Surprising Transformation of a Block Copolymer into a High Performance Polystyrene Ultrafiltration Membrane with a Hierarchically Organized Pore Structure

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We describe the preparation of hierarchical polystyrene nanoporous membranes with a very narrow pore size distribution and an extremely high porosity. The nanoporous structure is formed as a result of unusual degradation of the poly(4-vinyl pyridine) block from self-assembled poly(styrene)-*b*-poly(4-vinyl pyridine) (PS-*b*-P4VP) membranes through the formation of an unstable pyridinium intermediate in an alkaline medium. During this process, the confined swelling and controlled degradation produced a tunable pore size. We unequivocally confirmed the successful elimination of the P4VP block from a PS-*b*-P4VPVP membrane using 1D/2D NMR spectroscopy and other characterization techniques. Surprisingly, the long range ordered surface porosity was preserved even after degradation of the P4VP block from the main chain of the diblock copolymer, as revealed by SEM. Aside from a drastically improved water flux (~67% increase) compared to the PS-*b*-P4VP membrane, the hydraulic permeability measurements validated pH independent behaviour of the isoporous PS membrane over a wide pH range from 3 to 10. The effect of the pore size on protein transport rate and selectivity (α) was investigated for lysozyme (Lys), bovine serum albumin (BSA) and globulin- γ (IgG). A high selectivity of 42 (Lys/IgG) and 30 (BSA/IgG) was attained, making the membranes attractive for size selective separation of biomolecules from their synthetic model mixture solutions.

Introduction

Nanoporous membranes derived from various materials have gained significant attention owing to their high potential for advanced macromolecular separations as compared to the existing materials for challenging separation applications.^{1,2} Although various organic, inorganic, and hybrid materials have been explored in the development of nanoporous films, the fabrication of membranes with uniform nanopores and high pore density remains a challenge.³⁻⁸ Block copolymer self-assembly is an attractive method for manufacturing well-defined nanostructured films and membranes. For a long time, the manufacturing process for nanoporous membranes by block copolymer self-assembly was based on the creation of equilibrium morphologies. This membrane manufacturing process was tedious because solvent-annealing was required to obtain equilibrium structures and the etching of one block was necessary to create pores.⁹⁻¹¹ This time-consuming procedure hindered the up-scaling. To overcome this impediment, a fast non-equilibrium method combining self-assembly and non-

solvent induced phase separation (SNIPS) to manufacture asymmetric membranes with a highly ordered nanoporous surface was first described 2007.¹² Due to their high porosity and uniform pore size, these membranes surpassed the traditional permeability-selectivity trade-off.¹³⁻¹⁶ Moreover, the chemical nature of the polymer blocks often allowed the introduction of functional groups into the membrane pores without disturbing the nanoporous morphology.¹⁷ This feature enabled fine-tuning of the pores for specific separations.

The size selective separation performance of these membranes was favourably enhanced by tuning the pore size of the nanochannels. Recently, Yang et al. precisely controlled the pore size of spin-coated nanoporous BCP membranes through gold deposition and demonstrated their application in drug delivery systems.¹⁸ Kim et al. reported nanoporous polystyrene-block-poly(methyl methacrylate) membranes for the effective isolation of 30 nm human rhinovirus type 14 (HRV-14).¹⁹ Despite these efforts in fabricating nanoporous membranes with remarkable sieving ability, the difficulties in obtaining tunable pore sizes relevant to the separation/fractionation of biomolecules and other chemical products have severely limited their practical utilization. We and others have explored various routes for tuning the pore size of self-assembled nanoporous membranes without sacrificing their mechanical integrity as a potential pathway for efficient purification and separation of biomolecules.²⁰⁻²⁴ Annealing and selective chemical degradation of one block from BCP thin films has been studied to achieve a preferred morphology. However, these

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methods have been restricted to the formation of nanoporous thin films and monoliths.^{10,25,26} Phillip et al. prepared thin isoporous polystyrene films by self-assembly of poly-(styrene-*b*-lactide) followed by etching the polylactide block.²⁷ These films were supported by conventional ultrafiltration membranes and the water filtration performance of these composite membranes was evaluated. Here we use isoporous asymmetric PS-*b*-P4VP made by the fast SNIPS method and chemically degrade the PVP block in the pore interior to obtain asymmetric isoporous polystyrene membranes with a water flux more than 3 orders of magnitude higher than the previously described polystyrene membranes.

Experimental details

Materials

Polystyrene-*b*-poly-4-vinylpyridine block copolymer P9957-S4VP (PS-*b*-P4VP 139,000-*b*-40,000 g/mol; PDI = 1.1) was purchased from Polymer Source, Inc., Canada. Methyl iodide (CH₃I), lysozyme (Lys), bovine albumin serum (BSA), globulin- γ (IgG), hexane, Tween-80, and sodium dodecyl sulfate (SDS) were obtained from Sigma-Aldrich. *N,N*-dimethylformamide (DMF), 1,4-dioxane and ethanol (96%), were acquired from Sigma-Aldrich. Tetrahydrofuran (THF) was received from Fischer Scientific. All chemicals were used as received. Deionized (DI) water purified with a Milli-Q system (Millipore, Inc.) was used in this study.

Membrane fabrication

The isoporous membrane was prepared from a polymer solution containing 16.6 wt% PS-*b*-P4VP in a ternary mixture of 27.8 wt% DMF, 27.8 wt% 1,4-dioxane and 27.8 wt% THF. PS-*b*-P4VP was dissolved in the mixture of solvents by continuous stirring for 24 h at room temperature (RT). The solution was then cast on a glass plate using a casting knife with a gate height of 200 μ m. The glass plate along with polymer solution film was left for 10 s at RT to partially evaporate solvents and then immersed into a DI water coagulation bath and left until the membrane was detached. The membrane was taken out and subsequently stored in DI water bath for 24 h to remove traces of solvents. The PVP block lining the pores of these membranes was then removed in two steps. First the P4VP was quaternized (qPS-*b*-P4VP) by submersing the membrane into 1% methyl iodide (CH₃I) solution in ethanol at room temperature for 24 h. The qPS-*b*-P4VP membrane was then immersed into a 0.1 M NaOH solution in ethanol for different time intervals from 10 min to 24 h. Finally, the membranes were dried at RT for further modifications and characterizations.

Scanning Electron Microscopy

The surface morphology of the modified and unmodified membranes was observed under the field emission scanning electron microscopy (FESEM). The dried membrane samples were fixed on a SEM sample holder using a double-sided carbon tape and then sputtered with iridium for 60 s at 5 mA current in an argon atmosphere. After that, the samples were moved to the SEM stage and the images were recorded on a

FEI Quanta 200, scanning electron microscope at an accelerating voltage of 30 kV with varied magnifications.

Nuclear Magnetic Resonance

One-dimensional ¹H and ¹³C and two-dimensional ¹H-¹³C HMQC NMR experiments for polymer solution samples in deuterated DMF-*d*⁷ solvent at room temperature were performed on Bruker Advance III 700 MHz NMR spectrometer equipped with a TCI cryoprobe. The NMR spectrometer was operated at 700.13 MHz for ¹H and 176.05 MHz for ¹³C NMR experiments. The ¹H-¹³C HMQC NMR experiment was conducted with 4096 $\times$ 256 time domain points in the ¹H and ¹³C dimensions, whereas a 10 ppm $\times$ 180 ppm spectral window was used for ¹H and ¹³C, respectively. The relaxation delay was 1.5 s and the number of scans was 16. The ¹H-¹³C HMBC NMR experiment was carried out with 4096 $\times$ 256 time domain points in the ¹H and ¹³C dimensions, 13 ppm $\times$ 222 ppm spectral window for ¹H and ¹³C, the direct bond ¹H-¹³C J-coupling filter of 145 Hz, relaxation delay of 2 s, and 24 scans. Deuterated-DMF (DMF-*d*⁷) solvent was used to record the NMR spectra of the samples and the polymer concentration was 16 mg/ml. The room temperature two-dimensional ¹H-¹⁵N HMBC NMR experiment was performed on a Bruker Advance III 950 MHz NMR spectrometer equipped with a TCI cryoprobe operating at 950.3 MHz for ¹H and 96.3 MHz for ¹⁵N. The ¹H-¹⁵N HMBC NMR experiment was conducted with 2048 $\times$ 128 time domain points in the ¹H and ¹⁵N dimensions, 13 ppm $\times$ 600 ppm spectral window for ¹H and ¹⁵N, relaxation delay of 2 s, direct bond ¹H-¹⁵N J-coupling filter of 90 Hz, and 88 to 812 scans depending on the sample.

X-ray photoelectron spectroscopy

XPS analysis was conducted by using a Kratos Axis Ultra DLD spectrometer (Kratos Analytical Ltd, UK) equipped with a monochromated Al K α X-ray source (1486.6 eV) and a hemispherical analyzer with a resolution from 0 to 0.5 eV. All spectra were recorded at an aperture slot of 700 $\times$ 300 μ m.

Attenuated total reflection-Fourier transform infrared

ATR-FTIR spectra of membranes were recorded on a Thermo-Scientific spectrometer (Nicolet iS10 model). The spectra were recorded over a wide range from 4000 to 1000 cm⁻¹ for 16 scans with a resolution of $\pm$ 4 cm⁻¹.

Polymer molecular weight measurement

Gel permeation chromatography (GPC) measurements were carried out using Agilent module 1200 infinity series instrument with two PL gel 5 μ m MIXED-C columns equipped with a reflective index (RI) detector. HPLC grade tetrahydrofuran (THF) was used as an eluent at 1.0 mL/min flow rate. The RI detector was calibrated using various polystyrene standards; all molecular weight values quoted are relative to these standards. The polymer solutions (1mg/ml) were prepared using HPLC grade THF and filtered through a 5 μ m PTFE filter before injecting into GPC instrument.

PEG rejection studies

A 0.4 wt% PEG solution (0.1 wt% of 600, 3000, 10000, 35000 g/mol PEG) was used for PEG rejection. The feed, retentate and permeate samples were collected after filtration of PEG solution through the membrane at varied solution pH and the concentration of PEG in the respective samples was determined using a gel permeation chromatography (GPC) system (Agilent Co.) equipped with a dual column (Agilent PL Aquagel-OH 30 8 μm , PL Aquagel-OH 408 μm) and G1362A RID detector. The samples were filtered using a 5 μm PTFE filter before injecting into a carrier solution at 1 ml/min flow rate (ultrapure Milli-Q water, 18.2 M Ω).

Ultrafiltration of Oil-in-water emulsions and Proteins

The oil-in-water emulsion was prepared by mixing water and hexane (99:1 v/v) and the resulting mixture was sonicated using a bath sonicator at 40 kHz frequency for 2 h to obtain a white color homogeneous emulsion. The droplet size of the emulsion was in the range from nanometer to micrometer. A surfactant stabilized oil-in-water emulsion was prepared as follows: Tween 80 and SDS were alternatively added to the emulsion and the concentration of stabilizers was $\sim 0.2\text{mg/ml}$. Optical microscopic images of feed and permeate samples after ultrafiltration of surfactant stabilized oil-in-water emulsion were recorded with the help of a stereomicroscope (Nikon, SMZ 25) by dropping the respective samples on the biological counting board. The ultrafiltration of oil-in-water emulsion was performed at 1.0 bar for 1 h. Ultrafiltration experiments were conducted under stirring at 400 rpm to avoid concentration polarization and adsorption of oil droplets on the membrane surface. Total content of organic carbon (TOC) in feed and permeate samples was determined by using a total organic carbon analyzer (Teledyne Tekmer, USA). The ultrafiltration of protein solution through the membranes was assessed using 1mg/ml protein solutions. The concentration of protein in feed and permeate samples was analyzed using a UV-Vis spectrophotometer (Nano-Drop™ 2000/2000C, Thermo Fisher Scientific) at $\lambda_{\text{max}} = 280$. All ultrafiltration experiments were carried out on 3 different coupons of the same membrane and average values are reported. The observed transmission of the protein (τ_{obs}) through the membrane was determined using equation (2)^{28,29}

$$\tau_{\text{obs}} = \frac{C_p}{C_f} \quad (2)$$

where C_p and C_f are the concentration of protein in the permeate and feed sample solution.

The selectivity of the membranes towards proteins was calculated using equation (3)^{28,29}

$$\text{Selectivity} = \frac{(\tau_{\text{obs}})_i}{(\tau_{\text{obs}})_j} \quad (3)$$

where $(\tau_{\text{obs}})_i$ and $(\tau_{\text{obs}})_j$ are observed transmission values of two different proteins respectively.

Dynamic scanning calorimetry

DSC thermograms of the membrane samples were recorded on a differential scanning calorimeter (TA 2000, USA) under N_2 environment at a heating rate of 10 $^\circ\text{C/min}$.

Water contact angle

The hydrophilic characteristic of the membranes was examined by determining their contact angle values using a contact angle goniometer (FM40, Kruss GmbH, Germany) equipped with a video capture. For static and dynamic contact angle measurements 5 μl droplet of DI water was placed onto the surface of the membrane and the sessile drop method was applied to obtain water contact angle values. Images of the water droplets were captured on a camera (Stingray model, Allied Vision Technology, USA). To minimize the experimental errors, the contact angle was taken at least at four random locations of the membrane and the average values were reported.

Polyacrylamide gel electrophoresis (PAGE) analysis

SDS-PAGE sample loading buffer, tris/glycine/SDS electrophoresis migration buffer, 4-20% Mini-PROTEAN TGX Precast Gel, Precision Plus Protein Unstained Standards (10-250 kD), and Bio-Safe colloidal Coomassie Brilliant Blue G-250 protein staining solution were procured from Bio-Rad, USA. One part of the sample was diluted with one part of SDS-PAGE sample loading buffer. The resulting sample solution was heated at 70 $^\circ\text{C}$ for 15 min and then centrifuged at 20000 rpm for 5 min before loading into a well of the gel plate. SDS-PAGE was then carried out on a 4-20% precast polyacrylamide gel in a Mini-PROTEAN Tetra Cell (Bio-Rad, USA). The migration of the proteins was monitored at 100 V with an initial current of 20 mA/gel and at 1 W for about 1.5 h in a migration buffer solution. After the end of the electrophoresis experiment, the gel plate was stained in a colloidal solution of Coomassie Blue G250 with gentle agitation for 2 h and thereafter, the stained gel plate was kept in Milli-Q DI water until the background became clear.

Results and discussions

Membrane Preparation and Characterization

Scheme 1 illustrates the process for fabrication of the nanoporous PS membrane. Our approach started with the preparation of the BCP solution in a ternary solvent mixture. The BCP solution without air bubbles was then cast on a glass plate using a casting knife with gate height of 200 μm . The solvent from the “proto-membrane” was partially evaporated for 10 s, leading to a BCP concentration increase at the solution/air interface. The film was then immersed in a DI water coagulation bath where the hydrophobic PS block led to rapid precipitation. The well-defined nanopores were formed in the membrane top layer as a result of kinetic entrapment of self-assembled micelles after plunging the membrane into the water coagulation bath. The P4VP block was then quaternized (qPS-*b*-P4VP) by submersing the membrane into 1% methyl iodide (CH_3I)

Scheme 1. Schematic diagram of the SNIPS process combined with the selective chemical degradation of the P4VP block to produce isoporous PS membranes.

solution in ethanol at room temperature for 24 h.

We optimized the concentration of CH_3I to retain the isoporous structure of the membrane (**Fig. S1, ESI**). The qPS-*b*-P4VP membrane was immersed again into a 0.1 M NaOH solution in ethanol for different time intervals from 10 min to 24 h. It is reported that the N-alkyl-pyridinium ion converts into N-alkyl-2-oxy-1,2-dihydro-pyridine intermediates in alkaline medium due to the absence of α or β hydrogen. These intermediates hydrolyze in alkaline solution and as a result alkylamines are produced via a ring-opening reaction.³⁰ The alkylamines produced in the reaction subsequently undergo auto-oxidation in presence of dissolved oxygen to form alkylamines-oxide radicals. The resulting alkylamines-oxide radicals attack the polymer backbone and thus degrade the polymer backbone.³¹ We surmise that the pyridinium ion of the qPS-*b*-P4VP membrane decomposed in a similar pathway, where autoxidation of the BCP backbone led to the degradation of the P4VP block. Therefore, the P4VP block from the main chain of the diblock PS-*b*-P4VP was degraded under controlled alkaline conditions. The PS block remained unchanged in the membrane matrix whereas the quaternized P4VP block degraded in alcoholic alkaline solution and the resulting residue was highly soluble in ethanol. Finally, an asymmetric isoporous PS membrane with very high porosity and uniform pore size was successfully produced via a selective degradation of the P4VP block.

Fig. 1 shows the surface scanning electron microscope (SEM) images of the membranes after drying in a vacuum oven. These images confirm the retention of the nanoporous membrane morphology without defects, even after chemical degradation. The degradation of the P4VP block was assessed by 1D and 2D NMR (heteronuclear multiple quantum coherence spectroscopy: HMQC and heteronuclear multiple bond coherence: HMBC) spectroscopy.

Fig. 1. Top-surface and cross-section SEM images of: (a-b) PS-*b*-P4VP and (c-d) isoporous PS membranes.

Fig. 2 (a) illustrates the typical 2D (HMQC) spectrum for PS-*b*-P4VP and 2(b) for the qP4VP block with its characteristic peaks. Typically, the peak at 4.6 ppm in ^1H and at 42 ppm in ^{13}C ascertained the presence of the methylated pyridinium moiety. The degree of quaternization for PS-*b*-P4VP membrane was $\sim 78\%$ as deduced from ^1H -NMR spectrum. The 2D HMQC NMR spectrum of the qPS-*b*-P4VP membrane (**Fig. 2c**) after alcoholic alkaline treatment revealed the complete disappearance of peaks for aromatic protons and carbons of pyridine in the P4VP block. The disappearance of peaks is attributed to the degradation of quaternary pyridinium in an alcoholic alkaline medium. The ^1H and ^{13}C NMR spectra of the resulting membrane are in good agreement with reported NMR spectra for PS.²⁶ We did not observe any new peaks in the NMR spectra which clearly suggests that no degradation by-products have formed on the membrane. In addition, ^1H , 2D (HMBC) ^{15}N - ^1H NMR, and XPS analyses were done for nanoporous PS membrane samples. The peak for the nitrogen atom is absent in the 2D NMR and XPS spectrum of the PS membrane (**Fig. S2, ESI**). The characteristic peaks for the pyridine moiety (1000 and 1415 cm^{-1}) disappeared in the infrared spectrum of the PS membrane (**Fig. S3a, ESI**). These results confirm the removal of the pyridine moiety from the main chain of PS-*b*-P4VP membrane after chemical degradation.

The gel permeation chromatography (GPC) data for PS membrane samples (**Fig. 2d**) showed a considerable decrease in molecular weight compared to the PS-*b*-P4VP membrane (**Table S1, ESI**). The lower molecular weight of the isoporous PS membrane was due to the degradation of the P4VP block. Furthermore, the measured gravimetric weight loss of ~ 26 wt% for the resulting PS membrane was in good agreement with the calculated weight of P4VP in PS-*b*-P4VP (27.8 wt%) (**Fig. S3b, ESI**). Efforts were also made to degrade the unmethylated PS-*b*-P4VP membrane in the ethanolic 0.1M NaOH solution. However, the P4VP block was not degraded underscoring the importance of quaternization for alkaline degradation (**Fig. S4, ESI**).

The thermal behavior of the resulting PS membrane was evaluated and compared with a PS-*b*-P4VP membrane. The differential scanning calorimetry (DSC) data for the PS-*b*-P4VP membrane exhibited two glass transition temperatures corresponding to PS ($\sim 108^\circ\text{C}$) and the P4VP ($\sim 156^\circ\text{C}$) block (**Fig. S5, ESI**), representing a microphase separated state. The DSC thermogram of the resulting nanoporous PS membrane revealed the absence of a glass transition temperature for the P4VP block. The thermal decomposition of the resulting nanoporous PS membrane matched well with the earlier PS homopolymer.³²

Flux and pH-Responsive Characterization

The removal of the hydrophilic P4VP block changed the surface properties of the resulting PS membrane. As expected, the contact angle studies revealed a transformation of hydrophilic (60°) nanochannels to the hydrophobic regime (104°) (**Fig. S6, ESI**). This can be attributed to the hydrophobic nature of PS. The PS-*b*-P4VP membrane swells in alcohols. Since the pores of the membranes are lined with the hydrophilic P4VP block, this swelling leads to a strong decline in alcohol permeance. However, the PS membrane showed

minimum interaction with ethanol resulting in high ethanol permeability (**Fig. 3a**). The ethanol flux of the PS-*b*-P4VP membrane was 0.1 L m⁻² h⁻¹ bar⁻¹ whereas the PS membrane showed a very high flux (950 L m⁻² h⁻¹ bar⁻¹), which is almost 4 orders of magnitudes higher compared to the PS-*b*-P4VP membrane (**Fig. 3b**). To verify the reproducible high ethanol flux for the PS membrane, we performed 10 subsequent cycles with ethanol and DI water without measurable loss in ethanol flux. The hydrophobicity of the resulting PS membrane could be beneficial for the separation of oil/water

explained by excessive swelling of the P4VP block lined along the pore walls in the PS-*b*-P4VP membrane, thereby increasing the pore resistance for passage of the emulsion across the membrane. In the case of the PS membrane, the hindrance for passage of the emulsion was not observed because of the absence of the P4VP block. The purity of the water was determined by analyzing the carbon content in the filtrate using a Thermo-Fisher Scientific elemental analyzer. The purity of water was 99.9% with no detectable carbon in both cases with surfactant free or surfactant-

Fig. 2 2D NMR spectra of: (a) PS-*b*-P4VP; (b) qPS-*b*-P4VP and (c) isoporous PS membranes. Group A are the protons and carbons coupled in the P4VP block. The disappearance of group A in (c) is the result of chemical degradation of pyridinium in alcoholic alkaline medium. **Fig. 2** (d) shows GPC traces of PS-*b*-P4VP membrane before (black dashed line) and after chemical degradation (red dashed line) and molecular weight of membranes (inset).

emulsions.

Surfactant-free and surfactant-stabilized emulsions were explored for separations using PS-*b*-P4VP and PS membranes. The remarkable result is that the PS membrane allowed only water to pass whereas no measurable flux was obtained in the case of the PS-*b*-P4VP membrane under the same feed pressure. This can be

stabilized emulsions as feed. To validate the effective separation, we used optical microscopy to observe the emulsion droplets size in both feed and filtrate samples. We did not note any oil droplets in the filtrate samples, indicating the effective removal of oil from the oil in water emulsion (**Fig. S7, ESI**).

Fig. 3 (a) Schematic illustration of the PS-*b*-P4VP and the PS membrane in dry and wet states. The hydrophilic P4VP blocks are solidified, offering the least resistance in dry state. In the wet state, the swollen P4VP block decreased the pore diameter. In the case of the PS membrane, the pore size remained the same in either the dry or the wet state. (b) Ethanol flux and (c) pressure-dependent pure water flux of PS-*b*-P4VP and isoporous PS membrane.

Fig. 3c illustrates the pressure dependent water flux of the PS-*b*-P4VP and PS membranes measured using a dead-end stirred ultrafiltration cell. Remarkably, the PS membrane presented a linear change in water flux with the increase in pressure. This result demonstrates an excellent stability of the nanoporous PS membrane without pore compaction at high feed pressure. The water flux of the PS membranes in triplicate was $2000 \pm 150 \text{ L m}^{-2} \text{ h}^{-1} \text{ bar}^{-1}$, which is 67% higher than the flux of the PS-*b*-P4VP membrane ($1200 \pm 200 \text{ L m}^{-2} \text{ h}^{-1} \text{ bar}^{-1}$) (**Fig. S8, ESI**). The increase in flux was attributed to the pore expansion after chemical degradation of the P4VP block from the nanochannels of the PS-*b*-P4VP membrane and subsequently, the hydraulic resistance declined. The increase in pore diameter was quantitatively estimated using the Hagen-Poiseuille's equation, as given in equation (1).

$$dv/dt = (\pi R^4 \Delta p) / (8 \eta L) \quad (1)$$

where R is pore radius, Δp is the pressure drop across the membrane, η is water viscosity ($8.9 \times 10^{-4} \text{ Pa s}^{-1}$ at 25°C), and L is the length of the nanochannels. The effective pore diameter at different pH values is depicted in **Fig. 4a**. At all pH values, the calculated pore diameter of the PS membrane was higher compared to the PS-*b*-P4VP membrane, which is reflected in the observed water flux of the corresponding membranes.

In the case of the PS-*b*-P4VP membrane, the decrease in water flux at low pH is attributed to the protonation of the P4VP block lining the pore walls; the positively charged PVP chains extend and close the pores.³³ As expected, the water flux of the PS membrane was pH independent due to the elimination of the pH-responsive P4VP block. This feature can be beneficial for applications where constant flux is required at different pH values. The response to variations in pH of the original block copolymer membrane and the PS homo polymer membrane was also confirmed by measuring the rejection of polyethylene glycols (PEGs) of different molecular weights. The block copolymer membrane showed a low rejection at high pH and high rejection at low pH, whereas the rejection of the PS membrane was low over the entire pH range (**Fig. S9, ESI**).

Measurement of the water flux of the qPS-*b*-P4VP membrane as a function of NaOH/EtOH treatment time indicated a variation in the effective pore diameter estimated by Hagen-Poiseuille's equation (**Fig. 4b**, red symbols). As expected, the water flux and the effective pore diameter of the membranes increased with NaOH-EtOH treatment time. The effective pore diameter and the water flux of the membranes did not change after 24 h treatment, suggesting the complete removal of the P4VP block (**Fig. 4b**). The effective pore size can be tailored from about 5 nm to 30 nm by variation of the NaOH-EtOH treatment time.

Fig. 4. (a) Water flux and the effective pore diameter of the membranes at different pH values and (b) water flux and the effective pore diameter of the membranes treated with NaOH-EtOH for different time intervals. (c) Schematic of pore size tuning with NaOH-EtOH treatment.

Size Based Single and Mixed Protein Separation at Physiological pH

In line with other reported high flux hydrophobic membranes for size-based protein separation, we assessed the molecular separation ability of the isoporous PS membrane.² The biologically relevant proteins of different molecular weights (MW) and hydrodynamic diameters (D) lysine (MW = 14.3 kDa, D = 4 nm), bovine albumin serum (MW = 67 kDa, D = 6.8 nm) and γ -globulin (MW = 150 kDa, D = 14 nm) were selected. BSA and IgG were chosen because their separation is challenging.^{2,33} When we evaluated the single protein transmission for the permeate through a qPS-*b*-P4VP membrane treated with NaOH-EtOH mixture for different time intervals, we observed that our membrane allowed only Lys or BSA to pass and the transport of IgG was hindered (**Fig. 5a**). Even with the membrane with the largest pore size, IgG could not be detected in the permeate. It is apparent that the protein transport was highly dependent on the hydrodynamic diameter of the studied proteins. The protein content in the permeate sample increased with increasing pore size of the PS membrane. We adjusted the pore size in order to achieve the optimum selectivity for the separation of the proteins. The membrane treated with NaOH-EtOH for 4 h (27.3 nm pore size) exhibited the highest selectivity; Lys or BSA permeated through the membrane 42 and 30 times faster, respectively, than IgG (**Fig. 5b**). The observed transport of IgG was marginal even though the pore size of membranes treated with NaOH-EtOH for 4 h was two times larger than the hydrodynamic diameter of IgG. These results are in agreement with

data earlier reported by Striemer et al. and Qiu et al., that showed pore clogging due to protein adsorption via hydrophobic interactions which could lead to the diminution in the effective pore size of the membranes.^{2,33} Similarly, the protein transport was also influenced by other factors like ionic strength, solution pH, and protein structure. Contrary to the traditional flux-selectivity trade-off, our membrane displayed the highest selectivity (42 and 30) and very high fluxes of the protein containing solutions (Lys: 1096 L m⁻¹ h⁻¹ bar⁻¹, BSA: 1040 L m⁻¹ h⁻¹ bar⁻¹) compared to commercial membranes with similar pore size.^{33,34} In addition, the water flux of our nanoporous PS membrane was higher than the flux of BCP derived membranes reported in the literature (**Table ST2, ESI**).

Based on the encouraging results in the single protein transport study, we tested these membranes for separation of mixed proteins solutions. The membranes treated for different times with NaOH-EtOH were used for filtration of protein mixture solutions of Lys-IgG (**Fig. 5c**) and of BSA-IgG (**Fig. 5d**), and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was applied to examine the transport of the protein mixture components. Remarkably, only one strong band for the permeate sample was observed in each case. In each study, IgG did not cross the membrane, which confirmed the high selectivity of NaOH-EtOH-treated membranes for the smaller proteins. Moreover, the bands for permeate samples were detected at the same position in the gels, indicating that the original active structure of the protein was not altered by the filtration.

Fig. 5. (a) Single protein transmission data (permeate) for Lys, BSA, and IgG at 1mg/ml and 1 bar feed pressure. (b) The selectivity for Lys versus IgG and BSA versus IgG. SDS-PAGE analysis of: (c) Lys-IgG mixture and (d) BSA-IgG mixtures; lane 1 is the initial proteins mixture solution; lanes 2 to 6: elution samples (5 μ l) collected from membranes treated with NaOH-EtOH mixture for 10 min, 1 h, 4 h, 12 h and 24 h, respectively.

The transport of Lys and BSA increased gradually with increasing NaOH-EtOH treatment time. As expected, the band for the protein was not detected after only 10 min of treatment since the small pores completely hindered the protein transport. Moreover, we did not notice any bands for IgG in lanes 3, 4, 5, 6 and 7, showing that the transport of IgG was effectively hindered by the membrane. The results confirmed that the membrane selectivity was predominantly governed by size differences and was independent of the charge of proteins at the physiological pH 7.4 (Lys is positively charged: Lys⁺, and BSA is negatively charged: BSA⁻). However, IgG is electrically neutral at this pH and it is expected to have minimum charge interactions with the pores of the membranes.

Conclusion

In conclusion, we have demonstrated a unique method for preparation of polystyrene membranes with ultra-high porosity and uniform pore size through the combination of block copolymer self-assembly, non-solvent induced phase separation, and chemical degradation. Analyses by 2D NMR, FTIR, thermal degradation and GPC confirmed the decomposition of the P4VP block, whereas the nanoporous structure of the resulting membrane was validated via SEM. The P4VP-free nanoporous PS membrane was stable up to 300 kPa, demonstrating high mechanical stability, which facilitates its use in pressure driven filtration processes. The degradation time gives an unprecedented control over the final pore size, which can be tuned from 6.5 to 29.4 nm. The size-based selectivity of the membranes for proteins can be tuned by controlling the pore size. The effective separation of proteins especially BSA and IgG (2 times difference in MW) with high selectivity (> 30) suggests that our membranes can be effectively used for protein fractionation. A straightforward fabrication method (without annealing and harsh chemical treatments, no noble metal complexation/coating for pore size tuning, and no transfer to a porous support) integrated with high separation performance opens numerous possibilities for the use of these membranes in fractionation of nanoparticles and the purification and separation of biomolecules. The pore formation process presented here opens several avenues for future developments, including fabrication of synthetically challenging block copolymer-based nanoporous materials, surface functionalization, and manipulation of the PS matrix using well-established chemistry

Conflicts of interest

There are no conflicts to declare.

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