Preparation and Protein Separation Properties of the Porous Polystyrene/Ethylene–Vinyl Acetate Copolymer Blend Nanofibers Membranes

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ABSTRACT: To date, the preparation of a novel ultrafiltration membrane and the efficient separation and purification of protein solutions have gradually attracted widespread attention of many researchers. In this study, a hollow porous polystyrene/ethylene–vinyl acetate copolymer blend nanofibrous membrane (PS/EVA-BNM) was generated by electrospinning and chemical modification and then used to separate and purify proteins in solution. The BNM was characterized by scanning electron microscopy and specific surface area and pore size analyses. The membrane separation system was assembled using the BNM, which was overlaid to form the reaction layer. The optimal conditions for protein separation were determined by adjusting the operating pressure, filtration time, and pH. The results showed that the rejection rate of serum albumin and the membrane flux could reach 94.35% and 2.04 L/(m² min), respectively, under the following conditions: the operating pressure was 0.10 MPa and the processing time was 1.5 h. By comparing the parameters of the polyethersulfone commercial ultrafiltration membrane with the PS/EVA-BNM system, it could be inferred that the rejection rate of the latter decreased slightly, whereas its transport flux improved several times. At the same time, the experimental results indicated that the PS/EVA-BNM possessed excellent reusability and mechanical properties. Additionally, the BNM could retain its nanofibrous morphological structure after the separation of serum albumin several times in an aqueous environment.

1. INTRODUCTION

Proteins, an important constituent in organisms, usually exist in relatively complex systems. In the process of production, proteins are sensitive to changes in the pH and temperature of the external environment, which can easily affect the structure of proteins and cause denaturation.1,2 Currently, precipitation, ion exchange, electrophoresis, centrifugation, and chromatographic methods are commonly used to separate and purify proteins in solution. However, these methods also have many disadvantages, such as high production cost, complexity, and low separation and purification efficiency, which are not conducive to their popularization and application.3,4 In recent years, membrane separation technology has attracted increasing attention because of its many distinctive features, such as simplicity, mild treatment conditions, and requiring no reagents.5–7 In the process of membrane separation, proteins in complex systems can be separated and purified quickly and efficiently by choosing an appropriate separation membrane and adjusting operation pressure, filtration time, and other process parameters. Membrane separation technology mainly utilizes the impetus on both sides of the membrane to separate and purify the solution mixture through selective membranes. At present, nanofibrous membranes prepared by electrospinning are one of the hotspots in membrane separation.8 The nanofibrous membranes effectively improve the separation and purification efficiency and reduce energy consumption in the process of protein separation and purification because of their small fiber diameter, large specific surface area, and high porosity.9

Ethylene–vinyl acetate copolymer (EVA) is a commonly used plastic material that is prepared by high-pressure bulk polymerization or solution polymerization of EVA. In contrast to polyethylene, vinyl acetate monomer was introduced into the EVA molecular chain, which reduced the crystallinity and improved the flexibility, impact resistance, filler compatibility, and thermal sealing performance.10,11 It is well known that the properties of EVA resins mainly depend on the content of vinyl acetate (VA) in the molecular chain. Currently, EVA resin is

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widely used in various industrial fields, and the demand for EVA as a matrix resin in hot melt adhesives exceeds 80%. The reason is that EVA has excellent softness and heating fluidity and low temperature resistance. Compared with thermosetting, solvent-based and water-based adhesives, EVA materials generally have the characteristics of no solvent, no pollution, low-energy consumption, and convenient operation. The EVA hot melt adhesive is solid, and it can be processed into thin films, rods, strips, or particles according to different requirements under ambient temperature. However, the preparation of EVA nanofibers by electrospinning and their application in protein separation and purification have not been reported. In this study, EVA nanofibers were prepared by electrospinning for the first time and used as nanofiber mesh adhesive materials. At the same time, because nanofiber adhesives have the characteristics of small diameter, large specific surface area, and uniform distribution of bonding area, the melted EVA nanofibers can effectively and uniformly bond materials under certain pressure and temperature conditions, thus significantly improving the uniformity and strength of materials.

First, polystyrene/polyvinylpyrrolidone/EVA copolymer (PS/PVP/EVA) hybrid nanofiber membranes were prepared by electrospinning. The PVP components in the nanofiber membranes were removed by hydrolysis modification. The blend nanofibrous membrane (BNM) was heated by hot pressing under specific temperature and pressure conditions. Finally, the PS/EVA-BNM with a uniform and dense fiber surface and porosity was successfully prepared. Subsequently, these membranes and commercial polyethersulfone (PES) ultrafiltration membranes were used as separation layers to construct membrane separation systems for the same concentration of serum albumin solution (Figure 1).

Furthermore, the BNM obtained by electrospinning and physical modification has high porosity and is uniform and dense, which can effectively improve the membrane flux and antifouling ability in the process of protein separation and purification. In this paper, the separation performance of the PS/EVA-BNM in bovine serum albumin solution was systematically analyzed, which provided an experimental basis for the application of nanofiber membranes in protein solution post-treatment.

2. RESULTS AND DISCUSSION

2.1. Surface Morphology of PS/EVA-BNM. The PS/EVA-BNM was prepared by electrospinning and physical modification. It was used to isolate and purify serum albumin in solution. The PS/PVP/EVA-BNM, PS/EVA-BNM, and PES ultrafiltration membrane and the membranes after the isolation and purification of serum albumin were examined by scanning electron microscopy (SEM), and the results are shown in Figure 2. It can be clearly seen in Figure 2A that the PS/PVP/
EVA-BNM with a uniform diameter and good morphology was prepared by electrospinning technology. The diameters of the nanofibers are between 300 and 450 nm. Figure 2B shows that the dense and porous PS/EVA-BNM was formed by means of a chemical modification and hot-pressing process accompanied by dissolution of the PVP component and fusion bonding of the EVA component in the BNM. Moreover, the morphology of the blend nanofibers was distorted and deformed, as seen from the SEM images. Although the diameter of the blend nanofibers increased to a certain extent (400−600 nm), the diameter of the nanofibers became more uneven. In addition, it can be found that the morphology of the blend nanofibers remained stable during the process of separating and purifying the serum albumin solution, and a large number of pores between and within the fibers were covered with numerous protein molecules, as seen in Figure 2C. As shown in Figure 2D,E, the surface of the commercial PES ultrafiltration membrane showed uniform nanopores before protein separation. After separation of the protein solution, a large number of protein molecules entered the void of the fiber membrane and evenly covered the surface of the PES ultrafiltration membrane, resulting in the disappearance of nanopores in the ultrafiltration membrane.

**2.2. Specific Surface Area and Average Pore Size.** It is well known that the specific surface area and porosity values of the separation membrane are some of the most important factors in the protein separation process. In this paper, the test results of the specific surface area and average pore size of PS/PVP/EVA and PS/EVA nanofibrous membranes are shown in Table 1. Table 1 clearly shows that the specific surface areas of the electrospun PS/PVP/EVA and PS/EVA-BNM are 23.85 and 162.37 m²/g, respectively. The main reason for this phenomenon is that the PVP component in the PS/PVP composite nanofiber membrane was dissolved and removed during the hydrolysis process. Therefore, a large number of nanopores with different pore sizes are produced within and on the surface of the PS/PVP composite nanofibers. Although the voids of some composite nanofiber membranes are covered during hot pressing and melting bonding of EVA fibers, the specific surface area of the porous PS/EVA-BNM is still significantly higher than that of the electrospun PS/PVP/EVA nanofibers. In addition, the average pore diameter of the BNM was reduced from 28.46 to 21.5 nm after hydrolysis and hot-pressing modification. This is because the surface of the nanofibers produced many small-diameter nanopores during hydrolysis modification, and hot-pressing treatment further compressed the pores between nanofibers, resulting in a reduction in the average pore size of the nanofiber membrane. The PS/EVA nanofiber membrane after hydrolysis modification and hot-pressing treatment could efficiently separate a large number of purified serum albumin macromolecules because of its large specific surface area and small average pore diameter.

**2.3. Mechanical Property.** Porous nanofiber membranes generally have poor mechanical properties, mainly because of the uneven distribution of macromolecules and crystalline regions in the fibers, and the fibers are easily broken in many pore regions. The mechanical properties of electrospun PS/PVP/EVA-BNM and hot-pressed PS/EVA-BNM at a temperature of 80 °C are shown in Table 1. (Each sample in this experiment was tested three times under the same conditions, and their average values were calculated.) From Table 1, it can be found that the tensile breaking strength and elongation at break of hot-pressed PS/EVA-BNM reached 4.62 MPa and 20.72%, respectively. However, the maximum breaking strength and elongation at break of untreated electrospun PS/PVP/EVA-BNM were only 1.98 MPa and 24.93%, respectively, under the same conditions. The reason for this situation is that the EVA nanofibers in the BNM were melt-deformed under high-temperature conditions to bond many adjacent fibers, and multiple uniform and stable bonding points were formed inside and on the surface of the BNM. Therefore, the overall mechanical properties of the composite fiber membrane were enhanced to some extent due to the mesh-like adhesive structure and the topological entanglements among the nanofibers.

**2.4. Membrane Separation Performance.** In this experiment, a porous PS/EVA-BNM was prepared by electrospinning and physical modification to achieve the separation and purification of serum albumin solution. The membrane separation performance was characterized by the protein rejection and permeation flux of solution. During the membrane separation process, the operating pressure, treatment time, membrane pore size, and distribution, the solution flow rate and other factors greatly affected the membrane separation performance. This paper mainly studies the relationship between the operating pressure, number of membrane layers, separation time, protein rejection rate, and solution permeation flux in the process of membrane separation. Furthermore, the Coomassie brilliant blue method was used to determine the protein content in the solution.

**2.4.1. Effect of Membrane Number on Protein Separation Performance.** In the experiment, the effect of different layers of the nanofiber membrane on the separation performance of the protein solution was studied, as shown in Figure 3. As shown in Figure 3, the protein retention rate first increased rapidly with a gradual increase in the number of membrane layers and then reached equilibrium with approximately four membrane layers. At this time, the equilibrium value of the protein retention rate reached 94.35%. In contrast, as the number of layers in the separation membrane increased, the value of the membrane flux gradually decreased. When the number of membrane layers was less than 4, the separation membrane maintained a high membrane flux, and the number of membrane layers continued to increase, resulting in a rapid decrease in the membrane flux. The reason is that with the gradual increase in the number of membrane layers, the filtration resistance produced by the separation membrane increased slowly in the process of protein separation. According to the comprehensive analysis, when the number of nanofiber membrane layers in the separation device was 4, the separation and purification process still had excellent solution permeation flux while maintaining a high rejection rate. Therefore, the four-layer laminated PS/EVA-BNM was
selected as a separation layer to construct a membrane separation system, and the serum albumin solution was separated and purified in this study.

2.4.2. Effect of Membrane Separation Time on Protein Separation and Puriﬁcation. In this study, when the operating pressure was constant at 0.1 MPa, the relationship among the separation time of serum albumin, membrane permeation flux, and protein rejection rate was analyzed, as shown in Figure 4. The membrane flux of the PS/EVA nanofiber membrane and PES ultraﬁltration membrane decreased with the extension of the ﬁltration time. The relationship between protein retention and separation time was also analyzed. The retention rate of PS/EVA-BNM in a serum albumin solution gradually increased with the increasing separation time. When the ﬁltration time reached 1.5 h, the protein retention rate reached 93.59%, which is basically the same as that of the commercial PES ultraﬁltration membrane. When the ﬁltration time was further prolonged, the protein retention rate remained basically in equilibrium. However, the results of the membrane ﬂux measurements were the opposite. As the separation time increased, the solution permeability of two different nanofiber membranes decreased. When the ﬁltration time was 4 h, the solution permeabilities of the two separation membranes slowly reached a stable value. In addition, it can be seen that the self-made blend nanofiber membrane has a large solution permeation ﬂux during the 24 h ﬁltration process while maintaining a high protein retention rate from the ﬁgure. Such experimental results exhibited that the self-made nanofiber membrane has a good application prospect in practical applications.

The main reason for this phenomenon was that the average pore size of commercial PES ultraﬁltration membrane (3.2 nm) is signiﬁcantly smaller than that of self-made PS/EVA nanofiber membrane (21.5 nm). It was well known that the separation membrane presented that the smaller the pore size, the stronger the solute retention ability and weaker solution permeability it always exhibited. In addition, as the ﬁltration time was prolonged, on the one hand, many protein molecules accumulated on the surface or inside of nanofibers, which further reduced the pore size of the nanofibers and improved the protein retention rate. On the other hand, the protein concentration inside and on the surface of the separation membrane was signiﬁcantly higher than that of the top unﬁltered solution, thus causing the ﬂow resistance of the membrane to increase as the separation time increased. Furthermore, the adsorption resistance, deposition resistance, and concentration polarization on the surface of the ﬁber membranes also decreased the membrane ﬂux. Surprisingly, the permeation ﬂux of the PS/EVA nanofiber membrane was approximately 4–5 times that of the PES ultraﬁltration membrane (50 kD); in other words, the separation rate of serum albumin by the PS/EVA nanofiber membrane was faster than that of the PES commercial membrane under the same operating pressure.

2.4.3. Effect of Operating Pressure on Protein Separation and Puriﬁcation. In this article, the membrane ﬂux and rejection rate of PS/EVA-BNM and a PES ultraﬁltration membrane (50 kD) were calculated after 90 min of ﬁltration at 0.04–0.16 MPa pressure, as shown in Figure 5. The performances of separating and purifying serum albumin by the two ﬁber membranes show a certain deviation under different operating pressure conditions, as shown in Figure 5. When the operating pressure value was small, the membrane

Figure 3. Effect of membrane layer numbers on protein separation performance.

Figure 4. Effect of ﬁltration time on membrane separation performance.

Figure 5. Effect of operating pressure on membrane separation performance.
flux of the PS/EVA-BNM and PES ultrafiltration membrane were relatively low at the initial stage of separation because the power of the solution flow during the separation process was weak; with an increase in the pressure, the flow dynamics of the serum albumin solution were significantly stronger than the flow resistance generated by the separation membrane. Therefore, the flux of both nanofiber membranes increased. However, the permeation flux of the PS/EVA-BNM increased faster than that of traditional commercial ultrafiltration membranes. As shown in Figure 5, the permeation flux of PS/EVA-BNM was always higher than that of the PES ultrafiltration membrane under the same separation time and different operating pressures. The PS/EVA-BNM and PES ultrafiltration membrane maintained a high protein rejection rate with increasing operating pressure values at the same separation time, as seen by observing the change in the protein retention rate. Although the protein retention rate of nanofiber membranes fluctuated slightly, the overall data remained relatively stable. When the separation time was constant and the operating pressure was 0.1 MPa, the nanofiber membrane could effectively separate the serum albumin solution. With increasing operating pressure, slight damage to the nanofiber membrane resulted in the loss of some proteins in the separation process, which led to a decrease in the protein retention capacity of the membrane.

2.4.4. Reusability of BNM. In the actual protein separation and purification process, the membrane separation system not only exhibited significant protein retention performance but also showed excellent separation treatment efficiency; thus, it can be better applied to real life. Thus, electrospun PS/EVA-BNM can be used to efficiently separate and purify serum albumin under an operating pressure of 0.1 MPa and filtration time of 90 min. Figure 6 displays the reusability of different nanofiber membranes for the separation and purification of serum albumin solutions. It can be observed that the separation performance of the two membranes remained substantially stable with the increase in reuse times under an operation pressure of 0.1 MPa and a filtration time of 90 min. At the same time, after five times of repeated separation, the protein retention rate of the PS/EVA-BNM was slightly lower than that of the commercial PES ultrafiltration membrane, but the membrane flux was approximately 4 to 5 times the commercial PES ultrafiltration membrane flux. Therefore, the homemade PS/EVA-BNM has good reusability. The excellent protein separation performance compared to that of commercial membranes and other reported data illustrates the great potential of nanofiber membranes in improving the separation rate and energy consumption in the field of protein purification.

3. CONCLUSIONS

In summary, a PS/EVA-BNM was obtained by electrospinning; subsequently, a PS/EVA porous nanofiber membrane with a uniform diameter, high porosity, and uniform fiber membrane was manufactured by hydrolysis and hot pressing. The PS/EVA nanofibrous membranes not only can improve the inherent strength of the membrane but also have good uniformity and compactness after hot-pressing treatment. The membrane separation system based on the porous PS/EVA nanofibrous membrane can be used to efficiently separate serum albumin solution. The study shows that the protein retention rate of porous PS/EVA-BNM can reach 94.35% and that the membrane flux can reach 2.04 L/min. Compared with that of the PES ultrafiltration membrane, the solution permeation flux of PS/EVA-BNM can be greatly improved by maintaining a higher rejection rate. Moreover, the nanofiber membrane can be reused many times. The experimental results show that electrospun porous PS/EVA-BNM improves the separation rate and energy consumption during protein purification and has a significant application prospect in protein separation and purification pretreatment.

4. EXPERIMENT

4.1. Experimental Materials. PS, PVP, and EVA were obtained from China Pharmaceutical Group Chemical Reagent Co., Ltd. Coomassie Brilliant Blue (G-250), N,N-dimethylformamide (DMF), trichloromethane (CHCl3), tetrahydrofuran (THF), and sodium hydroxide (NaOH) were purchased from Aladdin Chemical Reagent Co., Ltd. Serum albumin was purchased from Shanghai Jinsui Biological Technology Co., Ltd.

4.2. Electrospinning Porous PS/EVA Nanofiber Membrane. Prior to electrospinning, lithium chloride nanoparticles were dried in a muffle furnace to remove the moisture contained therein. Then, 1.4 g of EVA resin particles, 0.04 g of lithium chloride nanoparticles, 13.02 g of CHCl3, and 5.58 g of THF were accurately weighed and placed in a conical bottle of proper size. The composite spinning solution with 7% EVA mass fraction was prepared by stirring for 15 min at 40 °C in a constant-temperature magnetic stirrer. The mass ratio of CHCl3 and THF was 7:3, and the mass fraction of lithium chloride was 0.2%. Afterward, 3.36 g of PS particles and 1.44 g of PVP powder were accurately weighed and dissolved in 35.2 g of DMF solvent. A homogeneous spinning solution with a 12% mass fraction was prepared by constant-temperature magnetic stirring at 40 °C. The mass ratio of PS to PVP was 7:3. The PS/PVP and EVA spinning solutions were placed in different syringes, and the syringes were connected to a high-voltage dc power supply. The PS/PVP/EVA blend nanofibrous was received by a rotating drum (the drum was connected to a ground wire). The spinning parameters were mainly as follows: the spinning speeds of PS/PVP and EVA were 0.8 and 0.2 mL/h, the distance between the roller and the injector was 20 cm, and the applied voltage was 18.5 kV. Under the above conditions, a PS/EVA-BNM was obtained by electrospinning; subsequently, a PS/EVA porous nanofiber membrane with a uniform diameter, high porosity, and uniform fiber membrane was manufactured by hydrolysis and hot pressing. The PS/EVA nanofibrous membranes not only can improve the inherent strength of the membrane but also have good uniformity and compactness after hot-pressing treatment. The membrane separation system based on the porous PS/EVA nanofibrous membrane can be used to efficiently separate serum albumin solution. The study shows that the protein retention rate of porous PS/EVA-BNM can reach 94.35% and that the membrane flux can reach 2.04 L/min. Compared with that of the PES ultrafiltration membrane, the solution permeation flux of PS/EVA-BNM can be greatly improved by maintaining a higher rejection rate. Moreover, the nanofiber membrane can be reused many times. The experimental results show that electrospun porous PS/EVA-BNM improves the separation rate and energy consumption during protein purification and has a significant application prospect in protein separation and purification pretreatment.

Figure 6. Effect of reuse times on membrane separation performance.
conditions, after continuous spinning for 20 h, the collected nanofiber membranes were dried in a 40 °C vacuum drying chamber for 2 h.

Subsequently, the above mentioned blend nanofiber membranes (0.1 g) were immersed in 200 mL of ethanol solution to react at room temperature for 24 h. The PVP component in the PS/PVP composite nanofibers were dissolved and removed to prepare porous PS/EVA nanofiber membranes (0.078 g).

4.3. Hot-Pressing Treatment of Nanofiber Membrane. The porous PS/EVA nanofibers were hot-pressed in a YM300 hot press for 4 h at a pressure of 4 MPa and a temperature of 80 °C. Thus, the EVA fibers in the composite membranes could be partially melted and uniformly and compactly bonded to multiple adjacent nanofibers, and a PS/EVA-BNM with good mechanical properties and porous morphology could be prepared.

According to the requirements of the mechanical properties test, the PS/EVA dried nanofiber membranes before and after hot pressing were first cut into 5 × 10 cm samples, and then, the mechanical performance was investigated using a table-top tensile tester (Instron 1185, USA). In this experiment, the sample drawing speed was set to 10 mm/min, and each group of samples was tested 3 times, and the average value was obtained.

4.4. Characterization Test of Nanofibers. An S-4800 scanning electron microscope from Hitachi Company was used to observe the morphology of various nanofiber membranes, and the samples were sprayed with gold before testing. According to the requirements of specific surface area and aperture analysis and measurement, the homemade BNM and commercial PES ultrafiltration membrane test samples were prepared, and the average pore diameter and BET specific surface area of the nanofiber membrane were characterized by measurement of the N₂ adsorption–desorption isotherms with a Conta NOVA 2000E specific surface area and pore size analyzer.

4.5. Construction of Membrane Separation System. To analyze the ability of the porous PS/EVA-BNMs to separate and purify protein solutions, homemade BNMs (the thickness of each nanofiber membrane was 0.22 mm) and commercially PES ultrafiltration membranes were used as separation membranes. The membrane separation system was constructed separately, and its structural schematic diagram is shown in Figure 7. A nylon diversion network was used as the support layer, and then, the separation membrane was covered on the upper layer. Then, a serum albumin solution was driven by a certain pressure, thus selectively permeating the separation membrane to achieve the separation and purification of proteins.

4.6. Separation Performances. A certain quantity of serum albumin was accurately weighed and dissolved in distilled water to prepare a 1 mg/mL serum albumin solution. Different layers of uniform dense PS/EVA nanofiber membrane and a widely used commercial PES ultrafiltration membrane (molecular weight of intercepted protein was 50 kD) were used as filtration layers to construct a membrane separation system for the separation and purification of serum albumin solution, respectively. The retention rate of serum albumin and the solution permeation flux of different membranes were measured to evaluate their separation performances by regulating the pressure and filtration time in the experiment. The protein rejection rate refers to the ratio of the amount of serum albumin intercepted by the membrane to the total amount of the solution, as shown in formula 1.

\[
R = \frac{C_0 - C_P}{C_0} \times 100\% \tag{1}
\]

\(R\) indicates the rejection rate of protein during membrane separation. \(C_0\) indicates the initial concentration of protein (mg/mL) in the solution before membrane separation. \(C_P\) indicates the concentration of protein in the filtrate after membrane separation (mg/mL).

The main definition of membrane flux (solution permeation flux) is the volume of solution penetrated by a separating membrane per unit time and area, as calculated by eq 2.

\[
J = \frac{V}{S \times t} \tag{2}
\]

\(J\) indicates the membrane flux (L/(m² min)), \(V\) indicates the volume of filtrate (L), \(S\) indicates the effective area (m²) of the separation membrane, and \(t\) indicates the operation time of membrane separation (min).

To analyze the separation and purification performance of nanofibrous membranes with different numbers of layers, 1, 2, 3, 4, 5, and 6 layers of PS/EVA-BNM were stacked together to form separation membranes. The permeation flux and interception performance of separation membranes were measured under the same operating conditions (the operation pressure was 0.1 MPa, and the filtration time was 90 min). Parallel experiments were carried out three times, and the standard deviation was calculated. In this study, when the operating pressure was 0.1 MPa, the rotating speed of the magnetic stirrer was set at 100 rad/min. The membrane flux and protein rejection rate of the PS/EVA-BNMs and PES ultrafiltration membranes were measured at different filtration times. A 1 mg/mL serum albumin solution was used as the filtrate. In addition, the effect of operating pressure on the membrane separation performance was studied experimentally. The effects of operating pressure on membrane flux and protein rejection rate were investigated when the filtration time was 90 min and the operating pressure was between 0.04 and 0.16 MPa. A serum albumin solution with a concentration of 1 mg/mL was separated by PS/EVA-BNMs and commercial PES ultrafiltration membranes separately. The nanofiber membrane was filtered and separated for 90 min under an operating pressure of 0.1 MPa. The nanofiber membrane was washed
with distilled water 3–5 times, and the nanofibrous membrane was backwashed with distilled water. The nanofiber membrane was reused 5 times under the operating conditions, and the membrane flux and protein rejection rate were calculated during each separation process.

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Notes
The authors declare no competing financial interest.

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