[
R_{\text{total}} = \frac{1}{V} \int _0 ^V R \, dV
\]  
(24)

According to Eq. (24), the total rejections for the three cycles were 0.765, 0.663, and 0.576, which remained in a high range.

In the other set of adsorption-desorption experiments, 10 mL BSA solution was permeated at the permeation rate of 5 mL/h, where the initial BSA concentration was 0.6 g/L. The concentration of the permeated solution was measured, and the BSA-adsorbed nanofiber mat was eluted. Then the collected permeation from the previous adsorption process continued to be permeated through the regenerated nanofiber mat again. After three rounds of dynamic adsorption, the BSA total rejection increased from 0.749 to 0.992, as shown in Table 2. Thus, the CB-modified PVA nanofibers can be recycled and reused.

| Cycle | 1st | 2nd | 3rd |
|-------|-----|-----|-----|
| \( R_{\text{total}} \) | 0.749 | 0.919 | 0.992 |

### 3.3.4 Comparison between static and dynamic adsorption

In order to provide a design basis for adsorption operation, the static and dynamic adsorption efficiencies were compared by the fitting curves according to the experimental data.

When the static adsorption reached equilibrium, the BSA adsorption amount calculation Eq. (1) is rewritten by:

\[
q_e = \frac{(C_0 - C^*)V}{W}
\]

(25)

where \( C^* \) (g/L) is the equilibrium concentrations of BSA in solution.

Substituting Eq. (25) into Eq. (4), a quadratic equation with respect to \( C^* \) is obtained. Solving the quadratic equation, then \( C^* \) can be expressed through the following equation:

\[
C^* = \frac{-(q_wK_w - C_qV_K + V) + \sqrt{(q_wK_w - C_qV_K + V)^2 + 4KC_qV^2}}{2KV}
\]

(26)

Substituting Eq. (26) for \( C_p \) into Eq. (2), the rejection \( R \) in the static adsorption at equilibrium can be expressed by:

\[
R = 1 - \frac{-(q_wK_w - C_qV_K + V) + \sqrt{(q_wK_w - C_qV_K + V)^2 + 4KC_qV^2}}{2KC_q}
\]

(27)

According to the study of 3.1.2, it is assumed that after 5 hours of PVA nanofiber mat being immersed into BSA solution of various volumes, the static adsorption has reached equilibrium. At the concentrations of 0.3, 0.6, and 1.2 g/L, through varying the volume from 0 to 25 mL, the static rejection values can be calculated according to Eq. (27). The total dynamic rejection values can be calculated by substituting Eqs. (20), (21) into Eq. (24). The theoretical rejection curves of static and dynamic conditions are shown in Fig. 12. For the initial concentrations of 0.3 and 0.6 g/L, all the rejections of the dynamic adsorption are greater than that of the static adsorption. It is worth noting that the dynamic adsorption takes the same 5 hours as the static adsorption only at the permeate volume of 25 mL. Under the other volume conditions of less than 25 mL, the dynamic adsorption takes a shorter time.
For example, the operating time with 12.5 mL BSA solution of the static adsorption and the dynamic adsorption is 12 and 6 hours; the latter provides higher rejection while saving half the time. For the initial concentration of 1.2 g/L, rejection of the dynamic adsorption is higher than the static adsorption at the beginning but reverses at the volume of 3.8 mL. Under high-concentration conditions, both of the rejections decrease rapidly. From the perspective of design optimization, very low rejection is meaningless for protein purification process. Taking 0.5 as the rejection design requirement, the disposable treatment volume of the static adsorption and the dynamic adsorption is 12.6 and 11.5 mL. The two treatment volumes for the 1.2 g/L solution are distinctly close, but the operating time of the static adsorption and the dynamic adsorption time is 5 and 2.3 hours. It can be concluded that dynamic adsorption is highly efficient and time-saving at the test concentrations. Furthermore, continuous operation of dynamic adsorption presents advantages for automated control, labor saving and integration with other continuous processes.

4. Conclusions

In this study, the electrospun PVA nanofiber fabrics were functionalized by CB as an affinity ligand. The PVA nanofibers under four different heat treatment temperatures were modified, and their adsorption behaviors for BSA were examined. The adsorption ability of the PVA nanofibers under the optimal heat treatment temperature before and after CB modification was analyzed by the Langmuir equation. Then, dynamic experiments were conducted to determine the effects of initial concentration and permeation rate. The pseudo-second-order model better fitted the experimental data than the pseudo-first-order model in both static and dynamic adsorption performance. Furthermore, the CB-modified PVA nanofibers possessed excellent regeneration ability and cycle performance. Finally, the adsorption efficiency by the static and dynamic methods was compared, and the results can provide a reference for process scale-up design. Considering the simple fabrication process, large binding capacity, and high adsorption efficiency, the CB-modified PV A nanofibers have great potential for the affinity adsorption and isolation of proteins.

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