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High flux membranes, based on self-assembled and H-bond linked triblock copolymer nanospheres

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Abstract

We developed composite membranes by stacking functionalized nanospheres (20 nm size) with a high density of H-bonds. The functionalized nanospheres were formed by a click-reaction in toluene between the polybutadiene segment of poly(styrene-*b*-butadiene-*b*-styrene) (PS-*b*-PB-*b*-PS) triblock copolymer and an azodicarbonyl (PTAD) compound. The strong hydrogen-bond interaction promoted by the pendant urazole groups of the PTAD-modified copolymer is an important parameter for obtaining stable and defect-free membranes, acting in analogy to self-healing systems. The hydrodynamic transport is facilitated by the high porosity of the membranes and the unique hourglass-shaped pores. The composite membrane has water permeation as high as $60 \text{ L m}^{-2} \text{ h}^{-1} \text{ bar}^{-1}$ and can exclude more than 95% of proteins with a molecular weight as small as 12 kg/mol. This novel class of nanoparticle-stacked membranes has therefore excellent separation properties for biomolecular separation.

1. Introduction

Water purification is essential for our society and reverse osmosis is one of the most successful and well-established methods for that, using cellulose acetate hollow fibers or polyamide-based thin-film composite membranes. Nevertheless, the search for even better membranes for demanding water purification processes and the recovery of valuable solutes in an aqueous medium is worldwide an important topic. Modern technologies are often inspired by natural biological systems [1] and the development of highly selective membranes can also benefit from this strategy [2-5]. The integration of aquaporin as part of synthetic membranes has been intensively investigated.[3] A more promising approach is the development of membranes with artificial channels. Barboiu's group[6] pioneered the field of artificial water channels based on self-assembled imidazole (I) quartets with dynamic H-bonding. Kumar and collaborators have recently proposed methods of preparation of membranes based on beta-barrel artificial channels.[7]

We have been preparing membranes with artificial channels for protein separation, mainly based on the self-assembly of block copolymers combined with a non-solvent induced phase inversion process.[8] The membranes that result from this method are integral asymmetric and have a high density of well-ordered pores with sharp size distribution. These characteristics contribute to a high water permeation in the range of ultra- to nanofiltration.[9] We have explored various block copolymers.[8, 10-14] A particularly promising approach to mimic biological channels uses copolymers with polypeptide blocks that exhibited α -helical conformation similar to natural protein channels.[15] Another example is the prepared self-assembled poly(styrene-*b*-4-hydroxystyrene-*b*-styrene) membranes containing channels functionalized with H-bond sites beneficial for protein separation.[10] Additives with H-bonds sites have been used to induce the

self-assembly during the membrane formation.[16] More recently, we took advantage of thiol-ene chemistry, to develop isoporous membranes based on poly(styrene-*b*-butadiene-*b*-styrene) (PS-*b*-PB-*b*-PS) copolymer and photomodification of the butadiene segments to increase the membrane water permeance.[11]

In this work, we developed membranes by a completely different method, forming nanoparticles with a high density of H-bonds and stacking them to form thin coatings. The water transport is promoted through the interstitia between nanoparticles. The fabrication of nanoparticle-stacked membranes, without the advantage of H-bond functionalization, has been previously demonstrated by McDonald and coworkers using polystyrene latex.[17, 18] Similar approaches using polystyrene nanoparticles have been investigated by other groups.[19-21] Matsuyama and co-workers developed ultrafiltration membranes based on silica [22] and functionalized silver-nanoparticle multilayers.[23] Meanwhile, Livingston and co-workers demonstrated the fabrication of nanoparticles-stacked membranes for organic solvent nanofiltration. The particles were constituted by cross-linked acrylate copolymers. They have also used poly(styrene-co-butadiene) nanoparticles to form tunable porosity membranes for aqueous nanofiltration.[24] Although the results reported by the different groups were promising for membrane manufacture, there are also drawbacks that have hindered further developments and the implementation of the membranes. The stability of the particle arrays has been one of the hurdles, as well as the scalability of the fabrication method, which requires an effective deposit the nanoparticles without the formation of cracks during the drying process. We believe that a breakthrough can be achieved with a proper selection of the particle material and chemical functionality.

Here, we demonstrate the fabrication of nanoparticle-stacked membranes based on self-assembled nanospheres obtained by the click reaction between PS-*b*-PB-*b*-PS triblock copolymer

and 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD). We observed for the first time that the attachment of the triazolinedione group on PS-*b*-PB-*b*-PS in toluene leads to the formation of nanospheres with a high density of H-bond sites. These sites promote a stable interparticle physical crosslinking, leading to thin coatings with self-healing properties and pores with unusual characteristics. The membrane potential for biological and pharmaceutical applications was demonstrated for the separation of small proteins, such as myoglobin, lysozyme, and cytochrome-c.

2. Experimental

2.1. Materials

Poly(styrene-*b*-butadiene-*b*-styrene) (average $M_w \sim 140,000 \text{ g mol}^{-1}$, styrene 30 wt%), 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD, 97%), toluene (anhydrous, 99.8%), bovine serum albumin ($\geq 96\%$), myoglobin, lysozyme (from chicken egg white), cytochrome-c (from bovine heart), vitamin B12, polyethylene glycol (PEG), N,N-dimethylformamide- d_7 (DMF- d_7) and chloroform- d (CDCl_3) were purchased from Sigma-Aldrich. Polyacrylonitrile (PAN) commercial membranes were supplied by GMT GmbH Germany.

2.2. Preparation of modified-poly(styrene-*b*-butadiene-*b*-styrene) nanoparticles

The modification of poly(styrene-*b*-butadiene-*b*-styrene) (PS-*b*-PB-*b*-PS) was performed by mixing a 5 wt% polymer solution in toluene and 2 wt% PTAD (equimolar ratio relative to butadiene) in toluene (Scheme 1), using a triazoline-ene reaction.[25-27] The solution turned from red to yellowish after overnight stirring (Figure S1). The stirring was continued for four more days to assure a complete reaction, whereby the color of the solution became less yellow with some turbidity, indicating the formation of a colloidal dispersion. The solution was

centrifuged at 7500 rpm for 1 h, resulting in a stable dispersion after decanting and removing the non-dispersed particles, and filtering the supernatant with a syringe (0.45 μm PTFE syringe filter). The filtered solution was then characterized and used for the membrane preparation.

Scheme 1. Chemical modification of the PS-*b*-PB-*b*-PS triblock copolymer, using 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD).

2.3. Composite membrane fabrication

Thin composite membrane films were prepared by spin-coating the polymer nanoparticle dispersion (2.5 wt% in toluene) on PAN support with a spinning speed and acceleration of 3000 rpm and 1000 rpm^{-1} , respectively. 1.8 mL of the solution was used to cover an area of 9 x 9 cm^2 of the PAN support.

2.4. Nuclear Magnetic Resonance (NMR)

Toluene was evaporated from the PTAD-modified PS-*b*-PB-*b*-PS copolymer solution at room temperature. The nanoparticles were then washed with methanol several times in a stirred beaker. Methanol was finally removed by vacuum filtration. The sample was further dried at room temperature under high vacuum overnight. ^1H -NMR and ^{13}C -NMR measurements were performed at room temperature in CDCl_3 and DMF-d_7 . NMR spectra were recorded in Bruker

Avance-III 400 MHz NMR spectrometers, equipped with a Z-axis gradient BBO probe and a B-ACS 60 automatic sample changer.

2.5. Dynamic Light Scattering (DLS)

Nanoparticle dispersions were diluted five times with toluene to obtain a polymer concentration of around 0.5 wt%. The hydrodynamic radii of the nanoparticles were measured by DLS using a Malvern Zetasizer. Measurements were performed using a glass cuvette to load the sample, a backscattering angle of 173° , 12-15 runs per measurement, and three repeating measurements for each sample.

2.6. Atomic Force Microscopy (AFM)

The equilibrium morphology of the PS-*b*-PB-*b*-PS copolymer was visualized by atomic force microscopy, after annealing PS-*b*-PB-*b*-PS thin films on silicon wafers. The polymer was dissolved in toluene (concentration of 5 wt%) and spin-coated on a silicon wafer at around 1500 rpm for 60 s. The spin-coated film was annealed in a closed chamber, saturated with toluene vapor at room temperature for a few hours. The AFM topography and phase profiles were acquired on an Agilent 5500 AFM in the tapping mode, controlled by PicoView 1.8 software. A silicon cantilever probe with a resonance frequency of 76-263 kHz and a force constant of 1.2-29 Nm⁻¹ was used, and the acquired images were post-processed, using Gwyddion software.

2.7. Transmission Electron Microscopy (TEM)

TEM images were obtained on a FEI Titan ST microscope, operating at 300 kV. Polymer nanoparticles (concentration of around 0.5 wt% in toluene) were dropped (3 μ L) onto a carbon-coated copper grid. The excess solution was blotted using a filter paper placed underneath the grid. After drying for a few minutes at room temperature, the sample was stained using osmium tetroxide (OsO_4) vapor for 3 – 4 hours, before visualization. For the cross-section images, the membrane was first stained with OsO_4 vapor for four hours, before embedding it in an epoxy resin. Ultrathin sections (80 – 60 nm) of the embedded membrane were prepared using a Leica ultramicrotome with a diamond knife.

2.8. Scanning Electron Microscopy (SEM)

The membranes were cut and mounted on an aluminum stub using an aluminum tape. Samples for the membrane cross-section images were prepared by fracturing the membrane in liquid nitrogen. The samples were sputter-coated leading to a 3 nm platinum layer to avoid charging, using a Quorum Technologies Q150T under argon atmosphere. SEM images were acquired on a FEI Novanano microscope at 5 kV accelerating voltage and 5 mm working distance.

2.9. Grazing-Incidence Small-Angle X-ray Scattering (GISAXS)

GISAXS 2D patterns of the PS-*b*-PB-*b*-PS thin films were obtained using synchrotron source X-ray with a beam energy of 10.6 keV and wavelength of 1.17 Å at the D1 beam line of the Cornell High Energy Synchrotron Source. The sample – detector distance used was 1.77 m, and an incident angle of 0.13 degree was used with an exposure time of 1 s. The horizontal projection was performed using Fit2D software to obtain scattering intensity versus the scattering vector (q) curve.

2.10. Fourier Transform Infrared (FTIR) spectroscopy

FTIR spectra of the membranes were recorded using a Nicolet spectrometer. The measurements were performed with 64 runs and 0.241 cm^{-1} resolution. The sample was first washed with methanol and dried under high vacuum overnight before the characterization.

2.11. Zeta potential

The zeta potential of the membrane surface was characterized using a SurPASS electrokinetic analyzer with a gap height of around $120\text{ }\mu\text{m}$. A NaCl aqueous solution (0.01 M) was used as the background, and the pH was adjusted using 0.1 M HCl and 0.1 M NaOH . The zeta potential was calculated using the Helmholtz-Smoluchowski equation.

2.12. Filtration tests

The water permeance at various transmembrane pressures ($\Delta P = 1\text{ to }5\text{ bar}$) was measured using an in-house stainless-steel cell with an effective membrane area (A) of 1 cm^2 and a dead-end Amicon ultrafiltration cell (effective membrane area = 4.1 cm^2). The permeance volume (V) collected during the filtration period (t) was used to calculate water permeance (J_w) as in equation (1).

$$J_w = \frac{V}{A \cdot t \cdot \Delta P} \quad (1)$$

The rejections of proteins and vitamin B₁₂ were analyzed using a dead-end Amicon ultrafiltration cell (effective membrane area = 4.1 cm^2) under continuous stirring at 300 rpm . A protein solution with a concentration of 1 g L^{-1} in phosphate buffered saline (PBS) solution (pH 7.4) was

used as feed. The vitamin B₁₂ feed was prepared by dissolving vitamin B₁₂ in milli-Q water with a concentration of 100 ppm. The permeate and feed concentrations were measured using a NanoDropTM 2000/2000c spectrophotometer (Thermo Fisher Scientific). The exclusion of PEG (200, 3000, 10 000, 35 000, and 100 000 g mol⁻¹) was studied using feed solutions of 1 g L⁻¹ PEG (total concentration) in water. The PEG concentrations were measured using an Agilent 1260 Infinity gel permeation chromatography with two columns in series (PL aquagel-OH 40 and 60), operated at 30 °C. The rejections (R) of proteins, vitamin B₁₂, and PEG were calculated using the equation (2), where c_p and c_f are the solute concentration in the permeate and the feed, respectively.

$$R = \left(1 - \frac{c_p}{c_f}\right) \times 100\% \quad (2)$$

3. Results and Discussion

*Morphological characterization of unmodified PS-*b*-PB-*b*-PS thin films.*

The membranes in this work were obtained by spin-coating solutions of a PS-*b*-PB-*b*-PS terpolymer, modified by reacting it with PTAD. The used terpolymer was commercially available in large-scale. The morphology of unmodified PS-*b*-PB-*b*-PS terpolymers in general has been previously investigated [29][30, 31], and it is highly dependent on the block lengths. To emphasize the effect of the chemical modification on the film morphology, we first investigated the morphology of the unmodified PS-*b*-PB-*b*-PS film obtained by spin-coating, similarly to the method used for the membrane fabrication. A continuous dense film with a uniform thickness of 400 nm was formed by spin-coating a 5% solution of PS-*b*-PB-*b*-PS in toluene. After the annealing with toluene, which is a nonselective solvent for PB and PS, a morphology close to the

equilibrium was obtained (Figure 1). The structure is mainly governed by the interaction between the blocks. Since PS and PB are both hydrophobic, the influence of their preferential interaction with the substrate and the air at the interfaces is less significant. The AFM measurement of the annealed film showed a highly ordered structure, as shown in Figure 1a. The AFM phase image (Figure 1b) clearly shows the microphase separation between the hard-spherical PS domains (bright phase) within the soft PB matrix (dark phase).[28] The formation of PS domains as spheres in PB matrix is expected since the PS block is the minor component (30 wt%). GISAXS measurements confirmed the formation of the well-ordered structure of the annealed PS-*b*-PB-*b*-PS thin film as indicated by the 2D pattern shown in Figure 1c. A horizontal cut along the Yoneda peak[32] of the GISAXS 2D pattern was translated into scattering intensity versus scattering vector (q), as shown in Figure 1d. The indexation was tested with the software indexGIXS (CHESS-Cornell) for simple cubic (sc), body-centered-cubic (bcc) face-centered cubic (fcc). [33][34] The best fitting was confirmed for bcc (110) (Figure S2, Supplementary information) with a lattice constant of 48 nm, corresponding to the average distances between the PS domains.

Figure 1. (a) AFM topological and (b) phase contrast image, as well as (c) GISAXS 2D pattern and (d) scattering curve of annealed PS-*b*-PB-*b*-PS thin film.

Without annealing, the structure is far from equilibrium and is trapped by the fast solvent evaporation as the spin-coating proceeds. The GISAXS pattern of the non-annealed PS-*b*-PB-*b*-PS film (Figure S3, Supplementary Information) corresponds to a more disordered structure, indicated by the presence of only two broad peaks. The spin-coating procedure was later applied to the membrane preparation using then modified PS-*b*-PB-*b*-PS copolymers. The structure in the membrane is analogously a non-equilibrium morphology.

Triblock copolymer modification and membrane fabrication

The pristine PS-*b*-PB-*b*-PS copolymer was characterized by NMR, as shown in Figure S4. For the membrane preparation, the copolymer was modified using the triazoline-ene click chemistry.[25-27] A 1:1 mol ratio of PTAD relative to butadiene was added into a dilute solution of PS-*b*-PB-*b*-PS (5 wt%) in toluene. After one day of reaction, the red color of the initial mixture faded to light orange, indicating a significant consumption of PTAD (red color) as it reacts with butadiene. The solution was stirred for five days in total to obtain a high conversion, and the color turned to light yellow with some undissolved solids, which were then centrifuged and filtered to obtain a stable dispersion (Figure S1, Supplementary Information). Gravimetric analyses were performed to estimate the final polymer concentration after centrifugation and filtration using samples from four different batches, which confirmed an average polymer concentration of 2.46 ± 0.04 wt%.

We found out that the attachment of PTAD onto butadiene groups promoted the formation of uniformly sized nanospheres in toluene (Figure 2a) with a diameter of around 20 nm, as presented in the TEM image (Figure 2b). DLS measurements of the dispersion (Figure 2c) showed a sharp distribution of particle hydrodynamic size (z-average) of around 60 nm (PDI = 0.09 ± 0.01). The diameter increase observed in the DLS measurements, compared to that of TEM, could be due to two factors: (1) the high swelling degree of the nanospheres in toluene and (2) the partial aggregation driven by the dense distribution of H-bond sites. An additional indication of the aggregation is given by the solution turbidity seen in Figure S1, Supplementary Information. We hypothesize that the formation of PTAD-modified PS-*b*-PB-*b*-PS nanospheres is induced by a hydrogen bonding between the original carbonyl (C=O) group of the PTAD and

the secondary amine (N-H) groups formed after the reaction. The TEM analysis of the nanospheres stained by osmium tetroxide (diene and carbonyl staining agent)[35], seen in Figure S5, showed no preferential order of the blocks within the nanosphere, suggesting a random distribution of the PTAD-modified butadiene through the entire volume. Since an equimolar ratio of PTAD was added (relative to moles of butadiene), we expected a high amount of urazole pendant group (PTAD) due to the fast triazoline-ene reaction. The polarity of the overall modified copolymer increased, while the presence of the non-polar PS and unreacted PB could still exhibit some compatibility with the phenyl group of the PTAD pendant groups, avoiding distinct microphase separation between the PS and PTAD-modified PB. The copolymer then adapted spherical morphology to minimize the surface energy affected by the polar-nonpolar interaction between the copolymer and the solvent, while forming the colloidal dispersion. Similar concepts have been recognized in the formation of micelles from amphiphilic block copolymers in selective solvents [36-38] or via polymerization-induced self-assembly in aqueous solution.[39]

Figure 2. (a) Schematic representation of the nanosphere formation induced by H-bond interaction and interparticle interaction after the modification; (b) TEM image and (c) Particle size distribution (DLS measurements) of the PTAD modified PS-*b*-PB-*b*-PS nanospheres in toluene.

The completion of the triazoline-ene reaction was confirmed by the FTIR spectra (Figure 3a) of the copolymer deposited on polyacrylonitrile (PAN) microporous support. The PAN peaks can be observed both in the PTAD-modified PS-*b*-PB-*b*-PS (SBS-PTAD) and the pristine PS-*b*-PB-*b*-PS (SBS) composite films. They include the nitrile stretching peak at 2240 cm^{-1} . The appearance of a strong carbonyl stretching frequencies at 1770 cm^{-1} and 1700 cm^{-1} in the SBS-PTAD spectrum confirms the presence of urazole pendant groups. Furthermore, the PTAD

attachment can also be confirmed by the appearance of the bonded N-H stretching band around 3190 cm^{-1} and N-H bending band around 1500 cm^{-1} , which also suggests the presence of hydrogen bond.[27] Unbonded N-H stretching can be observed at around 3450 cm^{-1} , which can be caused by the steric and conformational effects due to the high concentration of the urazole groups.[26] The successful modification of the copolymer was further supported by NMR spectroscopy. New peaks corresponding to the N-H and C=O fragments from the urazole appeared in the ^1H -NMR spectrum at $\delta = 10.67\text{ ppm}$ and in ^{13}C -NMR $\delta = 153.28$, respectively (Figure 4) after the modification (Figure S3, Supplementary Information). The newly formed C-N bond of the triazoline-ene reaction is observed at $\delta = 58.73\text{ ppm}$ in the ^{13}C -NMR copolymer modified spectrum (Figure 4). Additionally, a new set of peaks appeared in the ^1H -NMR in the range $\delta = 4\text{--}5\text{ ppm}$. These signals correspond to the protons from the rearranged modified polybutadiene polymer block. According to the integration areas from ^1H -NMR, it can be estimated that the modification yield is about 58 % of the total polybutadiene repeating units. The water contact angle (WCA) decreased from 90° to 60° due to the presence of polar urazole groups, indicating an increase of the hydrophilicity of the SBS-PTAD film, compared to that of the unmodified SBS film (Figure 3b). The SBS-PTAD hydrophilicity is still lower than that of PAN support (WCA around 40°).

Figure 3. (a) FTIR spectra and (b) water contact angle of PAN support, PS-*b*-PB-*b*-PS film (SBS), and PTAD-modified PS-*b*-PB-*b*-PS membrane (SBS-PTAD). The inset photographs in Figure 3b correspond to the water droplets on the film surface at 0, 30, and 60 s after depositing them; (c-d) SEM images of the membrane surface (c) and cross-section (d) of the PTAD-modified PS-*b*-PB-*b*-PS spin-coating on the PAN support; (e) TEM image of the PTAD-modified PS-*b*-PB-*b*-PS coating layer cross-section stained with OsO₄.

Figure 4. NMR spectra of the PTAD-modified PS-*b*-PB-*b*-PS triblock copolymer.

The SEM image of the porous surface of the uncoated PAN support is presented in Figure S6a (Supplementary Information). Figure 3c and d respectively show the surface and cross-section SEM image of the PTAD-modified PS-*b*-PB-*b*-PS composite membranes spin-coated on the PAN microporous support. The spherical particles homogeneously coat the support (Figure 3c), densely packed, forming a 500 nm thick layer (Figure 3d). Figure 3e clearly shows the interstitial channels formed between the stacked nanoparticles. These channels serve as preferential paths for molecular separation with H-bond interaction within the channel due to the presence of urazole groups.

It has been reported that previous membranes formed by particles arrays are prone to crack upon drying due to the stress release of the particle shrinkage during dehydration.[17, 19] For the membranes described here, cracks were observed when using a high polymer concentration (Figure S7, Supplementary Information), but at optimized lower polymer concentrations, the defects were practically non-existent and the particle assemblies were intact over the membrane cross-section, as illustrated by the SEM images in Figure 3 and S8 (Supplementary Information), with an excellent rejection of small proteins (Figure 5). The high density of sites for H-bonding on the particle surfaces might provide self-healing[40, 41] characteristics of the membranes. The dynamic physical crosslinking between particles permits to keep the continuity of the thin coating, keeping the high protein rejection.

The polymer concentration and spin-coating conditions are crucial parameters for the formation of defect-free membranes. Attempts to fabricate a thinner membrane, using a low polymer concentration solution (1 wt% in toluene), resulted in membranes with poor surface coverage. Dispersions with high polymer concentration lead to a thicker coating layer with severe cracks, as illustrated in Figure S7. An optimum polymer concentration should allow sufficient coverage

of the substrate while forming an adequate film thickness with good adhesion to the PAN support. The adhesion between the particles and the support is driven by the dipole-dipole and Van der Waals interactions, as well as hydrogen bond formation. The affinity between the particles and the support is therefore essential and any chemical modification that promotes it would increase the adhesion. Additionally, as mentioned before, for the membrane stability, the interaction between particles is even more important. The urazole groups, which are homogeneously distributed within the nanosphere, allow strong H-bonding interaction between the particles, as confirmed by the FTIR spectrum in Figure 3a, and promote the formation of a well-integrated film.

Membrane separation performance

We tested the membranes for ultrafiltration and analyzed the separation performance of the stacked nanospheres by comparing it with the pristine PAN support. Interestingly, the PTAD-modified PS-*b*-PB-*b*-PS (SBS-PTAD) composite membranes showed pure water permeance very similar to that of the PAN support alone (Figure 5a and Figure S6b). The membrane had good mechanical stability, and a stable water permeance was observed during seven-hour dead-end filtration under 3 bar pressure. The permeance profile of SBS-PTAD membrane (Figure 5a) shows a reasonably stable flux of around $50 - 60 \text{ L m}^{-2} \text{ h}^{-1} \text{ bar}^{-1}$ over a pressure range from 1 – 5 bar. A small decrease at high pressure was also observed for the water permeance profile of the PAN support (Figure S6b, Supplementary Information).

Figure 5. (a) Water permeance of the PTAD-modified PS-*b*-PB-*b*-PS composite membranes (SBS-PTAD) under different pressures with a schematic diagram of possible interstitial voids and hourglass-like slits accessible for water transport, (b) molecular retention of SBS-PTAD membranes and PAN support.

The results imply that the SBS-PTAD nanospheres contribute to a negligible hydrodynamic resistance, which would be unusual for a rigid system characterized by a flow-through packed-bed spheres. A possible explanation for the high flux is given below. The pressure-driven flow through the membranes can be expressed by the hydraulic flux (J) in terms of the pressure gradient (ΔP), fluid viscosity (η), and hydrodynamic resistance (R) presented in equation (3).

$$J = \frac{\Delta P}{\eta R} \quad (3)$$

Carman-Kozeny[18, 42] and Leva[17, 43] developed theories on flow through packed beds, which describe the flow resistance (R) in terms of porosity (ε) and particle properties as depicted in equation (4).

$$R = C \frac{1 - \varepsilon}{\varepsilon^3 d_p^2} \cdot \frac{m_p}{\rho_p A} \quad (4)$$

where, C is a constant (180 for Carman-Kozeny, 200 for Leva), d_p is the particle diameter, m_p is the mass of the packed bed, ρ_p is the particle density, and A is the filtration area. The equation (4) shows a high dependency of the flow resistance on the packed-bed porosity, which can be significantly reduced by third order as the porosity increases. The PTAD-modified PS-*b*-PB-*b*-PS membranes exhibited a high porosity since the particle contact points are not covalently crosslinked, but only stabilized by H-bonds. Hence water can pass through the small slits at the joint between particles (in addition to the interstitial voids) as illustrated in Figure 5a. In contrast, other groups have attempted to stabilize analogous nanoparticle-stacked membranes by merging the particle joints through thermal annealing or forming covalent linkages between the particles via chemical cross-linking, making the paths less accessible for water transport.[17, 18, 20, 23] In our case, the stabilization is done by strong but relatively dynamic hydrogen bonds between the particles, without compromising the flux. Furthermore, the geometrical structure of the slits and the interstitial voids can be an advantage for water transport. Gravelle et al. [44] examined the viscous dissipation at the conical entrance of the hourglass-shape channels similar to aquaporin. An optimum opening angle for the conical entrance can provide a large increase of permeability. Therefore, not only the high porosity but also the hourglass-like channel geometry in our membranes may play a role in the negligible flow resistance through the packed-bed nanospheres.

We confirmed that the water permeance of the SBS-PTAD membrane does not arise out of the defects since the membranes exhibited remarkably higher rejection of proteins than that of PAN support (Figure 5b). The rejections were confirmed by up to four measurements for each protein, using different membranes. Water might swell the particles during filtration due to the presence of polar urazole groups, which can then enhance the separation performance and heal minor cracks.

Figure 5b shows that while the PAN support has a BSA (66.5 kg mol^{-1}) rejection similar to that of the SBS-PTAD membrane, the rejections of myoglobin (16.7 kg mol^{-1}), lysozyme (14.3 kg mol^{-1}) and cytochrome-c (12 kg mol^{-1}) were much lower than that of the coated membrane. The higher rejection of the PAN support for cytochrome-c than for myoglobin and lysozyme might be due to adsorption effects. Both the PAN support and the SBS-PTAD membrane could, however, transport vitamin B₁₂ with low rejection ($< 5\%$). The SBS-PTAD membrane was characterized by a molecular weight cut-off around 35 kg mol^{-1} (Figure S9, Supplementary Information), estimated by filtration of PEG with different molecular weights. PEG is a highly hydrophilic linear molecule and might be able to permeate between the slits between particles. For the protein transport, in addition to the size exclusion, the transport through the membrane pores is highly dependent on electrostatic interactions.[45, 46] The rejection can also be influenced by the hydrogen bond interaction as proposed in our previous work.[10] The SBS-PTAD membrane is negatively charged surface over a wide range of pH, as presented in Figure S10 (Supplementary Information), which could be attributed to the high content of carbonyl groups. The rejection of cytochrome-c (isoelectric point (pI): 9.6) and lysozyme (pI: 11) can be affected by the adsorption due to electrostatic attraction between the negatively-charged membrane and the positively-charged proteins. The SBS-PTAD membrane still exhibited a high

rejection of myoglobin, which is neutral (pI: 6.8-7.4) and has a hydrodynamic diameter of 3.5 nm, very close to that of cytochrome-c (3.1 nm).[47] This observation suggests that the membrane has an effective diameter of around 3 nm. The high rejection of myoglobin and cytochrome-c were also visually indicated, as shown by the feed and permeate images displayed in Figure S11 (Supplementary Information).

The results highly indicate that the deposition of SBS-PTAD nanospheres on the PAN support significantly enhance the membrane separation performance by having a higher rejection of proteins, while maintaining water permeance. The membranes fabricated in this work would be highly beneficial for biomolecule separation in pharmaceutical and biotech industries. We anticipate the possibility of tailoring the pore sizes, shifting towards the nanofiltration range, by using a low molecular-weight triblock copolymer, which could yield nanospheres with smaller sizes. However, further investigation would be required to optimize the manufacturing conditions. We believe that this work could be further leveraged if analogous approaches would be extended to other chemically-tunable nanoparticles, keeping the exceptional water permeance, and high porosity, but leading to thinner hourglass-shaped channels with even higher selectivity.

Conclusions

Self-assembled nanospheres functionalized with urazole groups were synthesized, by applying the triazolene-ene click chemistry between PS-*b*-PB-*b*-PS terpolymer and an azodicarbonyl compound (PTAD) in toluene. The nanospheres exhibited a sharp size distribution of around 20 nm as confirmed by TEM. The particles were deposited on microporous PAN support via spin-coating to form a composite membrane. Strong hydrogen-bond interactions between the particles play a significant role in stabilizing the nanoparticle-stacked membrane and healing any defect potentially formed during operation. We confirmed the formation of defect-free membranes by

demonstrating the effectiveness of the nanosphere stacks in discriminating small proteins and vitamin B₁₂. High rejection of myoglobin, lysozyme, and cytochrome-c was measured, in contrast to the pristine PAN support. More interestingly, the water permeation of the coated nanosphere-stacked membrane was similar to the unmodified PAN support, due to 1) its high porosity resulted from the voids between the particles and passable slits between the particle, and 2) the hourglass-shaped channels. Our work is expected to contribute to the development of ultrafiltration membranes for biomacromolecule separations, which can be further optimized for nanofiltration, as a new method for the preparation of self-assembled artificial channels.

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Supplementary Information

High flux membranes, based on self-assembled and H-bond linked triblock copolymer nanospheres

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Figure S1. Preparation steps of the PS-*b*-PB-*b*-PS (SBS) modification, using 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) in toluene, obtaining a stable dispersion (light yellow) after centrifugation and filtration.

Figure S2. GISAXS indexation fitting for bcc arrangement, using the software indexGIXS (CHESS, Cornell).

Figure S3. (a) GISAXS 2D pattern and (b) horizontal projection of scattering intensity (I) vs scattering vector (q) of a non-annealed PS-*b*-PB-*b*-PS thin film spin-coated on a silicon wafer.

Figure S4. NMR spectra of the PS-*b*-PB-*b*-PS terpolymer.

Figure S5. TEM image of the PTAD-modified PS-*b*-PB-*b*-PS particles stained with OsO₄.

Figure S6. (a) SEM image and (b) water permeance of polyacrylonitrile (PAN) microporous support.

Figure S7. SEM image of the surface of a PTAD-modified PS-*b*-PB-*b*-PS membrane prepared using a dispersion with a higher concentration than optimized for the membranes reported in this work. The other conditions were the same as adopted for the optimized membrane: spin-coating on PAN support (3000 rpm, 60 s).

Figure S8. SEM images of PTAD-modified PS-*b*-PB-*b*-PS membranes (a-c) surfaces with different magnifications and (c-d) cross-sections.

Figure S9. Gel permeation chromatography of PEG mixtures in water obtained at the feed and permeate sides after dead-end filtration using PTAD-modified PS-*b*-PB-*b*-PS membrane. Peaks are labeled with the corresponding PEG molecular weight in kg mol^{-1} .

Figure S10. Surface zeta potential of PTAD-modified PS-*b*-PB-*b*-PS composite membrane.

Figure S11. Photographs of the feed and permeate in dead-end filtration experiments of myoglobin (left) and cytochrome-c (right), using PTAD-modified PS-*b*-PB-*b*-PS membranes.

Highlights

High flux membranes, based on self-assembled and H-bond linked
triblock copolymer nanospheres

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- Membrane with self-healing properties
- Coatings based on particles modified by click chemistry
- Pores with hourglass shape with high water permeation and selectivity