Modification of commercial ultrafiltration membranes for improved performance

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Modification of commercial ultrafiltration membranes for improved performance

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Preface

This thesis presents the work conducted during my PhD project Feb 2018 to Jan 2021 at the Department of Chemical and Biochemical Engineering, Technical University of Denmark (DTU).

This work was carefully supervised by Professor Manuel Pinelo, and co-supervised by Professor Jianquan Luo, Associate Professor Anders E. Daugaard, and Professor John Woodley.

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From the bottom of my heart, I really appreciate that my supervisor Manuel Pinelo gave me the opportunity to study in his group for three years. His patient supervision, scientific guidance, cheerful attitude, and warm encouragement accompanied me throughout my entire PhD. I spent pleasurable and unforgettable time with Manuel in our group. This is not to forget the part played by Professor Jianquan Luo, Associate Professor Anders E. Daugaard and Professor John Woodley in ensuring the successful completion of my PhD project.

Thanks to my all colleagues Zhibo Zhang, Enrico Mancini, Konstantinos Asimakopoulos, Anna Burniol Figols and Ziran Su, who helped me in various ways, either direct or indirectly, in contributing to fruitful discussions on the analytical work and sharing ideas and information.

Thanks to my officemate Sigyn Björk Sigurdardóttir, Antonio Grimalt Alemany and Antonios Melas, we spent three years of good time together.

Finally, I would like to thank my family for their kind understanding and encouragement of my scientific research. Without the support of my family, I cannot devote myself to the PhD study.
Abstract

Ultrafiltration (UF) is widely used in food, medicine, water treatment, bio-separation and other fields due to its advantages of low energy consumption, simple operation, mild conditions and environmental friendliness, etc. However, UF also faces huge challenges, such as low permeability, weak selectivity, and severe membrane fouling. These shortcomings are mainly related to the chemical properties of the membrane material and membrane structure. In this thesis, a polysulfone (PSf) membrane substrate with excellent mechanical and thermal properties was modified using three methods: pre-treating the membrane with ethanol or NaOH solution, depositing polyelectrolytes on the membrane surface, and grafting of charged small molecules on the membrane surface. These modifications effectively improved the chemical properties and structural morphology of the UF membrane and brought about a substantial enhancement in membrane permeability, selectivity and fouling resistance.

First, we studied the effect of ethanol or NaOH pre-treatment on membrane structure and performance. Using analysis of FTIR spectra, we found that after pre-treatment with hot water, ethanol or NaOH, the preservatives in membrane were completely removed and the flux of membrane was improved. In particular, water flux of membrane treated with absolute ethanol increased by nearly 5-fold. Polymeric materials swell in organic solvents, resulting in large pore size and porosity, which was confirmed by the increase in membrane thickness and the decrease in dextran rejection. However, ethanol treatment causes the hydrophilic additives (PVP) in the membrane to be leached out, resulting in a decrease in membrane hydrophilicity and hence more membrane fouling, especially for protein solutions. For membrane pre-treated with NaOH, membrane permeability was also improved. The PVP additive in the membrane had undergone a hydrolytic reaction to generate more hydrophilic groups, which was verified by the decreased water contact angle and the increase in negative charge. The NaOH treated membrane had a high flux, while retaining a similar fouling resistance to the original membrane.

Although pre-treatment enhanced the permeability of membrane, it had no significant effect on the retention of the membrane. In order to further improve the selectivity of UF
membranes, we coated different polyelectrolyte layers on the membrane surface and used lysozyme in ultrafiltration experiments to study membrane performance. We found that positively charged lysozyme was adsorbed on membrane surface and pores when the surface was coated with negatively charged polyanions PDA or PAA, which led to sharp flux decline and membrane fouling. In contrast, when the membrane surface was coated with a positively charged polycation PDADMAC, positively charged surface/pores prevented contact with the same charged lysozyme. The modified membrane exhibited a higher flux to protein solution and showed better fouling resistance. As positive charge on the surface of the membrane increased, membrane fouling resistance was also improved. Furthermore, the rejection of lysozyme could be regulated by adjusting surface charge of the membrane.

Although polyelectrolyte coating improved the selectivity and fouling resistance of membrane, it severely damaged membrane permeability. In order to retain high permeability, we grafted the small molecules taurine, cysteine and PEI on PSf membrane surfaces. Binary solutions of BSA and bovine hemoglobin (Hb), which have similar molecular weights but different isoelectric points, were used for ultrafiltration experiments. We found that the permeability of the membrane was significantly enhanced after taurine or cysteine grafting. The PEI grafted membrane suffered severe fouling during the filtration process and exhibited undesired transmission rate and selectivity. However, there was pH-related protein transmission and selectivity for taurine and cysteine grafted membranes. Under acidic conditions, a lot of proteins were adsorbed on the membrane surface/pores due to charge attraction, and severe membrane fouling occurred that resulted in low transmission of protein. When pH was higher than the isoelectric point of Hb, membranes grafted with taurine and cysteine exhibited excellent fouling resistance and allowed more protein to pass through. Strong charge repulsion facilitates protein permeation through negatively charged PSf membranes. However, the highest selectivity was obtained at a pH slightly higher than the isoelectric point of Hb for cysteine grafted membrane. Furthermore, under low transmembrane pressure and suitable stirring rate, cysteine modified PSf membrane with a TA coating time of 6 h achieved better selectivity while maintain good permeability and fouling resistance.
Resumé

Ultrafiltrering (UF) anvendes i vid udstrækning inden for mad, medicin, vandbehandling, bio-separation og andre områder på grund af dens mange fordele såsom lavt energiforbrug, enkel betjening, milde forhold og miljøvenlighed. UF har imidlertid også store udfordringer når man ser fremad, såsom lav permeabilitet, svag selektivitet og alvorlig tilsmudsning af membranen. Disse mangler er hovedsageligt relateret til de kemiske egenskaber ved membranmaterialet og membranstrukturen. I denne afhandling er en polysulforon (PSf) membran med fremragende mekaniske og termiske egenskaber modificeret ved hjælp af tre metoder: Forbehandling af membranen med ethanol- eller NaOH-opløsninger, deponering af polyelektrolytter på membranoverfladen og binding af små ladede molekyler på membranoverfladen. Disse modifikationer forbedrede effektivt UF-membranens kemiske egenskaber og strukturelle morfologi, hvilket medførte en væsentlig forbedring i membranpermeabilitet, selektivitet samt reducerede tilsmudsningen af membranen.

Først studerede vi effekten af ethanol eller NaOH-forbehandling på membranens struktur og ydeevne. Ved hjælp af FTIR analyse blev det konstateret at konserveringsmidlerne i membranen blev fjernet effektivt efter forbehandling med varmt vand, ethanol eller NaOH, og membranstrømmen blev forbedret. Især øgedes vandstrømmen af membran behandlet med absolut ethanol næsten 5 gange. Polymermaterialer svulmer op i organiske oplosningsmidler, hvilket resulterer i forøget porestorrelse og porøsitet, hvilket blev bekræftet ved en stigning i membrantykkelse og et fald i dextranseparation. Behandling med ethanol bevirker imidlertid, at de hydrofile additiver (PVP) i membranen udvaskes, hvilket resulterer i et fald i membranhdrofilicitet og dermed en øget tilsmudsning af membranen, især med proteinopløsninger. For membraner forbehandlet med NaOH blev membranpermeabilitet også forbedret. Her konstateredes PVP-additivet i membranen at have gennemgået en hydrolyse, hvilket genererede flere hydrofile grupper på membranen, som blev verificeret af den tilsvarende nedsættelse i vandkontaktvinkel og stigningen i negativ ladning på overfladen. Den NaOH-behandlede membran havde en høj flux, mens den bibeholdt en sammenlignelig beskyttelse mod tilsmudsning i forhold til den oprindelige membran.

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List of Publications

The thesis was based on the publications as listed as below:

1. **Commercial polysulfone membranes pretreated with ethanol and NaOH: effects on permeability, selectivity and antifouling properties**
   
   **Mingbo Ji**, Jianquan Luo, Jiang Wei, John Woodley, Anders Egede Daugaard, Manuel Pinelo
   
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2. **Charge exclusion as a strategy to control retention of small proteins in polyelectrolyte-modified ultrafiltration membranes**
   
   **Mingbo Ji**, Xianhui Li, Maryam Omidvarkordshouli, Sigyn Björk Sigurdardóttir, John Woodley, Anders Egede Daugaard, Jianquan Luo, Manuel Pinelo
   
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3. **Grafting of charged small molecules on a commercial membrane surface to improve both permeability and selectivity**
   
   **Mingbo Ji**, Jianquan Luo, John Woodley, Anders Egede Daugaard, Manuel Pinelo
   
   **In preparation.**
Chapter 1-Introduction

1.1 Membrane technology

A membrane is a film with selective permeability, which can be used to perform the separation, purification and concentration of different components of the feed liquid [1]. Its separation ability is directly related to the chemical properties of the membrane material and the membrane structure [2]. Due to the wide variety of materials, separation ability and structure of existing membranes, it is difficult to classify membranes uniformly. Generally, there are several commonly used classification methods. Membranes can be divided into artificial membranes and natural membranes according to type of membrane materials. Membranes can also be divided into microfiltration membranes (MF), ultrafiltration membranes (UF), nanofiltration membranes (NF) and reverse osmosis membranes (RO) according to pore size differences or molecular weight cut-off (MWCO). Membranes may be divided into symmetric membrane and asymmetric membranes based on membrane structure. The pores in symmetric membranes have an almost uniform diameter of the pores throughout the depth of the membrane, which allows the entire membrane to act as a selective barrier for particles or molecules permeation. Asymmetric membranes are comprised of an ultra-thin (0.1–1 μm) skin layer with very small pores supported by a thick macro-porous support, so its pores change considerably with the depth of the membrane. Membranes may be divided into flat membranes, roll membranes, tubular membranes, and hollow fiber according to the membrane module [3]. Flat membranes are mostly used for laboratory research. Compared with traditional separation methods, such as distillation and extraction or centrifugal precipitation, membrane separation technology has become more and more popular due to its advantages of high efficiency, energy saving, environmental protection, and simple operation [4]. Consequently membrane technology is now widely used in biomedical separation, in petrochemical, gas separation and food processing, in sewage treatment, seawater desalination and in other fields [5–9].
1.2 Ultrafiltration (UF) membranes

1.2.1 Definition and mechanism

UF is a pressure-driven membrane separation technology mainly based on a size exclusion mechanism [10]. The separation principle is shown in Figure 1.1. Under a certain pressure, the feed solution flows across the surface of the UF membrane and larger molecules are rejected, while water/solvent and small molecular substances pass through the membrane to the other side; in this way the separation, concentration, or purification of substances with different molecular weights or particles of different sizes is achieved. Generally, a UF membrane has a pore size of 2-100 nm, a MWCO of 3000-500000, and an operating pressure of 0.1-0.5 MPa [11]. UF technology is widely used to remove proteins, bacteria, colloids and other macromolecular substances in a solution [12].

![Figure 1.1 Schematic of the ultrafiltration process (from Synder Filtration)](image)

1.2.2 Membrane material

Membranes can be divided into inorganic membranes and polymeric membranes according to the nature of the material. Although inorganic membranes have advantages of good heat resistance, high strength and large flux, they are limited by high preparation cost and limited raw materials [13]. In contrast, the preparation process of polymeric membranes is simple, and modification of polymer materials is relatively easy. Therefore, polymeric membranes are used to a greater extent nowadays. The polymeric membrane materials that have been
industrially produced include cellulose esters, polyarylether, polyvinylidene fluoride (PVDF), polyolefins and polyamides (PA). Some polymer materials are temporarily at the laboratory research stage, such as polybenzo imidazole, polyaniline, polyphenylene ether, etc [14]. Polyarylether materials, especially polyarylether sulfone polymers such as polysulfone (PSf) and polyethersulfone (PES), are now being produced industrially to make UF membranes [16,17] due to their excellent thermal stability, mechanical strength, chemical resistance and oxidation resistance etc. [15]. However, PSf membranes still face many problems that greatly limit membrane separation efficiency due to the constraints arising from the membrane preparation process (typically phase inversion method) and the chemical properties of the polymeric material itself (hydrophobic).

1.2.3 Advantages and applications

The advantages of UF technology are simple operation, low cost, and that no chemical reagents need to be added to perform the separation. In particular, the operation conditions of UF technology are mild, there is no phase change compared with evaporation and freeze-drying, and there are no temperature and pH changes. The denaturation, inactivation and autolysis of biological macromolecules can therefore be prevented. UF is mainly used for the desalination, dehydration, and concentration of biological macromolecules. We will introduce the application of UF technology in different fields below.

1.2.3.1 Dairy

In the dairy industry, UF is used for a wide range of applications. UF allows the smaller lactose, water, mineral, and vitamin molecules to pass through the membrane, while the larger protein and fat molecules (key components for making cheese) are retained and concentrated [16]. The following are some applications of UF in the dairy industry,

➢ Whey protein concentration/separation

Membranes with MWCO of 3 kDa, 5 kDa and 10 kDa are commonly used for the separation of whey protein concentrate and whey protein isolate from cheese whey, and such UF membranes can achieve a desired balance of flux, separation efficiency and membrane durability. In order to increase the removal efficiency of small non-protein species into the
permeate, a continuous UF system is usually established [17].

➢ Milk protein concentration/isolate
Concentrated milk proteins and isolates can be separated from skimmed milk using UF membranes (such as PES 30 kDa) [18].

1.2.3.2 Biotechnology and pharmaceutical technology
UF has the advantages of high throughput, mild operating conditions and easy scale up, and is therefore particularly suitable for the separation of biological compounds and biologically active macromolecules. The following will introduce some applications of UF in biotechnology.

➢ Protein concentration
UF can concentrate protein solutions and recover enzymes. Due to the mild operating conditions, membrane filtration is selected to concentrate the bio-compounds. The UF filtration has proven to be a cost-effective way to concentrate and purify proteins and enzymes [19]. Nowadays the use of UF for enzyme concentration has been accepted as a standard separation process that makes possible the concentration of enzymes and proteins while allowing salts and water to pass through the membrane [20].

➢ Pyrogen removal
It will induce a fever when pyrogen is injected into humans and animals. The molecular weight of the pyrogen is 10-120 kDa, and the maximum is 1,000 kDa [21]. Based on size exclusion and charge interactions, UF technology can produce pyrogen-free solutions for pharmaceutical industry [22].

1.2.3.3 Juice process
In the juice industry, UF is used to improve product yield and quality. UF removes proteins, suspended colloids, polyphenolic compounds, starch, pectin, and microorganisms from feed solution. Therefore produce high-quality juices [23].

1.2.4 Limitations
Although UF technology has achieved industrial production and application, with the cross-development of various disciplines, the use of UF still faces many problems. The first is poor permeability. Due to the constraints of preparation method, the prepared membrane has a thicker skin layer and low surface porosity, resulting in poor membrane permeability [24].
which seriously affects the separation efficiency of the membrane. The second is low selectivity. Because of the large pore size distribution of the prepared membrane, the separation factor for the separated compounds is low, which affects the separation efficiency of the membrane. It is been found that the maximum separation efficiency of UF can be achieved for compounds with a 10-fold difference in the size or molecule weight [25]. The third problem is that the membrane faces serious fouling. Membrane fouling will cause a significant degradation of membrane performance and affect service life. In industrial applications, membranes will need to be cleaned regularly, which greatly increases operating cost. Generally, by adjusting the conditions of the casting solution (e.g., the types of solvents and non-solvents, the temperature, and the solidification bath etc.), we can control the membrane structure and improve membrane permeability and selectivity. However, there is little scope to adjust the membrane fabrication process and it is difficult to optimize selection performance; high permeability membranes lack selectivity, and membranes with good selectivity have low permeability. In addition, changing only the process conditions without changing the chemical properties of the membrane itself cannot improve membrane anti-fouling ability [26]. It is commonly accepted that by changing the chemical properties of the membrane, the membrane can be significantly improved in terms of permeability, selectivity and fouling resistance. Development of new membrane materials and modification of existing materials is therefore fundamental for solving this problem.

1.3 Membrane features

There are three important indicators for evaluating the performance of membrane, namely permeability, selectivity, and fouling resistance. The permeability and selectivity of UF membranes are mainly determined by the pore structure of the membrane. The selectivity of UF membranes depends on the pore size and pore size distribution of the membrane surface. The permeability depends on the structure of the skin and the support layer. However, there is more and more evidence that the permeability and selectivity of the membrane are not controlled only by the pore structure but also by the chemical properties of the membrane [27]. The fouling resistance of the membrane mainly depends on the surface chemistry of the membrane. In addition to the above three main indicators, a UF membrane in actual use
will encounter a variety of liquids, and therefore the mechanical strength, dimensional stability, thermal stability, oxidation resistance, and acid and alkali resistance of the membrane must also be considered.

1.3.1 Permeability and selectivity

A larger surface pore size and higher volume porosity will lead to a better permeability and lower rejection rate. A narrow pore size distribution results in higher rejection rate under the same permeability, and a better permeability under the same rejection rate [28]. Hydrophilic membranes have low resistance for water, resulting in increased membrane permeability. At the same time, a membrane with good hydrophilicity will form a tight hydration layer on the surface of the membrane, and will thus prevent contact of hydrophobic foulants with membrane [29]. Furthermore, the charged membrane repels solutes with the same charge in the feed, resulting in increased selectivity [30].

1.3.2 Antifouling capacity

Membrane fouling indicates that the solute in the feed is adsorbed on the surface and the pores of the membrane due to mechanical or physical action, and a cake layer may even be formed on the membrane surface. The fouling reduces the effectiveness of pore size and greatly increases the permeation resistance of the membrane, and finally brings about irreversible changes in membrane permeability and selectivity. In industrial applications, frequent cleaning or replacement of membrane cores is necessary due to membrane fouling, which greatly increases the operation and production costs [31]. In addition, serious membrane fouling may even cause secondary water fouling. Therefore, the fouling resistance of UF membranes is a key factor that determines whether a membrane can be efficiently used for a long time. The fouling behavior of the membrane is mainly affected by its surface properties, including hydrophilicity/hydrophobicity, charge, roughness, and pore structure.

1.3.2.1 Pore size and porosity

Recent studies have shown that the pore size of the membrane surface also affects the degree of membrane fouling to a certain extent [32]. When the material liquid flows through the surface of the membrane under pressure, some substances smaller than the pore diameter of the membrane will easily enter the inside of the membrane pores and cause blockage and
narrowing of the pores. This kind of fouling depends on the degree of matching between the membrane surface pore size and the hydrodynamic radius of the separated substance in the feed liquid. A small pore size with a narrow distribution limits the solute entering the membrane pores and prevents fouling. Generally, the MWCO of the membrane should be an order of magnitude smaller than the size of the contaminants to be separated. Hong S. P [33] et al. found that decrease in rate of permeate flux increased with increase of membrane pore size. The porosity of the membrane affects the flux of the solution. From the Hagen-Poiseuille formula, combined with the Spiegler-Kedem equation, the spatial obstruction pore model and the TMS model, the solute and solvent are found to have a small permeation flux for membranes with great thickness, bending factor, and small porosity [34].

1.3.2.2 Hydrophilicity/hydrophobicity

The hydrophobic effect of foulants and membrane surface mainly comes from van der Waals forces. The Hamek constant Hamaker (H) is commonly used for representing the van der Waals force between substances [35], the formula is:

\[
H_{213} = \left[ \sqrt{H_{11}} - \frac{4}{\sqrt{H_{22} \times H_{33}}} \right]^2
\]

(1)

where \(H_{11}\), \(H_{22}\), and \(H_{33}\) represent the H constants of water, solute, and membrane, respectively. As the hydrophilicity of the solute and membrane increases, \(H_{22}\) and \(H_{33}\) increase while \(H_{213}\) decreases, which means that the van der Waals force of the foulant and membrane decreases, resulting in decreased membrane fouling. For hydrophilic membranes, the presence of hydrophilic functional groups on the membrane surface have a strong affinity for water, which makes it is easy for a stable hydration layer to be formed. It is a process of entropy decrease that the compound gets close to the membrane surface and replaces the hydration layer. This process cannot proceed spontaneously and requires a certain amount of energy. However, the force between the surface of the hydrophobic membrane and the water molecules is very small and there is a strong hydrophobic interaction with the hydrophobic substance. The compounds to be separated squeeze through the water molecule layer is a process of increasing entropy, which can proceed spontaneously. Therefore, the more hydrophobic the membrane surface, the more serious the membrane fouling.
1.3.2.3 Charge

When a membrane with ionizable groups is in contact with the compounds to be separated, the surface of the membrane will be charged due to ionization. At the same time, the substances to be separated in the compounds are also charged. When a substance with the same charge approaches the surface of the membrane, the charge on the membrane will repel the substance [36]. On the contrary, when a substance with a different charge approaches the membrane surface, it will be attracted to the membrane. Therefore, a membrane with the same charge (as the compounds to be separated) does not easily foul, while a membrane with a different charge will increase fouling of the membrane. Under the pH value of natural water, most of the foulants in the water are negatively charged, so a negatively charged UF membrane can help alleviate membrane fouling.

1.3.2.4 Roughness

A rougher membrane surface has many grooves and ridges. The compounds to be separated in the feed liquid will stay on the grooves and ridges when the feed liquid flows through the membrane surface [37]. Due to the blocking effect of this structure, the foulants (compounds to be separated) are difficult to remove by simple hydraulic cleaning, leading to severe fouling. The smoother membrane surface has no such blocking effect on foulants, so the fouling behavior is relatively light. Increasing the surface roughness of the membrane can increase the surface area of the membrane. Therefore, increasing the surface roughness of the membrane increases the water flux of the membrane. There is a certain linear relationship between the roughness and the water flux [38]. On the other hand, increase in the surface roughness of the membrane further increases the possibility of adsorbing foulants (proteins, sugars etc.) on the membrane surface. At the same time, the surface roughness of the membrane also affects the flow state and flow rate of the influent water on the membrane surface, which thus affects the deposition rate of foulants on the membrane surface and hinders formation of foulants on the membrane surface.

1.4 Protocols for membrane pretreatment

Membranes are commonly treated with chemicals or solvents (e.g. ethanol, methanol, acetone etc.) before use, either for removal of preservative or for cleaning [39][40]. After
use, for foulant removal or disinfection, other chemical treatments are also generally applied, such as NaOH, sodium hypochlorite (NaClO) or acids, e.g. HCl, HNO₃, H₂SO₄, H₂O₂ [41][42]. However, polymers are sensitive and undergo severe chemical changes during chemical or solvent exposure because polymer chains may not be stable in extreme environments.

Besides pore enlargement and polymer chain hydrolysis, other physical and chemical effects have been observed when membranes are in contact with other organic solvents. Indeed, swelling or re-dissolving of the membrane polymers in organic solvents have also been also previously observed [43]. Swelling of polymeric membranes is additionally affected by solvent concentration and contact time, which significantly alter the membrane pore structure and permeability. Rezzadori [44] et al. reported membrane surface free energy and polar components depletion after n-hexane permeation, which indicated that the membranes become more hydrophobic. Study of membrane physical and chemical structure by Scanning Electron Microscopy (SEM) and Fourier Transform Infrared (FTIR) analysis also showed slight changes due to clustering effects. With regard to this effect, Bruggen [45] et al. also found reorganization of the membrane structure due to the clustering of hydrophobic and hydrophilic zones within the active layer of the membranes. These changes were verified by SEM images of the membranes treated by different organic solvents (methylene chloride, acetone, hexane, ethyl acetate and ethanol). Lencki [46] et al. reported that if the membrane pore structure was naturally homogeneous, the polymeric structure would easily swell with miscible solvents such as ethanol and result in expansion of the pore network, increase in pore size, and decrease in permeability resistance.

1.5 Membrane modification protocol

Researchers have made great efforts in membrane modification and have proposed many feasible methods, including blending modification, bulk modification, and surface modification etc. There are also many membrane modifications schemes that are a combination of several modification methods. Through these modification methods, the performance of membranes has been significantly improved. The following introduces some of the commonly used modification methods for UF membranes.
1.5.1 Blending modification

Blending hydrophilic materials (e.g. hydrophilic polymers or inorganic nanoparticles) with polymer is one of the most convenient and universally accepted techniques to increase membrane hydrophilicity [47]. Yasin et al. [48] successfully incorporated thermo-exfoliated vermiculite (VMT) in a PES membrane via a phase inversion technique. The resulted membranes showed improved flux, reduced pore size distribution and improved solute rejection. The antifouling properties of the modified membranes were also improved due to increased hydrophilicity, reduced surface free energy, and reduced pore size. According to flow cytometry results, enrichment of the PES UF membrane matrix by 10 wt % thermo-exfoliated VMT particles lead to obvious bacterial damage of Bacillus subtilis 168 (by 98.10%) and Escherichia coli DH5 alpha (by 99.39%). The results of the static BSA adsorption test and the bacterial attachment test indicated that the membranes with macro roughness on their surface showed better antibiofouling resistance. Zhang et al. [49] reported the successful employment of a salt coagulation bath to enhance the morphological structures and properties of PVC/Pluronic F127 UF membranes. The resultant membrane without macrovoids exhibits a high surface porosity (7.2%) and surface hydrophilicity (water contact angle of 40°), which endows the membrane with a remarkable flux (1405 L/m2 h/bar) and antifouling performance. The biggest advantage of blending such methods is that it is simple and convenient, and the process is fast. However, the disadvantages are also obvious. Blending modification has poor durability, and the blending element is also prone to migration. The added anti-fouling properties tend to migrate to the inside of the membrane and fail to act on the surface [50].

Instead of modifying the membrane material to change the properties of the membrane itself, modifying the membrane surface may be a better approach. Surface modification changes only the surface properties of the UF membrane that are directly in contact with the external environment. Therefore surface modification can effectively improve the original properties of the surface or impart new properties to the surface, such as hydrophilicity, biocompatibility, antistatic properties, surface resistivity and dyeing properties etc. [51]. At the same time, surface modification can basically ensure that the physical and mechanical properties, chemical stability and thermal stability of the membrane body are not be affected.
1.5.2 Adsorbed coating

Surface coating is a simple and inexpensive method of surface modification of the membrane, which can be done easily in industrial operations and on a large scale.

1.5.2.1 Dip-coating

The membrane surface can be modified by coating with a functional layer. This layer is adsorbed on the surface of the membrane through a secondary reaction (hydrogen bonding, van der Waals force, hydrophobic force and electronic attraction etc.) without chemical coupling [53]. Coating the membrane surface usually reduces the permeability of pure water, but the flux of the modified membrane in relation to the protein solution will increase. For instance, Kim et al. [54] coated poly(vinyl) acetate (PVA) onto UF membranes by simple treatment methods (passive or convective adsorption), which produced a benefit in flux enhancement for BSA ultrafiltration. Average flux improvements of 20 to 40% relative to untreated membranes can be achieved.

Polyelectrolytes (PEs) are also widely used in membrane surface coating. Nyström et al. [55] observed that the severity of fouling was reduced during protein filtration when PEs were adsorbed on UF membranes. This reduction in fouling was attributed to the shielding effect provided by the charged PEs against charged proteins. Fouling was further reduced when the ionic strength of the feed was increased, which presumably increased the charge density on the modified membrane surface and on the protein. Although fouling alleviation might only be expected when the membrane surface bears the same charge as the foulant, Mahlicli et al. [56] found a significant improvement in fouling tendency when a negatively charged membrane was challenged with a feed containing positively charged proteins. The hydrophilicity of the membrane (which is heightened when it is either positively or negatively charged) may play a greater role in fouling resistance than the sign of its charge.

Polyelectrolytes have been employed in many other studies of layer-by-layer (LBL) deposition of surface coatings. A variety of substrates have been modified by the application of alternating layers of oppositely charged polyelectrolytes. The layers are held together by the electrostatic interactions between the layers, and the rigidity of the assembly increases as more layers are added [57]. LBL surface modifications offer good adhesion because of the
large number of charged groups on each molecule while also being relatively insensitive to small surface imperfections [58]. Zhu et al. [36] deposited poly (acrylic acid) (PAA) and poly (diallyl dimethylammonium chloride) (PDADMAC) on membrane surfaces using LBL methods. Surface charge tuning is achieved by controlling the degree of ionization of the weak polyelectrolytes at various pH values in the penultimate layer and subsequent manipulation of the amount of polyelectrolyte deposited in the last layers. The procedures proposed allow surface isoelectric points of LbL architectures to be adjusted to achieve optimal antifouling performance of a given material while taking into account specific pH values of the environment and the character of the fouler.

1.5.2.2 Vacuum assisted coating

Vacuum assisted filtration is a new method for preparing separation membranes that has been developed in recent years. The general idea is to put the base membrane to be modified on a vacuum filtration device, and then pour the solution or suspension of the modifying agent on the surface of the base membrane. When most of the solution has been filtered, a small amount of solution is left behind on the surface of the membrane and solidifies into the membrane. The advantage of this method is that the prepared separation layer is very thin, and the pore size is relatively uniform, so that which high permeability and high selectivity can simultaneously be achieved. For example, Zhang et al. [59] coated graphene oxide (GO) nanosheets onto a PES membrane using a vacuum filtration strategy. The static protein adsorption results revealed that the modified membrane had much lower adsorption propensity than the pristine membrane. Foulants (BSA) were tested using dynamic filtration experiments. The GO coated membrane had much higher FRR than the pristine PES membrane for all three foulants. In addition, contact killing and antibiofouling experiments revealed the antibacterial property of the modified membrane.

1.5.2.3 Bio-inspired coating

Recently, mussels and other sessile marine organisms inspired coating has aroused the interest of researchers. These species can anchor themselves firmly to almost any underwater surface. Messersmith and coworkers [60] reported the coating of dopamine onto a variety of substrates, including ceramics, metals, and synthetic polymers, from a mild aqueous dopamine solution. The polydopamine (PDA) layer was super hydrophilic. In addition, PDA
layers have been demonstrated to react with amino- and thiol-containing compounds such as biomacromolecules, commercially available small molecules and polymers, and amino-containing synthetic polymers [61]. Davenport et al. [62] grafted zwitterionic polymer brushes on UF membranes and pore surfaces via dopamine initiated ATRP. Fouling on the membrane surface can be prevented by grafting thick zwitterionic polymer brushes (greater than 100 nm). Li et al. [63] immobilized PEG-NH₂ via dopamine coating. The modified membranes had less flux reduction in filtration and lower adsorptive amount of BSA in isothermal adsorption tests. The PD-g-PEG modification improves the stability of the PES membrane and the absorbability for BSA more significantly. Zhou et al. [64] hydrophilized the membrane surface via simple deposition of PSBMA and dopamine. Significant improvement was demonstrated for surface hydrophilicity from the results of water contact angle and pure water flux. Dynamic protein filtration experiments confirmed the excellent antifouling property of the resulting membranes. Furthermore, the utilization efficiency reached 9.13 wt% for PSBMA, 10 times higher than that of SBMA for UV-induced grafting.

Tannic acid (TA) is another kind of plant polyphenol that can be directly extracted from lots of plants, including tea, wood and galls. The cost of such TA is then much lower than that of dopamine [65]. Similar to the dopamine coating, TA layers also can be formed spontaneously in a weak alkaline buffer aqueous solution at room temperature [66]. Because there the presence of large numbers of hydrophilic units (-OH), the TA layer will make the membrane surface more hydrophilic. Chen et al. [67] constructed an antifouling surface based on use of tannic acid and zwitterionic polymers. The prepared surface could effectively resist protein adsorption, bacterial attachment, and platelets adhesion.

1.5.3 Chemical treatment
To improve coating stability, surface-reactive agents may be covalently coupled to the membrane polymer. These agents may be either small molecules or, more commonly, polymers. There are many pathways through which covalent coupling to the membrane surface may be achieved. Sulfonation is a popular way of introducing hydrophilic groups on hydrophobic polymers, such as poly(aryl sulfone)s (e.g. PSf and PES) that are often used in water purification membranes [68]. A common method to introduce aryl sulfonic acid groups
onto poly(aryl sulfone)s is through contact with chlorosulfonic acid which, after neutralization, yields highly hydrophilic sulfonate groups directly connected to the aromatic rings of the polymer backbone [69]. Garry et al. [70] prepared negatively charged PVDF microfiltration membranes using direct sulfonation with chlorosulfonic acid. The pure water flux of the treated membranes increased significantly and fouling of modified membrane decreased while rejection values increased with increasing degree of sulfonation, mainly as a result of effective electrostatic repulsion between negatively charged compounds and negatively charged membrane. Amination is another approach for improving membrane performance. For instance, Wu et al. [71] soaked commercial PVC hollow fiber UF membranes in trimethylamine (TMA) solution to prepare aminated UF membranes. By the modification, the membranes changed gradually from negatively charged to positively charged, the hydrophilicity of PVC membrane was enhanced, and the membrane pore shrunk, which resulted in simultaneous the improvement of permeability and rejection. Moreover, antibacterial research testified that the PVC membrane was endowed with antibacterial properties after modified of quaternization.

1.5.4 Surface Grafting

1.5.4.1 Plasma-initiated grafting

Plasma-initiated grafting is a method that has developed rapidly in recent years. Plasma is usually a substance in an ionized gas state composed of atoms deprived of electrons and of electrons generated after atoms are ionized. These high-energy particles can generate free radicals, such as active groups of carbonyl and hydroxyl, or form a cross-linked layer structure after contacting the surface of the hydrophobic membrane material. The free radicals generated on the surface can directly initiate the monomer graft polymerization reaction or can generate peroxide groups after being oxidized by oxygen to initiate the subsequent graft polymerization reaction [72]. This method has the advantages of simple operation, short processing time, less damage to the surface of the material, processing only the surface in the range of tens to hundreds of nanometers, and a processing process that is non-toxic, pollution-free, and radiation-free. The disadvantages are that the method requires relatively expensive and complicated equipment, and it is difficult to effectively control the grafting rate and the grafting rate is low, which is not suitable for large-scale industrial
production. The main gases suitable for plasma are oxygen, argon, nitrogen, hydrogen and ammonia [73]. Moghimifar et al. [74] modified the PES membrane by corona air plasma and coating TiO$_2$ nanoparticles. The experimental results showed that the corona treatment and coating TiO$_2$ nanoparticles led to a significant enhancement of the surface hydrophilicity and improvement of the antifouling properties and permeation fluxes for all modified membranes without significant changes in membrane cross sectional morphology. The modified membranes had a lower fouling tendency and long-term flux stability in comparison to the untreated PES membranes. Wang et al. [75] immobilized PEG on a PVDF membrane surface using argon plasma-induced grafting. Protein adsorption experiments revealed that the PEG-g-PVDF membranes with a PEG graft concentration defined as the [CO]/[CF2] ratio above 3.2, exhibited good anti-fouling property.

1.5.4.2 UV-initiated grafting

The most widely used method is UV-initiated surface graft modification. This method generates free radicals on the surface of the membrane by direct ultraviolet light to initiate monomer graft polymerization [76]. The advantages of this method are fast reaction rate, high initiation efficiency, simple and convenient processing method, and low equipment cost. Ultraviolet rays are electromagnetic waves with a wavelength of 40 ~ 400 nm. They have high energy but poor penetration. This is an advantage because the grafting reaction is strictly limited to the surface or subsurface of the membrane and does not cause damage to the membrane itself. Quartz is usually used as a filter and 280 nm wavelength ultraviolet light is selectively used to initiate the graft polymerization reaction [77]. Yu et al. [78] grafted zwitterionic molecules on a PSf membrane surface using UV-initiated polymerization. The results showed that static contact angle decreased from 75° to 50.5° with increase in grafting degree, implying an improvement of membrane surface hydrophilicity. The ultrafiltration experiment results indicated that the value of $R_t$ significantly decreased after UV-initiated grafting polymerization. The modified membrane with a grafting degree of 374 $\mu$g/cm$^2$ showed the best performance in ultrafiltration process, achieving a 30% reduction in the value of $R_t$, and a 44% decrease of $R_t$. Dai et al. [79] grafted cyclic sugars on to PAN membrane by UV-initiated grafting polymerization. The results revealed that the hydrophilicity was enhanced and the adsorption of BSA was inhibited significantly. The flux
recovery ratio was also increased after modification, indicating that the anti-fouling performance of PAN membrane was improved.

### 1.5.4.3 Irradiation-induced grafting

In surface grafting initiated by high-energy radiation, free radicals are generated on the surface of the membrane under the action of high-energy rays. These free radicals can polymerize with vinyl monomers to achieve the purpose of grafting on the membrane surface [80]. This method is also one of the effective approaches of membrane modification. The radiation sources used in radiation grafting technology mainly include natural radiation sources and artificial radiation sources (such as cobalt-60, cesium-137, strontium-90 and other gamma rays) and different types of accelerators (such as X-ray tubes, linear accelerator, cyclotron and other high-energy equipment) are required [81]. The high-energy radiation-induced grafting method has the advantages of simple operation and a controllable grafting rate and is thus more suitable for industrial production. But there are also unsatisfactory situations, such as complicated equipment maintenance and high radiation intensity, which lead to serious degradation of the membrane material itself while the grafting rate will also be affected by the morphology of the membrane surface [82]. Wei et al. [83] reported a modified poly (vinylidene fluoride) (PVDF) hollow fiber membrane prepared by γ-radiation-induced grafting of acrylic acid (AA). When the grafting degree was 4.4%, the maximum pure water flux reached 1496.3 L/m² × h which was 1.79 times that of the original membrane. The pure water flux, flux recovery rate and rejection ratio for bovine serum albumin could also be improved simultaneously at a low grafting degree (<4.4%).

### 1.5.4.4 Ozone initiated grafting

When the polymeric membrane is in an ozone atmosphere, the surface will be oxidized by ozone to form hydroperoxide, carbonyl and carboxyl groups. These hydroperoxides are extremely unstable, easily decompose into surface free radicals, and can smoothly initiate the graft polymerization reaction of double bond-containing monomers such as vinyl or acrylate monomers on the membrane surface [84]. The mechanism of ozonation is currently relatively mature, and the entire grafting process is roughly divided into three steps. First, ozone molecules decompose into active oxygen free radicals which then make contact with the polymer material on the surface of the membrane to capture their hydrogen atoms. The
free radicals are transferred to the surface and further oxidized to hydroperoxyl radicals. Finally, reactive ethylene graft polymerization occurs on the membrane surface under the action of peroxide [85]. In general, if the time of ozonation treatment increases, more peroxide groups will be produced on the surface, which ensures sufficient grafting efficiency. However, if the ozonation treatment time is too long, the body of the membrane material will be degraded to a certain extent. Therefore it is especially important to find the best treatment time for the specific experimental process. Compared with the plasma method, the ozonation method has the advantage that it can introduce peroxide groups more uniformly on the surface of the polymeric membrane, the experimental procedure is simple, the equipment is cheaper than the plasma equipment, and the general applicability is very good as it is not limited by the membrane material and the complex morphology of the membrane surface. However, this method also has obvious shortcomings. The ozone generated in the experiment will pollute the environment and cause a certain amount of degradation of the processed membrane materials [86].

Chaing et al. [87] grafted the zwitterionic sulfobetaine methacrylate (SBMA) on the surface of PVDF membrane via ozone surface activation and surface-initiated atom transfer radical polymerization (ATRP). Hardly any albumin adsorption was observed, as the grafting density exceeded 0.4 mg/cm² of polySBMA. The adsorption of γ-globulin was also greatly reduced. The cyclic filtration test for BSA yielded an extremely low irreversible membrane fouling ratio (Rir) of 13% in the first cycle, and apparently no irreversible fouling was observed in the second cycle. A more stringent test was carried out by passing γ-globulin solution. The virgin PVDF membrane was found to be continuously fouled by γ-globulin after three cyclic operations, while the polySBMA-modified membrane had the Rir value as low as 4.7% in the third cycle.
Chapter 2- Purpose, Hypotheses, and Objectives

2.1 Purpose and hypothesis

2.1.1 Commercial membrane pre-treatment for improved permeability

Traditional commercial polymeric UF membranes face some shortcomings, such as poor permeability and low selectivity, and can easily be fouled. To solve these problems, various membrane modification methods have been studied to improve membrane performance. Membrane pretreatment is a simple and cheap method which is beneficial for enhancing membrane performance. Polymeric UF membranes are generally prepared by the phase inversion method. The polymer powder and additives are first fully dissolved in solvent (N-Methyl pyrrolidone, Dimethylacetamide etc.). Next, the casting solution is spread on the surface of the plate with a spatula. After a few seconds, the plate is immersed in the non-solvent (such as water). The solvent dissolves in the water from the polymer network and forms membrane pores. After the solvent is completely dissolved, the polymeric membrane is prepared. Generally, the polymers for membrane preparation are soluble or slightly soluble in organic solvents. Therefore the polymer chain will swell in an organic solvent environment and thus increase the pore size. In other cases, if some chain segments in the polymer are compatible with organic solvents, chain rearrangement may occur. After the organic solvent pretreatment, some segments may be re-distributed on the surface of the membrane or pore walls. We used different concentrations of ethanol aqueous solution to pretreat commercial PSf membrane, and the membrane pores may change significantly. NaOH solution is also used for the membrane pretreatment. Alkali may damage the polymer structure. Alkali-catalyzed lactam ring hydrolysis of PVP occurs to form a charged hydrophilic group (-COO-), which enhances the hydrophilicity of the membrane and improves the permeability and fouling resistance. In addition, both organic solvent and NaOH solution can leach out the preservatives or additives inside the membrane, thus increasing the effective pores of the membrane. The general study hypotheses were the following:

- The solvent/chemical pre-treatment affects the membrane structure by enlarging the pore size or damaging the polymer chain
Commercial membranes pre-treated with suitable solvent/chemical have enhanced separation performance compared to virgin membrane, such as flux, selectivity, and fouling resistance.

2.1.2 Selective polyelectrolytes coating to enhance membrane selectivity

Membrane surface modification is an important method for enhancing membrane performance. It is generally accepted that a hydrophilic surface can improve membrane permeability and inhibit membrane fouling because the hydration layer formed on the surface prevents non-specific contact/adsorption of foulants (protein) on the membrane. However, single hydrophilic modification has little effect on the selectivity of the membrane, and the improvement of permeability may even reduce the selectivity. Non-charged hydrophilic functional groups (such as hydroxyl) inhibit membrane fouling so more substances can penetrate the membrane, resulting in low rejection. Therefore, another modification must be applied to enhance membrane selectivity. The introduction of charges on the membrane surface is a common method for enhancing the selectivity of UF membranes. The selective permeation of compounds can be achieved by controlling the electrostatic interaction between the membrane surface and the compounds by changing the pH of feed solution. In theory, electrostatic attraction between the compounds and the membrane can promote the penetration of the compounds to be separated, while electrostatic repulsion can enhance the rejection of the compounds.

Polyelectrolytes (PEs) deposition is a simple method of constructing charged membrane surfaces. Commercial PSf membranes usually have a weak negative charge in the range of pH 3.0 – 11.0. Through charge attraction, a PEs layer can be deposited on the membrane to give a strongly charged surface. Layer-by-layer PEs self-assembly has been widely used in membrane surface modification. However, the deposition of multiple layers of PEs will seriously block the membrane pores and turn the UF membrane into a NF membrane. To reduce the impact of PEs deposition on membrane pores, we chose to deposit only a single layer or double layer of PEs on the surface of a commercial PSf UF membrane. Hydrophilic polyanionic PAA and polycationic PDADMAC were used for membrane surface modification. To further improve the hydrophilicity and charge of the surface, dopamine was also used for surface coating. By selectively coating different types of PEs, the membrane
pore size and charge can be changed. On the other hand, ionic strength affects PEs deposition and will significantly alter membrane pore size. In the low ionic strength range, the charges of the PEs experience low screening from salt ions of the deposition solution, and the oppositely charged PEs deposited on the membrane dominate the charge balance, leading to a dense PEs layer with enhanced resistance to water and solute transport. As the ionic strength is increased beyond a certain value, salt counterions from the deposition solution dominates the charge balance. As a consequence, the PEs layer becomes thicker but less dense, which limits PEs adsorption and results in larger pores. The effect of ionic strength on PDADMAC deposition was investigated by varying the background ionic strength during deposition of PDADMAC. The general study hypotheses were the following,

- The deposition of selective PEs on commercial PSf membrane surface can significantly improve membrane selectivity
- PEs layer structure can be controlled by adjusting the ionic strength in PEs deposition process

2.1.3 Grafting of small size charged molecules to improve both the permeability and selectivity

Although PEs deposition can construct a charged surface, the presence of a macromolecular PEs seriously blocks the membrane pores: the compounds retention is increased while the permeability of the membrane is severely damaged. On the other hand, the presence of positive and negative PEs will adsorb a large amount of protein, causing a sudden decrease in membrane flux and irreversible fouling. General proteins such as BSA carry negative charges in a neutral aqueous environment and are easier to adsorb on the positively charged membranes. Therefore constructing a negatively charged surface is more promising for improving both the selectivity and fouling resistance of the membrane. Since the presence of macromolecular PEs may reduce the permeability of the membrane, we can consider grafting small molecules with charged groups such as cysteine and taurine on the membrane surface.

The charged groups are usually hydrophilic, so the presence of these molecules can effectively prevent and reduce contact between foulants and the membrane surface. On the
other hand, electrostatic repulsion between the same charges can also inhibit the contact of foulants with the membrane surface and further reduce membrane fouling. However, the surface of the PSf membrane is inert, and there are no active sites that can be used to initiate the grafting. Therefore, to graft these charged small molecules, we must introduce active groups onto the membrane surface. Tannic acid is a hydrophilic molecule containing many hydroxyl groups, which can be oxidized to form catechol groups under weakly alkaline conditions and adhere to the surface of various materials. The generated catechol groups can further undergo Michael addition or Schiff base reactions with molecules containing -NH₂ and -SH groups, and can be used for further grafting or immobilization of functional molecules.

We selected tannic acid coating to assist the grafting of hydrophilic charged small molecules of cysteine, taurine, and PEI600 onto PSf membrane surface. The presence of these small molecules does not affect membrane pores but enhances membrane hydrophilicity, which improves permeability of the membrane. The presence of the hydration layer combined with the electrostatic repulsion will further inhibit the protein adsorption, thus improving membrane fouling resistance. In addition, electrostatic interactions can also enhance membrane selectivity and in favor of protein separation. The general study hypotheses were the following,

- **Grafting of charged small molecules can simultaneously improve membrane permeability, selectivity, and fouling resistance**
- **Protein has a higher transmittance at the isoelectric point, so maximum membrane selectivity can be obtained at this point**

### 2.2 Research objectives

Specific objectives linked to each of the chapters are as follows,

**Chapter 4 Commercial polysulfone membranes pre-treated with ethanol and NaOH:**

**Effects on permeability, selectivity, and antifouling properties**

- Study the membrane permeability and selectivity performance after ethanol and NaOH pre-treatment using protein and dextran as characterization agents in ultrafiltration
- Investigate fouling resistance and stability of ethanol/NaOH pre-treated commercial
membranes

- Elucidate the mechanism of pre-treatment methods on polymer and membrane structure in relation to change of membrane pore size or degradation of polymer chains

Scheme 2.1 Graphic summary of the influence of three pre-treatment methods on membrane performance

Chapter 5 Charge exclusion as a strategy for controlling retention of small proteins in polyelectrolyte-modified ultrafiltration membranes

- Study permeability and selectivity performance of commercial membranes modified by selective PEs deposition
- Explore the effect of charge interaction on protein permeation using lysozyme and dextran as characterization agents
- Investigate the fouling behaviour of PEs deposited membranes and clarify the fouling resistance mechanism
Scheme 2.2 Workflow of positively charged membrane fabrication using PAA/PDA co-deposition and subsequent PDADMAC deposition

Chapter 6 Grafting of charged small molecules on a commercial membrane surface to improve both permeability and selectivity

- Study permeability, selectivity and fouling resistance of the modified membranes and compare the difference between these membranes
- Explore the effect of charge interaction on membrane separation and fouling performance and verify that charge transmittance is highest at the isoelectric point
- Optimise the modification protocol and define the optimal operational conditions for attaining high selectivity and fouling resistance for a BSA/Hb binary solution
Scheme 2.3 Mechanism of charged membrane surface construction via tannic acid coating and subsequent charged molecules immobilization
Overview of the Different Progressive Research Steps Carried Out During the Thesis

1. Paper 1
Commercial membrane pre-treated with ethanol/NaOH solution for improved permeability and selectivity

2. Paper 2
Charge exclusion as a strategy to control retention of small proteins in polyelectrolyte-modified ultrafiltration membranes

3. Paper 3
Grafting of charged small molecules on the commercial membrane surface to improve both permeability and selectivity

I. Improved permeability by ethanol or NaOH pre-treatment

II. Selective polyelectrolytes layer coating via charge attraction

III. Charged small molecules grafting via tannic acid coating
Chapter 3-Materials and method

3.1 Reagent

Biomolecules of BSA (66.7 kDa, pI 4.8), pepsin (34.5 kDa, pI 2-3) and lysozyme (14.3 kDa, pI 10.7) hemoglobin (64 kDa, pI 6.8) were purchased from Sigma-Aldrich. Dopamine, tannic acid, poly (acrylic acid) (PAA), PDADMAC, PEI, cysteine and taurine were also obtained from Sigma-Aldrich. Ethanol absolute was obtained from VWR CHEMICALS (France). All reagents are analytical grade with no further purification.

3.2 Liquid

Deionized (DI) water was used for all solutions in the experiment if not otherwise indicated. Milli-Q water (with resistivity of 18.2 MΩ cm at 25°C) was used as ultrapure water for preparing protein solutions.

3.2.1 Phosphate buffer solution (PBS)

10 mM PBS (pH 7.4) preparation. 8.0 g NaCl, 0.2 g KCl, 1.44 g Na$_2$HPO$_4$ and 0.24 g KH$_2$PO$_4$ were dissolved in 800 mL Milli-Q water. The solution was adjusted to pH 7.4 and then made up to 1000 mL.

100 mM PBS (pH 7.4) preparation. 80 g NaCl, 2.0 g KCl, 11.49 g Na$_2$HPO$_4$ and 2.59 g KH$_2$PO$_4$ were dissolved in 900 mL Milli-Q water. The solution was adjusted to pH 7.4 and then made up to 1000 mL.

3.2.2 Acetic acid/sodium acetate (Ac/NaAc) buffer solution

10 mM Ac/NaAc (pH 4.7) solution preparation. 0.3339 g sodium acetate was dissolved in 900 mL Milli-Q water. Then 0.304 mL acetic acid was added to the NaAc solution and the pH was adjusted to 4.7. The solution was made up to 1000 mL.

3.2.3 Tris-HCl buffer solution

50 mM Tris-HCl (pH 8.5) buffer solution preparation. 6.057 g Tris(hydroxymethyl)aminomethane (Tris) was dissolved in 900 mL Milli-Q water and the pH was adjusted to 8.5 using 1 M HCl solution. The solution was made up to 1000 mL.

3.2.4 Sodium carbonate/sodium hydroxide buffer solution

100 mM Na$_2$CO$_3$/NaOH (pH 11.0) buffer solution preparation. 10.6 g Na$_2$CO$_3$ powder was dissolved in 900 mL Milli-Q water and the pH was adjusted to 11.0 using 1 M NaOH solution.
The solution was made up to 1000 mL.

3.2.5 Citric acid/sodium citrate buffer solution

10 mM citric acid/sodium citrate buffer solution preparation. 0.2 M Na$_2$HPO$_4$ and 0.1 M citric acid solution were prepared in stock.

Table 3.1 Citric acid/sodium citrate buffer solution preparation using stock solution

<table>
<thead>
<tr>
<th>pH</th>
<th>0.2 M Na$_2$HPO$_4$ (mL)</th>
<th>0.1 Citric acid (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0</td>
<td>7.71</td>
<td>12.29</td>
</tr>
<tr>
<td>4.8</td>
<td>9.86</td>
<td>10.14</td>
</tr>
<tr>
<td>6.8</td>
<td>15.45</td>
<td>4.55</td>
</tr>
<tr>
<td>7.5</td>
<td>18.17</td>
<td>1.83</td>
</tr>
</tbody>
</table>

The buffer was prepared with the stock solutions and diluted to the target concentration.

3.3 Membrane

A polysulfone membrane with MWCO of 100 kDa was purchased from Alfa Laval (Sweden). Another PSf membrane (100 kDa) was obtained from Ande membrane separation technology engineering (Beijing, China) Co. Ltd. A polyacrylonitrile (PAN) membrane (100 kDa) was purchased from Sterlitech Corporation (Kent, USA). A hydrophilic modified PSf membrane (100 kDa) was obtained from Lenntech Water Treatment Solutions (Delfgauw, Europe).

3.4 Experimental device

The main properties of UF membranes include permeability, rejection and fouling resistance. The ultrafiltration experiments of all solutions were performed in a dead-end filtration device (Figure 3.1) and the effective area of the test membrane was 11.34 cm$^2$. The main steps were as follows: (1) The membrane was fixed in the cell and the pressure was adjusted to 2 bar to pre-press the membrane for 30 minutes. Then the pressure was adjusted to 1.0 bar for pure water permeability measurement. The entire experimental procedure was performed at room temperature. The pure water permeability, rejection and fouling resistance were calculated by the following formulas,

$$ J = \frac{V}{A \times t} $$

(1)
$J$ represents the pure water permeability, $V$ represents the volume of pure water that passes through, $A$ represents the effective area of the membrane, and $t$ represents the filtration time. (2) Next, the deionized water was replaced with the protein solution to be separated and the ultrafiltration experiment was performed at 1.0 bar. The filtration time of the permeate was recorded and the UV absorption of permeate was measured.

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

(2) $R$ represents the rejection rate, $c_p$ and $c_f$ represent the protein concentration in the permeate and feed solution, respectively. (3) The fouled membrane was washed with deionized water several times, and the pure water permeability was measured again. The flux recovery rate (FRR) was used to characterize the fouling resistance of the membrane. $FRR$ indicates the degree of membrane permeability recovery after fouling and cleaning.

$$FRR(\%) = (\frac{J_{w,2}}{J_{w,1}}) \times 100$$

(3) $J_{w, 1}$ and $J_{w, 2}$ are the initial water permeability of the membrane and the water permeability of the membrane after the fouling-cleaning process, respectively.

Figure 3.1 Schematic diagram of the ultrafiltration experiment setup
3.5 Analytical methods

3.5.1 Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR)

Membrane samples were cleaned and dried. ATR-FTIR was performed at 2 cm\(^2\) resolution with a Thermo Nicolet IR 200 spectroscope (Thermo Nicolet Corporation, USA) in the range of 400 – 4000 cm\(^{-1}\). Typically, 64 scans were signal averaged to reduce spectral noise. At least three replicate spectra were obtained for every membrane type. These spectra were subsequently corrected for the wavelength-dependent penetration depth and background subtraction using OMNIC™ software.

3.5.2 Scanning Electron Microscope (SEM)

Field emission scanning electron microscopy (FE-SEM, Hitachi S-4800, Japan) with an accelerating voltage of 20.0 kV was used to study the morphology of the membrane. Before introduction to the electron beam, the membrane sample was coated with a thin layer of gold by gold sputtering. SEM images were taken at a magnification of 5000 and 50,000 at 20 kV. At least six replicate SEM images were obtained for each membrane type.

3.5.3 Water contact angle

Water contact angle (WCA) is a direct characteristic for evaluating the hydrophilicity of membranes. The water contact angle (WCA) of the membranes was measured using a contact angle goniometer (OCA20, Dataphysics Instrumente, and Germany) in the room environment. 1 µL of MiliQ water was carefully dropped on top surface of the membrane and the contact angle between the water and membrane was recorded on video for 120 s. To minimize experimental error, the contact angle was measured for at least six random locations for each sample and the average was reported.

3.5.4 Zeta potential

Membrane surface charge was characterized using SurPASS™ 3 electrokinetic analyzer (Anton Parr, Austria). Before measurement, the membranes were equilibrated with 1 mM KCl for 2 h. Then the zeta potential measurement was performed using 1 mM KCl as electrolyte solution. The pH of the solution was adjusted by an automatic titrator from pH
3.0 to pH 11.0. For each pH value, four data records were taken and the average was reported.

### 3.5.5 Ultraviolet spectrophotometer

An UV-1280 visible spectrophotometer (Shimadzu Company, Japan) was used to conduct quantitative analysis of proteins in the feed solution and permeate solution under UV light (280 nm). Ultrapure water was used as the blank control sample. In addition, hemoglobin concentration could be measured at a wavelength at 406 nm.
Chapter 4-Commercial polysulfone membranes pre-treated with ethanol and NaOH: Effects on permeability, selectivity, and antifouling properties

In order to maintain the stability of the membrane structure, the storage of commercial polymeric membranes usually requires the addition of some preservatives, such as glycerin, sodium dodecyl sulfate (SDS) etc. Therefore, before using membranes, organic solvents such as ethanol and propanol are used to pre-treat/clean membranes. This pretreatment has a significant impact on membrane performance. Commercial polymeric UF membranes are usually prepared by the phase conversion method which uses organic solvents to dissolve polymers. Polymeric membranes can generally therefore not withstand long-term organic solvent soaking because small molecules of organic solvent penetrate the membrane and lead to membrane swelling which has an impact on the micro and macro structure of the membrane. In the case of swelling, the membrane thickness and pore size change, and the additives in the membrane may be washed out. In addition to organic solvent pre-treatment, NaOH solution is also commonly used for membrane pre-cleaning. However, the alkaline solution may destroy the structure of the polymer, especially certain groups such as ester groups, and finally generate hydrophilic charged groups that will affect membrane performance.

We used ethanol and NaOH solutions to pre-treat membranes and studied the effects of pre-treatment methods on the polymer, membrane structure, and membrane performance. We clarified and experimentally verified the mechanism of change in membrane performance.

4.1 Hypotheses

- Organic solvent and NaOH pre-treatment have different effects on membrane performance, due to the effect of solvent and alkaline on polymer structure
- Pre-treatment can improve the permeability and fouling resistance of the membrane

4.2 Experimental considerations

Permeability, selectivity, and fouling resistance are the main indicators for evaluating
membrane performance. In our study, we employed BSA, pepsin and dextran ultrafiltration experiments to investigate the performance of the original membrane and the pre-treated membranes. Three pretreatment methods were used, namely hot water treatment, ethanol treatment, and NaOH solution treatment. Next, the performance of the membranes and their stability after treatment were tested. To clarify the effect of pre-treatment on membrane structure and chemical properties, FTIR spectra, water contact angle and zeta potential tests were used to characterize the membrane. Infrared spectroscopy can directly show functional group changes in the membrane surface, while the water contact angle reveals the changes in membrane hydrophilicity/hydrophobicity. Changes in potential energy can also characterize the changes of groups (i.e. -OH, -COOH) on the membrane surface. The original membrane is relatively hydrophobic and carries only a small amount of negative charge, in the range of pH 3.0 - 11.0. After pre-treatment, the membrane structure and chemical properties changed, resulting in different hydrophilicity/hydrophobicity and zeta potential compared to the original membrane. In particular, changes in membrane structure, i.e., changes in membrane pores, can be characterized by solute (dextran) retention. Lastly, we investigated the stability of the membrane after pre-treatment, i.e., the flux of the storage membrane in deionized water over time.

4.3 Highlights

First, the three pre-treatment methods could effectively remove preservatives (such as glycerin) and have a certain effect on the additives in the membrane. Ethanol and NaOH solution pre-treatment had different effects on membrane performance. In terms of permeability, the pure water flux test shows that ethanol pre-treatment can significantly improve the permeability of the membrane. For example, pure ethanol pre-treatment for 2 h increased the PWF of the membrane 3.5-fold (as shown in Fig. 4.1), and the flux of the pre-treated membrane for BSA, pepsin and dextran solutions were also greatly improved. Through the rejection test of different molecular weight dextrans, the membrane pre-treated with ethanol was found to allow more molecules to pass through (as shown in Table 4.1), which indicates that the membrane pores may become larger after treatment. According to our hypothesis, the larger membrane pore size may be due to membrane swelling. On the
other hand, ethanol pre-treatment washes out the preservatives and additives, thus promoting membrane permeability. However, the washing out of hydrophilic additives will reduce the hydrophilicity of the membrane, which will cause serious membrane fouling. As shown in the Table 4.2, we see that the water contact angle of the ethanol pre-treated membrane increased, indicating that the membrane has become more hydrophobic. In Fig.4.2 b, the recovery of membrane flux after protein filtration is also shown to be low.

For the membrane pre-treated with NaOH, the permeability of the membrane also improved, which is due to the increase in the hydrophilicity of the membrane. The alkaline solution can catalyze the degradation of ester bonds, so the PVP additive in the membrane may undergo a hydrolytic reaction that increases the hydrophilicity and charge of the membrane. The greater hydrophilicity and the negative charge maintain the fouling resistance of the NaOH pre-treated membrane. As shown in Fig. 4.2 d, there was no significant FRR difference after the protein was filtered and cleaned.

Finally, we tested the membrane stability after pre-treatment. By measuring the pre-treated membrane permeability at different times, we found that the permeability of the membrane remained stable over the storage time. However, after multiple measurements of the same membrane, the permeability of the membrane was found to decrease with increase of the number of tests. This may be because pre-treatment has changed the structure of the membrane and that multiple pressure tests will cause the membrane pores to collapse; the membrane structure thus becomes denser, resulting in a decrease in permeability.
Figure 4.1 Pure water flux of pristine, hot water, EtOH, NaOH treated PSf membranes

Table 4.1. Retention of absolute EtOH and 1.0 M NaOH treated PSf membrane with different sizes of dextran. Superscript letters indicate significant differences as determined by one-way ANOVA analysis

<table>
<thead>
<tr>
<th>Membrane type</th>
<th>T 70</th>
<th>T 100</th>
<th>T 229</th>
<th>T 2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot water treated PSf</td>
<td>1.63 ± 0.08a</td>
<td>4.52 ± 0.54a</td>
<td>23.69 ± 1.73a</td>
<td>66 ± 1.82a</td>
</tr>
<tr>
<td>EtOH treated PSf</td>
<td>0.84 ± 0.22b</td>
<td>2.31 ± 0.22c</td>
<td>11.62 ± 2.35b</td>
<td>49.32 ± 5.78b</td>
</tr>
<tr>
<td>NaOH treated PSf</td>
<td>1.28 ± 0.12b</td>
<td>3.07 ± 0.63b</td>
<td>25.84 ± 0.14a</td>
<td>65.26 ± 1.23a</td>
</tr>
</tbody>
</table>
Table 4.2. Static water contact angle of virgin, hot water, pure ethanol and NaOH treated membranes. Superscript letters indicate significant difference as determined by one-way ANOVA

<table>
<thead>
<tr>
<th>Treatment type</th>
<th>Static contact angle (°)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>After 120 s</td>
<td></td>
</tr>
<tr>
<td>Virgin membrane</td>
<td>87.4 ± 2.3</td>
<td>66.6 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>Hot water treatment</td>
<td>84.2 ± 1.2</td>
<td>62.2 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>Absolute ethanol treatment</td>
<td>84.7 ± 1.5</td>
<td>74.4 ± 2.4</td>
<td></td>
</tr>
<tr>
<td>1.0 M NaOH treatment</td>
<td>72.4 ± 2.1</td>
<td>52.5 ± 2.2</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.2. Protein ultrafiltration performance of PSf membrane treated with hot water, Absolute EtOH and 0.1 M NaOH: (a) flux of BSA and pepsin for EtOH treated PSf membrane, (b) retention and flux recovery ratio for EtOH treated PSf membrane, (c) flux of BSA and pepsin for NaOH treated PSf membrane, (d) retention and flux recovery for NaOH treated PSf membrane
4.4 Significance of the study

This study explored the effects of different pre-treatments on the performance of polymeric membranes and has significance for guiding the choice of membrane pre-treatment methods. First, the pre-treatment could remove the preservatives in the membrane and improve the permeability and stability of the membrane. Ethanol pre-treatment expanded the polymer chain and enlarged the membrane pores, which greatly enhanced membrane permeability. However, the treatment process caused the hydrophilic additive PVP to wash out, which reduced the hydrophilicity of the membrane and reduced membrane fouling resistance. In the protein separation process in particular, proteins were more likely to block the membrane pores and cause serious fouling due to non-specific adsorption. Therefore this kind of membrane pre-treatment is more suitable for separation of non-protein mixtures, such as polysaccharides or nanoparticles. The NaOH pre-treatment process resulted in the hydrolysis of PVP and formation of hydrophilic negatively charged groups, which not only enhanced the permeability of the membrane but also maintained the membrane fouling resistance. This kind of pre-treatment can be used for protein solutions separation.

4.5 Paper

This chapter was based on the following paper,

Commercial polysulfone membranes pretreated with ethanol and NaOH: effects on permeability, selectivity and antifouling properties

Mingbo Ji, Jianquan Luo, Jiang Wei, John Woodley, Anders Egede Daugaard, Manuel Pinelo

Published: accepted in Separation and Purification Technology (5.774), Sep, Purif. Technol. 2019, 219: 82-89.
Chapter 5-Charge exclusion as a strategy for controlling retention of small proteins in polyelectrolyte-modified ultrafiltration membranes

In the previous chapter, we found that although pre-treatment improved membrane permeability, membrane selectivity and fouling resistance were still poor. Among them, the NaOH pre-treatment degraded the ester bonds and generated hydrophilic groups that slightly improved the hydrophilicity and maintained the membrane fouling resistance. We can therefore consider enhancing membrane performance by introducing hydrophilic groups and charged groups. In an aqueous environment, a hydrophilic surface will form a hydration layer to prevent the substance/foulant from contacting the membrane and in this way inhibit membrane fouling. Electrostatic interaction can be used in separation process to further enhance membrane selectivity. In theory, the electrostatic attraction between opposite charges can promote the transmission of the substance, whereas the charge repulsion between similar charges prevents contact of the substance with the membrane surface, thus increasing the rejection rate of the membrane. However, for protein solutions the electrostatic adsorption between the protein molecules and the membrane may clog the membrane pores, and hence cause serious membrane fouling and ultimately increase the rejection rate. We selectively coated layers with different charges on the surface of membranes and conducted an in-depth investigation on the performance of the modified membranes.

5.1 Hypotheses

- Selective coating significantly changes the performance of the membrane. Coating membranes with different PEs, significantly enhances selectivity and fouling resistance of the membrane
- The hydrophilicity and charge of the membrane surface have a significant impact on membrane performance. A surface with the same charge as the protein inhibits membrane fouling, while a surface with the opposite charge to the protein aggravates fouling due to electrostatic attraction.
5.2 Experimental considerations

Our target product was lysozyme, which has a molecular weight of 14.3 kDa and an isoelectric point of about 10.8. In a neutral aqueous environment, lysozyme carries a positive charge. Therefore we planned to construct a positively charged surface for the separation of lysozyme.

Polyacrylic acid and PDADMAC are common polyanions and polycations, which can be adsorbed on the surface of the membrane by electrostatic attraction. Generally, this kind of electrostatic adsorption requires multilayers that will block the membrane pores, cause a sudden decrease in membrane flux, and convert the ultrafiltration membrane into a nanofiltration membrane. To reduce the influence of these PEs on the membrane pores, we planned to coat only a single layer of PEs on the membrane surface. Since the surface of the original membrane carries a negative charge, we further introduced a dopamine coating method to coat more PAA on the membrane surface. This method relies on hydrogen bonding between dopamine molecules and PAA molecules to deposit more PAA molecules on the surface of the membrane and enhance the hydrophilicity and charge of the membrane.

To investigate the effect of different coatings on the membrane performance, we coated the membrane surface with PAA/PDA, PDA+PDADMAC and PAA/PDA+PAADMAC layers. Next, we investigated the permeability, selectivity, and fouling resistance of the modified membranes and in particular the influence of coating on membrane charge and its further influence on membrane selectivity and fouling resistance.

5.3 Highlights

While retention in commercial UF membranes is commonly governed by size exclusion, addition of charged PEs to the membrane surface has been proposed as a facile and inexpensive method of modulating retention during filtration of charged compounds, such as lysozyme. Our study demonstrated that selected combinations of common PEs can be efficiently used to control the retention of proteins. We show how the retention of positively charged lysozyme increased from ~7% to ~50% when a negatively charged PEs was deposited on a commercial PSf membrane; this increase was most probably due to a combination of an associated pore narrowing effect and protein adsorption onto the
membrane. By contrast, the retention decreased again (from ~50% to ~30%) upon further addition of a positively charged PEs on the membrane (as shown in Fig. 5.1 a). The electrostatic repulsion between the positively charged surface and proteins prevented protein adsorption onto the membrane (as shown in Fig. 5.2), while pore size was still large enough to ensure that size exclusion was limited. The retention decreased further to ~22% when increasing amounts of PEs of the same charge as the protein were deposited on the membrane. As shown in Fig. 3a, modified membrane exhibited high rejection for lysozyme both at high and low pH. Variations in pH revealed that the negative effect of PEs of the same charge as the protein on lysozyme retention was reversed when the charge density of lysozyme reached a certain level, beyond which the positively charged electrolyte promoted dramatic increases of rejection. The high recovery of permeate flux with simple flushing at pH 3.0 compared to higher pH indicates that it is mostly reversible fouling that occurred at low pH, compared to more irreversible fouling when pH was increased. The results of this study suggest that use of PEs-modified UF membranes with a much larger pore size than the charged solute to be filtered could be a strategy to control retention of such solutes by minimizing the effect of size exclusion and fouling.

Figure 5.1 Protein ultrafiltration performance of virgin and modified membranes; (a) Lysozyme flux and retention, where – represents charge, – – represents strong negative charge, + represents positive charge, ++ represents strong positive charge, (b) Flux decline ratio after lysozyme filtration and index of flux decline versus retention. Means with different letters (a, b) are significantly different (ANOVA, p < 0.05).
5.4 Significance of the study

Modification of UF membrane surfaces by deposition of PEs layers was a facile approach for controlling protein retention. PEs-modification can be easily implemented in membranes with much larger pore size than the charged solute to minimize the size exclusion effect and fouling while retaining high retention and water flux. In our study, size exclusion was not the main separation mechanism, and retention of charged proteins can be effectively controlled by membrane surface charges. Membranes with a similar charge to the protein undergo low fouling during the protein filtration process, while severe fouling occurs for membranes with an opposite charge to the protein. Furthermore, the structure of PEs layers
can be adjusted by the deposition conditions, such as ionic strength. This approach can be easily integrated into commercial polysulfone-based membranes for obtaining product streams specially designed for retention of proteins, for example in the case of lactose separation from whey protein.

5.5 Paper

This chapter was based on the following paper,

**Charge exclusion as a strategy to control retention of small proteins in polyelectrolyte-modified ultrafiltration membranes**

*Mingbo Ji*, Xianhui Li, Maryam Omidvarkordshouli, Sigyn Björk Sigurdardóttir, John Woodley, Anders Egede Daugaard, Jianquan Luo, Manuel Pinelo

**Published:** accepted in Separation and Purification Technology (5.774), Sep, Purif. Technol. 2020: 116936.
Chapter 6-Grafting of charged small molecules on a commercial membrane surface to improve both permeability and selectivity

In the previous chapter, we found that although PEs coating could adjust the substance rejection rate and improve the membrane fouling resistance, the permeability of the membrane is greatly compromised. This is because the coated macromolecular PEs block the membrane pores, making it difficult for substance and water molecules to penetrate the membrane. We must therefore find other functional molecules for membrane surface modification. Hydrophilic charged small molecules may be a better modifier because they have little effect on membrane structure. To introduce these small molecules onto the membrane, we need to introduce an active layer on the membrane surface. Tannic acid (TA) is a polyphenolic substance like dopamine. It can be adsorbed on the surface of various materials. The phenolic group in its structure can be oxidized and further react with molecules containing amino groups. It can be used for the immobilization of functional molecules. Unlike dopamine, TA molecules do not undergo self-polymerization to form large particles, so they have little effect on the membrane structure. The large number of phenolic groups contained in the molecule can greatly enhance the hydrophilicity of the membrane. Therefore, through TA coating, we can expect to graft different types of charged small molecules on the membrane surface.

6.1 Hypotheses

- Tannic acid-assisted grafting of charged small molecules can significantly enhance membrane performance, including permeability, selectivity, and fouling resistance
- The best selectivity of the modified membrane to the protein solution may not be at the isoelectric point of the protein

6.2 Experimental considerations

A binary solution of BSA (66.7 kDa, pI 4.8) and bovine hemoglobin (Hb, 64.5 kDa) was used for the ultrafiltration experiment. The two proteins have similar molecular weights but have different isoelectric points. It is therefore possible to consider the separation of the two proteins by charge. In a neutral aqueous solution, BSA and Hb carry negative charges, and
our previous study found that charge repulsion can improve the fouling resistance of the membrane. In this study we were thus more inclined to construct negatively charged membrane surfaces. Cysteine, taurine and PEI are used for membrane surface modification. These small molecules have good hydrophilicity, carry a large amount of charge and can improve the performance of the membrane after successfully being grafted onto the membrane surface. Commercial PSf membranes were first coated with a layer of TA, and then grafted with cysteine, taurine and PEI respectively. Finally, the modified membranes were immersed in a glutaraldehyde solution to enhance the stability of grafting. Characterization methods such as FTIR, water contact angle and zeta potential measurement confirmed that small molecules were successfully grafted to the surface of the membrane. Then we did a detailed study of the permeability, selectivity, and fouling resistance of the modified membranes. Finally, we optimized the selectivity of the ultrafiltration process by adjusting the pH of the solution, the operating pressure and the rotating speed.

6.3 Highlights

After grafting with small molecules, all membranes showed increased water permeability. As shown in Fig 6.1, the cysteine grafted membrane had the highest PWP of 525.88 L/m² h bar, which is 1.71-fold higher than virgin PSf membrane (308.02 L/m² h bar). This result possibly indicates that cysteine is more hydrophilic than taurine and PEI. Binary solution filtration was then conducted to investigate membrane performance. The PEI grafted membrane had almost no transmission both for Hb and BSA because of membrane fouling. However, membranes grafted with cystine and taurine had different transmissions at different solution pH. As shown in Fig. 6.2, all membranes exhibited low transmission and selectivity under acid conditions. Although it is widely accepted that protein has high transmission at its isoelectric point, we did not observe high solute transmission in our experiment. In fact, protein is easy to aggregate at its pI due to the exposure of hydrophobic groups that will block the membrane pores and result in low solute transmission. It is therefore better to perform the ultrafiltration in weak alkaline conditions. We observed the highest Hb and BSA transmission at pH 9.0. However, the cysteine grafted membrane showed the highest selectivity at pH 7.5, which is close to the pI of Hb. When pH increased
to 9.0, the modified membranes had higher protein transmission but lower selectivity. The flux loss in Fig. 6.3 demonstrates that better fouling resistance can be obtained under alkaline conditions due to the strong charge repulsion. However, to achieve the best selectivity it is better to perform the filtration close to the pI of the protein.

![Figure 6.1 Pure water permeability of virgin PSf and small molecules grafted membranes](image1)

Figure 6.1 Pure water permeability of virgin PSf and small molecules grafted membranes

![Figure 6.2 Observed transmission of Hb and BSA for virgin PSf and for Cys and Tau-grafted membranes](image2)

Figure 6.2 Observed transmission of Hb and BSA for virgin PSf and for Cys and Tau-grafted membranes
6.4 Significance of the study

In this study, a variety of small molecules were successfully grafted on the surface of polymeric membranes using tannic acid coating assisted grafting. This modification method had almost no effect on the membrane structure, but greatly improved the hydrophilicity and chargeability of the membrane. The cysteine grafted membrane exhibited excellent hydrophilicity, selectivity, and fouling resistance, which was mainly attributed to the hydrophilic charged groups carried by the membrane. In the binary solution ultrafiltration experiment, we found that the higher the pH, the more the protein permeates, but the best selectivity occurs at a pH slightly higher than the pI of the protein. This may be because proteins at the pI are more likely to aggregate into macromolecules, blocking the membrane pores and causing membrane fouling. This result, which does not support the consensus that the protein will have the highest transmittance rate at its pI, is of significance for guiding the selection of protein filtering conditions. Our research provides a simple and cheap method for membrane modification. Due to the versatility of tannic acid coating, this method can be used to modify almost all material surfaces.
6.5 Paper

This chapter was based on the following paper,

**Grafting of charged small molecules on a commercial membrane surface to improve both permeability and selectivity**

**Mingbo Ji, Jianquan Luo, John Woodley, Anders Egede Daugaard, Manuel Pinelo**

*In preparation.*
Chapter 7-Conclusions and Future Perspectives

Membrane modification is an important method for improving membrane performance. In this thesis, commercial polysulfone based membranes were used as the substrate. The membranes were modified by pre-treatment with ethanol or NaOH, coating with polyelectrolytes on the membrane surface, and surface grafting with small molecules, respectively. We conducted a detailed study on surface properties, pore structure, permeability, selectivity and fouling resistance of the modified membranes, which led to the following conclusions.

1. Pre-treatment with ethanol or NaOH solution greatly improved the permeability of membranes. For ethanol treated membrane in particular, pure water permeability increased by nearly 5-fold compared to virgin PSf membrane. Membrane thickness measurement and dextran rejection experiment revealed that the polymer segment in the membrane matrix expanded after ethanol treatment, resulting in increased pore size and porosity, which promoted membrane permeability and reduced rejection. Analysis of FTIR spectra demonstrated that ethanol treatment leached out the hydrophilic additive (PVP) in the membrane matrix and reduced membrane hydrophilicity, leading to more severe membrane fouling during protein filtration process. For NaOH treated membrane, permeability of membrane was increased while maintaining a fouling resistance similar to virgin PSf membrane. Water contact angle and Zeta potential measurements showed that hydrophilicity and surface charge of NaOH treated PSf membrane were both enhanced. PVP is subject to a hydrolysis reaction in strong alkaline conditions, which generated a more hydrophilic group (-COOH) and resulted in improved membrane permeability. Furthermore, the negatively charged surface/pores prevented contact of protein and membrane material and retained membrane permeability. Compared to ethanol treatment, NaOH treatment is more suitable for membranes which are used in protein separation processes.

2. By selectively coating different polyelectrolytes on the membrane surface, a series of anti-fouling membranes with controllable rejection were obtained. Charge interaction played a vital role in the protein filtration process. When the membrane surface was coated with
polyanions PAA or PDA, a lot of positively charged lysozyme would adsorb onto the modified membrane surface and pores due to charge attraction, resulting in a high rejection rate and severe membrane fouling. On the contrary, when the membrane surface was coated with the polycation PDADMAC, charge repulsion prevented the protein from contacting the membrane surface and pores. Since membrane pores were relatively larger than lysozyme, the modified membrane allowed more proteins to pass through, and exhibited low rejection and good fouling resistance. Further adjustment of the coating conditions of polyelectrolytes led to enhanced charge on the membrane surface and a controllable rejection rate. In addition, membrane fouling was prevented through adjusting solution pH. Under acidic conditions, the modified membranes and the lysozyme both carried positive charges which repelled each other and hence brought about excellent fouling resistance.

3. Although PEs coating could control solute transmission, modified membranes suffered great permeability loss. To retain membrane permeability, we grafted hydrophilic, charged small molecules (taurine, cysteine, PEI) onto PSf membrane surfaces, which simultaneously enhanced membrane permeability, selectivity and fouling resistance. Water permeability of cysteine grafted PSf membrane increased by 1.71-fold compared to virgin PSf membrane. Zeta potential measurement showed that the surface of the modified membrane carried numerous negative charges which affected protein transmission. PEI grafted membranes showed low transmission and selectivity to BSA and Hb, which is unsuitable for a protein separation process. However, taurine and cysteine grafted membranes exhibited pH-related transmission and selectivity. Under acidic conditions (below protein isoelectric point), severe fouling caused by charge attraction prevented protein from passing through the membrane, and resulted in low flux, high rejection rate and low selectivity. In an alkaline environment, the modified membrane and the protein both carried negative charges; membrane fouling was consequently suppressed due to charge repulsion and the membrane allowed more protein to pass through and exhibited excellent fouling resistance. It is noteworthy that fouling affected selectivity of the membrane to BSA and Hb. Cysteine grafted PSf membrane achieved highest selectivity at a pH slightly higher than the isoelectric point of Hb. When we further adjusted operation conditions, we found that low transmembrane pressure and a suitable stirring rate improved membrane selectivity while
maintaining good fouling resistance.

In this thesis, different methods were used to modify PSf membranes and performance of the membranes was greatly improved. In particular, grafting of cysteine on the membrane surface resulted in good selectivity with improved membrane permeability and fouling resistance. However, there are some challenges that need to be addressed. Modified membranes still suffered fouling because our modification merely altered the surface properties of membranes whose internal pores were hydrophobic and uncharged. Furthermore, selectivity of modified membrane was low and not ideal. Therefore, in future work, we will focus more on modifying the internal pores of the membrane and improving membrane selectivity. In addition, due to long running times in industrial processes, the stability of the modified membrane also needs to be studied.
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Appendix

Paper 1

Commercial polysulfone membranes pretreated with ethanol and NaOH: Effects on permeability, selectivity and antifouling properties

Mingbo Ji\textsuperscript{a}, Jianquan Luo\textsuperscript{b\textsuperscript{*}}, Jiang Wei\textsuperscript{a}, John Woodley\textsuperscript{a}, Anders Egede Daugaard\textsuperscript{b}, Manuel Pinedo\textsuperscript{c}\textsuperscript{*}

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\textbf{A R T I C L E  I N F O}

Keywords:
Ethanol pretreatment
Sodium hydroxide pretreatment
Polysulfone ultrafiltration membrane
Protein separation
Fouling

\textbf{A B S T R A C T}

This study explores the effect of three simple pretreatment methods – hot water, ethanol (EtOH) and sodium hydroxide (NaOH) – on the performance of commercial polysulfone (PSF) membranes. 100% EtOH and 1 M NaOH pretreated membranes exhibited increases of, respectively, 600% and 50% of water permeability compared to the hot water pretreated membrane (control). Such increases were partially ascribed to the removal of preservatives i.e. glycerol and polyvinylpyrrolidone (PVP) in the chemically pretreated membranes, as confirmed by FTIR spectra. The dramatic increase of water permeability in the ethanol pretreated membranes was also explained by pore enlargement, which was confirmed by decreased retention of dextran of selected molecular weights. Protein (BSA and pepsin) filtration experiments confirmed the occurrence of higher flux during filtration when using ethanol and NaOH pretreated membranes, though fouling caused during filtration was more severe and more difficult to remove in ethanol pretreated membranes compared to the control. Static protein adsorption measurements confirmed that ethanol pretreated membranes had an enhanced propensity to adsorb more protein. Water permeability tests performed after maintaining the pretreated membranes in water for several days confirmed that the changes induced by the selected pretreatments persist after at least 7 days. In general these results show that the studied pretreatments method can be used to tailor the properties of commercial polysulfone membranes in terms of permeability and retention to a certain extent and in a simple, inexpensive manner.

1. Introduction

Polysulfone (PSF) ultrafiltration membranes are widely used in industrial processes due to their excellent properties, such as chemical inertness across the entire pH range (0-14), compressive strength and thermal stability [1-3] (Fig. 1). Such membranes are commonly treated with chemicals or solvents before use (ethanol, methanol, acetone, etc.), either for preservative removal or cleaning [4,5]. After use, for fouling removal, or disinfection, other chemical treatments are also generally applied, such as NaOH, sodium hypochlorite (NaClO) or acids e.g. HCl, HNO\textsubscript{3}, H\textsubscript{2}SO\textsubscript{4}, H\textsubscript{2}O\textsubscript{2} [5-7]. PSF membranes, however, are sensitive and undergo severe chemical changes during chemical or solvent exposure, since polymer chains may not be stable in extreme environments.

Indeed, much literature has reported significant changes in membrane properties after cleaning with NaClO solution, which as one of the most aggressive cleaning treatments is regarded as the reference for membrane cleaning, Zhang et al. [8], for instance, found that pores were enlarged significantly when PSF membranes were treated with NaClO, which resulted in major changes in dextran and humic acid retention. Such treatment resulted in surface charge density increases and significantly affected membrane separation performance and fouling behavior. Rouaix et al. [9] also reported that exposure to high concentrations of organic solvents may lead to PSF chain breaking, which leads to changes of the membrane structure at the microscopic scale and in turn to changes in performance. A few chain scissions may have notable effects on the elongation and tensile strength of the membrane, which for NaClO solutions showed a loss of 80% in elongation at break point for a percentage of chain scissions lower than 2.8%. These results illustrate some of the most dramatic effects that exposure to organic solvents may exert on PSF, and it is easy to infer that such changes will also have a notable effect on membrane...
Although previous literature has reported chemical and physical effects (along with some effects on membrane performance) of the exposure of polymeric membranes to organic solvents, a comprehensive, systematic study of the effects on the membrane performance is lacking. Our work proposes a systematic experimental strategy to determine how selective treatments with hot water, ethanol and NaOH affect the performance of a commercial PSf membrane in terms of water permeability, retention and antifouling properties. To this end, filtration performance, pure water flux recovery and separation mechanisms were studied in detail, together with the interconnection between these factors and the underlying surface chemical changes caused by ethanol/NaOH pre-treatment.

2. Materials and experimental section

2.1. Materials and reagents

A PSf membrane with molecular weight cut off (MWCO) of 100 kDa (Alfa Laval, Denmark) was used in this study. The filtration experiments were performed in an Amicon Stirred Cell Model 8050 (Millipore, Germany) with effective membrane area of 13.4 cm². Bovine serum albumin (BSA, 67 kDa, isoelectric point (pl) 4.7) and pepsin (35 kDa, pl 1.0) were obtained from Sigma Aldrich, Denmark. Dextran with molecular weight of 70, 100, 229, 20000 kDa, that is T70, T100, T229, T2000, respectively, were kindly donated by Pharmacosmos, Denmark. Absolute ethanol (99.9%), NaOH and polyvinylpyrrolidone (30 kDa) were obtained from Sigma Aldrich. All the water used in this research was deionized (DI) water.

2.2. Membrane treatment

All membranes were treated with hot water, according to the instructions of the manufacturer. Virgin membranes were immersed in hot DI water at 70 °C for 90 min at 130 rpm. Hot water pretreated membranes without further chemical treatment were used as a control in this study. The treatment condition is shown in Table 1.

For ethanol pretreatment, hot water pretreated membranes obtained as above were subjected to two different treatments: (1) 60% EtOH solution at 35 °C for 75 min at 130 rpm, or (2) Absolute EtOH solution at 50 °C for 120 min at 130 rpm.

For the NaOH treatment, the hot water treated membranes were immersed in (1) 0.55 M NaOH solution at 35 °C for 75 min at 130 rpm or (2) 1.0 M NaOH solution at 50 °C for 120 min at 130 rpm.

After EtOH or NaOH pretreatment, membranes were rinsed with deionized water several times to remove residual chemicals and finally stored in deionized water at 4 °C for further use.

2.3. FTIR spectra analysis

Membrane surface chemical characteristics were determined by FTIR spectroscopy using a Nicolet 6700 FTIR Spectrometer (Thermo Fisher Scientific, USA). Before measurement, membranes were dried for 24 h. The wavenumber between 400 cm⁻¹ and 4000 cm⁻¹ was recorded with 64 interferograms for each sample. As a standard, pure PVP powder was also measured.

### Table 1

<table>
<thead>
<tr>
<th>Treatment solution</th>
<th>Time (min)</th>
<th>Temperature (°C)</th>
<th>Stirring ratio (rpm)</th>
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<tbody>
<tr>
<td>#1 DI water</td>
<td>90</td>
<td>70</td>
<td>130</td>
</tr>
<tr>
<td>#2 60% EtOH</td>
<td>75</td>
<td>35</td>
<td>130</td>
</tr>
<tr>
<td>#3 Absolute EtOH</td>
<td>50</td>
<td>120</td>
<td>130</td>
</tr>
<tr>
<td>#4 0.55 M NaOH</td>
<td>75</td>
<td>35</td>
<td>130</td>
</tr>
<tr>
<td>#5 1.0 M NaOH</td>
<td>50</td>
<td>120</td>
<td>130</td>
</tr>
</tbody>
</table>
2.4. Pure water flux and water contact angle measurement

Water contact angle (WCA) was tested in a goniometer (OCA 20, Dataphysics, Germany) using the sessile drop method to determine the hydrophilicity/hydrophobicity of the membrane surface. 1 mL of MilliQ water was slowly settled onto the drier membrane surface using a microsyringe, and the water contact angle was recorded by video for 120 s. Water contact angle measurements were performed a minimum of 6 times for each membrane and the average determined. Pure water flux (PWF) was measured using an Amicon Stirred Cell Model 8050 with an effective filtration area of 13.4 cm². First, the membrane was pre-pressured for 30 min at 2.0 bar by DI water filtration (to ensure stable membrane compaction and stable flux). Next, the time was recorded for each 2 mL of permeate until 12 mL permeate was collected at 1.0 bar. The average flux, \( J \), was obtained as follows:

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

where \( V \) represents filtration volume, \( S \) represents effective filtration area and \( t \) represents the filtration time.

Finally, water flux was measured again at 2.0 and 3.0 bar, and the pure water flux, \( J_w \), was calculated by the slope of flux against pressure. PWF was measured in duplicate.

2.5. Dextran and protein filtration experiments

Dextran solution was prepared at a concentration of 1 g/L by dissolving dextran powder of 70, 100, 220 and 2000 kDa in DI water. The membrane was initially pre-pressured for 30 min at 2.0 bar by DI water filtration and then the PWF was calculated. 50 mL dextran solution was filtered at 1.0 bar and 500 rpm. Time was recorded for each 5 mL of permeate until 45 mL of permeate was collected. Then the membrane was rinsed three times using DI water and PWF measured again. The flux recovery ratio was calculated as follows:

\[
FRR = (1 - \frac{J_{w\, \text{after}}}{J_{w\, \text{initial}}}) \times 100\%
\]

where \( J_{w\, \text{initial}} \) represents the initial pure water flux, and \( J_{w\, \text{after}} \) represents the pure water flux after solute filtration and simple cleaning.

The concentration of dextran was determined using the phenolsulfuric acid method [21]. 2 mL of sample was added to a colorimetric tube and 1 mL of 0.5% phenol solution was added. The mixed solution was vortexed for 30 s to obtain a uniform mixture. Next, 5 mL of concentrated \( \text{H}_2\text{SO}_4 \) was added to the mixture and vortexed for 30 s. The final mixture was incubated in a fume hood for 30 min and the absorbance was determined at 490 nm using a Shimadzu UV-1280 spectrophotometer. The retention was calculated as follows:

\[
\text{Retention} = (1 - \frac{C_{\text{water}}}{C_{\text{initial}}}) \times 100\%
\]

where \( C_{\text{water}} \) represents the absorbance of permeate solution and \( C_{\text{initial}} \) represents the absorbance of feed solution.

For the protein filtration experiments, BSA or pepsin solution was prepared at a concentration of 1 g/L using PBS buffer solution (0.01 M, pH 7.4). The membrane was initially pre-pressured for 30 min at 2.0 bar using DI water and then the PWF was measured. Next, 50 mL BSA solution was filtered at 1.0 bar and 500 rpm. The time was recorded for each 5 mL of permeate until 45 mL of permeate was collected. Finally, the membrane was rinsed three times using DI water and the PWF was measured again. The flux recovery ratio was calculated using Eq. (2). Retention of proteins was determined by measuring the solution absorbance at 280 nm and using Eq. (3).

2.6. Static protein adsorption

The antifouling properties of the membranes were also evaluated by static protein adsorption measurements using BSA solution. Membrane was fastened at the bottom of the filtration cell and 20 mL of 1 g/L BSA solution (PBS, 0.01 M, pH 7.4) was added. Then the cell was kept in a fridge (4 °C) and incubated for 24 h to obtain adsorption equilibrium. Protein adsorption (µg/cm²) was determined by the concentration difference of BSA solution before (\( C_{\text{initial}} \)) and after the adsorption (\( C_{\text{final}} \)) experiment as follows:

\[
\text{Adsorption amount} = \frac{(C_{\text{initial}} - C_{\text{final}}) \times V}{S}
\]

where \( V \) and \( S \) are the BSA solution volume (20 mL) and the effective membrane area (15.4 cm²), respectively. The values were determined by testing at least three membranes for each type and finally averaged.

2.7. Storage and stability

Modified membranes were stored in deionized water at 4 °C and the pure water flux was measured after 1, 3, 5 and 7 storage days.

2.8. Statistical analysis

All experiments were performed at least in triplicates, so the values given are the average of such replicates. Significant difference between values was determined by one-way ANOVA (Mini-Tab In. Pennsylvania, USA).

3. Results & discussion

3.1. Water permeability and contact angle

PWF of membranes treated with hot water, EtOH or NaOH changed significantly during pretreatment (Fig. 2). The pristine PSf membrane had the lowest water permeability, approximately 110 L/m² h bar. After hot water treatment, the permeability increased to 175 L/m² h bar, probably due to preservatives removal. Likewise, treating the membrane with EtOH solution led to dramatic increase in water permeability. Indeed for the 60% EtOH treated membrane, the water flux increased to 600 L/m² h bar, which is three times that of hot water treated membranes, and the increase was fivefold when the membrane was treated with absolute EtOH. These results reflect fairly closely those from experiments by Argyle et al. [16] who pretreated the PSf membrane with the pure EtOH for 24 h in an ambient environment and also found a threefold increase in the water flux values. They postulated that
the mechanism for performance improvement could be attributed to swelling of membrane skin-layers and additives removal (such as PVP, which is a pore formation reagent during membrane manufacture). The higher permeability observed by us can be ascribed to the higher temperature used during ethanol pretreatment, which may more easily solubilize the PVP, changing membrane structure and composition [22]. For NaOH treatment, the PWF of membranes treated with 0.55 M and 1.0 M NaOH reached 205 and 250 L/m2 h bar, respectively. Removal of residual preservatives and additives has also been reported to contribute to the increase of water flux in NaOH treated membranes, even though swelling and chemical interactions between NaOH and surface polymers have also been found to have a more dramatic effect on water flux [17,23].

To evaluate the preservatives removal hypothesis, membranes were subjected to FTIR analysis. FTIR spectral bands at 921.56 and 1411.02 cm⁻¹ were absent after each of the selected pretreatments – hot water, ethanol and NaOH – were applied on the membrane (Fig. 3). Additionally, the bands at 3331.21 cm⁻¹ were weakened. Such bands are characteristic of the preservative glycerol [16] which seems to be removed in all three cases. Additionally, membranes treated with hot water, EtOH and NaOH showed similar but weaker peak intensity at 1655.42 cm⁻¹, which is characteristic of the PVP molecule and indicates that some PVP may be leached out. Leaching of PVP, which is commonly used as a pore formation reagent and hydrophilic modification reagent for membrane fabrication [24], has been observed by previous researchers [25,26]. FTIR thus confirmed that removal of some of the most common preservatives used during membrane casting occurred at least partially during the applied pretreatments.

WCA measurements also shed some light on the explanation of higher water permeability. Table 2 shows the WCA of virgin PSf, hot water treated PSF, and ethanol and NaOH treated PSF membranes. Virgin PSF membranes showed the highest WCA (87.4° and 66° after 120 s), while WCA decreased to 84.2° (62.2° after 120 s) with hot water treatment. WCA is affected by the hydrophilicity/hydrophobicity, roughness and charge of the material of the surface of the membrane [27]. Preservatives removal has also been reported to have an influence on WCA reductions because preservative removal contributes to making the membrane smoother and in turn more hydrophilic [28], although in some cases special additives are added that can contribute to enhance hydrophilicity [29]. EtOH treated membranes displayed similar initial WCA to hot water treated membranes, though the reduction in WCA lineafter 120 s was significantly lower compared (74.4°), which indicated a reduced hydrophilicity (especially when the increased pore size is taken into account). Previous literature has reported swelling and enlargement of pore size when membranes are treated with EtOH, which may explain the higher water flux for this treatment compared to hot water treated membranes [30]. The highest decrease of contact angle was observed for NaOH treated membranes (72.4°), which was even more apparent after 120 s (52.5°). Sydney [23] postulated that hydrolisis of the lactam ring of polyvinylpyrrolidone (PVP) may occur during alkali exposure, which results in generation of hydrophilic groups and a significant reduction of the WCA when PSf membranes are in exposed to NaOH. Therefore each of the three pretreatment methods evaluated seemed to have a positive effect on enhancing the hydrophilicity of the membrane. However, whilst the hot water and EtOH effect could mainly probably be ascribed to removal of preservatives – and also pore enlargement in the case of EtOH – a chemical reaction also seemed to be involved in the hydrophilicity increase of the NaOH treated membrane.

### Table 2

<table>
<thead>
<tr>
<th>Treatment type</th>
<th>Static contact angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td>Virgin membrane</td>
<td>87.4 ± 2.3</td>
</tr>
<tr>
<td>Hot water treatment</td>
<td>84.2 ± 1.2</td>
</tr>
<tr>
<td>Absolute ethanol treatment</td>
<td>84.7 ± 1.5</td>
</tr>
<tr>
<td>1.0 M NaOH treatment</td>
<td>72.4 ± 2.1</td>
</tr>
</tbody>
</table>

3.2. **Dextran filtration performance**

The swelling hypothesized in the previous section was tested by filtering a dextran solution through the virgin and pretreated membranes and determining flux and retention during filtration. The so-called macroporous swelling was expected to lead to dilution of the entire structure and cause enlargement of pores, which would result in lower retentions [15]. Membranes treated with either EtOH or NaOH showed higher filtration fluxes than the virgin membrane or the one treated with hot water when filtrations were performed using the same MW dextran solute (Fig. 4). Filtration of dextran 220 resulted in much lower fluxes compared to the fluxes observed for smaller MW dextrans. This result was ascribed to the formation of a cake layer of dextran on the surface of the membrane, which hindered high fluxes right from the very start of the filtration. It is also interesting to note that whilst the flux during filtration remained stable after an initial decrease for membranes treated with hot water and NaOH, a progressive decrease of the flux was observed for EtOH treated membranes, which suggested a higher affinity between the membrane treated with EtOH and the solute. Such higher affinity would impede back-diffusion of solute to the feed solution during filtration and cause accumulation. If this hypothesis is correct, it should be more difficult to remove the fouling layer.
created on EtOH treated membranes using a mild cleaning treatment e.g. water rinsing after filtration. This hypothesis was evaluated in further experiments (see next sections).

The slightly higher flux observed for Dextran 100 compared to Dextran 70 (when the opposite was expected) for EtOH treated and pristine membranes may be explained in terms of osmotic pressure. For the same concentration of solute, a larger MW molecule exerts a lower osmotic pressure which in turn would cause more flux to pass to the permeate. It is also possible that the size of 70 kDa dextran is similar to the membrane pore size of the EtOH treated membrane, and thus severe pore blocking may be occurring during dextran filtration and lead to low solute flux.

Retention of dextran increased with increasing dextran MWs, as expected (Table 3). For dextran of 70, 100 and 229 kDa, the hot water treated membrane exhibited a retention of 1.63, 4.52 and 25.69%, respectively. Even for dextran of 2000 kDa, hot water treated PSf had a retention of 66%, which is much lower than the values stated by the manufacturer. The results show that the EtOH treated membranes have a lower retention for all of the dextran sizes compared with the hot water and NaOH treated membranes, which additionally supports the hypothesis of pore size enlargement postulated above.

NaOH treatment did not seem to have an effect on retention compared to the hot water treated experiments, despite the significantly higher flux during filtration. This observation suggests that while treatment with NaOH probably does not cause pore enlargement, it does cause an enhanced hydrophilicity of the membrane, which should also have a positive effect on the removal of solute from the membrane surface. Such results are somehow not surprising. Polysulfone is used as a membrane material precisely because it has high resistance to thermal and acid/alkali exposure, and thus not much change would be expected. Industrial processes using polysulfone membranes often subject membranes to harsh chemical cleaning daily and the membranes are sometimes used for almost three years.

3.3. Protein filtration performance

While dextran filtration allows a good characterization of the pore size of the membranes, since dextran is a linear molecule that does not aggregate, filtration of proteins is much more challenging. Proteins tend to aggregate, which results in a much larger size in many cases, and have a strong tendency to foul the membranes because they contain charged functional groups and hydrophobic moieties that interact with the membrane material. BSA (67 kDa) and pepsin (35 kDa) were then filtered through the treated membranes (Fig. 5).

The flux during filtration in the EtOH and NaOH pretreated membranes was higher than in the hot water treated membrane, except for filtration of BSA in NaOH treated membrane, but the difference decreased in step with increasing concentration of the feed solution (Fig. 5a). This is expected because the effect of swelling or enhanced hydrophilicity is expected to be offset by increased concentration polarization and resulting fouling during concentration. In general, BSA filtration caused lower fluxes than pepsin filtration, which can probably be ascribed to the bigger size of BSA and the tendency to aggregate.

This postulation was confirmed by the retention results; in contrast with what was observed during dextran filtration, no significant differences in the values of retention were observed either for pepsin (23.04% and 21.22%, respectively) or BSA (99.5% and 94.7%, respectively) between hot water and EtOH treated membranes. Protein aggregation, and interactions between protein and membrane material favoring fouling, seemed to offset the pore enlargement effect of treating the membrane with EtOH, and only showed a slight decrease in retention values was observed. No significant differences of retention were likewise observed between membranes treated with hot water and NaOH (24.02% for pepsin and 91.6% for BSA), which was probably also caused by an enhanced effect of fouling.

To evaluate the hypothesis of enhanced protein fouling on EtOH and NaOH treated membranes, the membranes were water rinsed after filtration and the flux recovery ratio (FRR) was determined. Hot water treated PSf membranes reached FRR values of 74% and 60% for pepsin and NaOH treated membranes reached FRR values of 74% and 60% for Pepsin.

<table>
<thead>
<tr>
<th>Membrane type</th>
<th>T 70</th>
<th>T 100</th>
<th>T 229</th>
<th>T 2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot water treated PSf</td>
<td>1.63 ± 0.06*</td>
<td>4.52 ± 0.54*</td>
<td>23.69 ± 1.72*</td>
<td>66 ± 1.82*</td>
</tr>
<tr>
<td>EtOH treated PSf</td>
<td>0.84 ± 0.22*</td>
<td>2.31 ± 0.22*</td>
<td>11.62 ± 3.92*</td>
<td>49.52 ± 5.74*</td>
</tr>
<tr>
<td>NaOH treated PSf</td>
<td>1.28 ± 0.12*</td>
<td>2.07 ± 0.83*</td>
<td>25.84 ± 0.14*</td>
<td>65.26 ± 1.23*</td>
</tr>
</tbody>
</table>

Table 3: Retention of absolute EtOH and 1.0 M NaOH treated PSf membrane with different sizes of dextran. Superscript letters indicate significant differences as determined by one-way ANOVA analysis.
and BSA, respectively. For EtOH treated membranes, the FRR decreased to 45% and 22%, respectively (Fig. 5b). Such dramatic decreases may be explained not only by adsorption of BSA to the membrane pores but also by the entrapment of BSA in the pores, which would cause internal fouling and would explain the sharp flux decline. The fact that the initial flux during filtration was much higher for EtOH than for NaOH treated membranes confirms the hypothesis of pore enlargement with the former treatment. Such pore enlargement also explains the sharper decrease of flux for EtOH treated membranes, which may be caused by internal fouling. FRR values for NaOH treated membranes, which were slightly lower compared to the hot water membranes (Fig. 5d), also confirm that NaOH pretreatment caused fewer physical changes than EtOH to the membrane but also affected the interaction membrane-solute. It is interesting to observe that the FRR value of the NaOH pretreated membrane after BSA filtration was the same as was observed for hot water treatment, whereas a decrease was observed for pepsin, which may indicate that pepsin more readily causes internal fouling.

Static protein adsorption experiments confirmed the higher affinity of proteins for the pretreated membranes, particularly for those pretreated with EtOH (Table 4). A protein adsorption of 8.35 µg/cm² was observed for the hot water treated membranes, while for membranes treated with 60% and absolute EtOH, the adsorption increased to 11.12 and 13.02 µg/cm², respectively. No change in protein adsorption was observed in NaOH pretreated membranes at low concentrations of NaOH, though a slight increase was observed at 1.0 M concentration. As expected, there was correspondence between the results of protein adsorption and the FRR values: the higher the protein adsorption (EtOH pretreated membranes), the lower the values of FRR. Recent literature has proposed other different membrane modifications to improve the antifouling properties, like using a CeO₂ photocatalyst on a polysulfone membrane [11] or use of mPEG on PVDF-co-HFP membranes [23], which are also good examples of simple treatments that can be further applied to the pre-treated membranes (particularly EtOH) to improve the performance in terms of fouling.

The results presented in this section show that treating the membranes with EtOH or NaOH, unlike in the case of dextran, did not have any influence on the retention of the selected proteins. This outcome may indicate that fouling and aggregation effects outweigh the physical and chemical changes that take place in the membranes during chemical treatments. While both EtOH and NaOH pretreatments promoted increases of flux during the initial stages of protein concentration, the fouling generated during the process is higher (since the initial fluxes are also higher) and more difficult to remove than in the case of hot water pretreatment.
Table 4
Static protein adsorption on PES, EIOH and NaIOH treated PES membrane.

<table>
<thead>
<tr>
<th>Membrane treated with Protein</th>
<th>60% EIOH treated PES</th>
<th>NaIOH treated PES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein amount (μg/cm²)</td>
<td>0.35 ± 0.25</td>
<td>11.12 ± 0.12</td>
</tr>
<tr>
<td>Absolute EIOH treated PES</td>
<td>13.02 ± 0.45</td>
<td>9.01 ± 0.22</td>
</tr>
<tr>
<td>0.55 NaIOH treated PES</td>
<td>10.22 ± 0.33</td>
<td></td>
</tr>
</tbody>
</table>

Table 5
Evolution of pure water flux of EIOH and NaIOH treated PES membranes subjected to compression after storage in water for 1, 5, 6 and 7 days.

<table>
<thead>
<tr>
<th>Time (day)</th>
<th>Day 1</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>EIOH treated PES</td>
<td>880.95 ± 27.82</td>
<td>563.14 ± 3.87</td>
<td>465.9 ± 9.72</td>
<td>388.3 ± 4.95</td>
</tr>
<tr>
<td>NaIOH treated PES</td>
<td>379.79 ± 0.73</td>
<td>338.26 ± 14.88</td>
<td>297.73 ± 5.96</td>
<td>238.85 ± 1.28</td>
</tr>
</tbody>
</table>

Table 6
Overview of the effect of the selected pretreatments on several characteristic membrane parameters. + represent increased, – represent reduced, no represent no significant change.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Hot water treated PES</th>
<th>EIOH treated PES</th>
<th>NaIOH treated PES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water permeability</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Water contact angle</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Density Flux</td>
<td>+</td>
<td>+ + +</td>
<td>+</td>
</tr>
<tr>
<td>Flux retention</td>
<td>no</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Flux recovery</td>
<td>no</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>BSA Flux</td>
<td>+</td>
<td>+ + +</td>
<td>+</td>
</tr>
<tr>
<td>Flux retention</td>
<td>no</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Flux recovery</td>
<td>no</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Peptin Flux</td>
<td>+</td>
<td>+ + +</td>
<td>+</td>
</tr>
<tr>
<td>Flux retention</td>
<td>no</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Flux recovery</td>
<td>no</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Change in pore size</td>
<td>no</td>
<td>+</td>
<td>no</td>
</tr>
</tbody>
</table>

3.4. Storage stability and resistance to compaction

Storage stability experiments were performed in order to evaluate whether the effects of the applied pretreatments were retained over time or whether the membrane recovered its initial properties with time. The experiments were performed by using either the same membrane subjected to water filtration after 1, 5, 6 and 7 days storage in water at room temperature, or by subjecting a different membrane to water filtration on each of the days. In the former case, the membrane did not show significant changes in PWF, which confirmed that the effects of EIOH and NaIOH pretreatments persisted with time. However, when the same membrane was subjected to several water filtration experiments, PWF was observed to decrease with increasing number of days water storage (Table 5). Such PWF decrease supports the hypothesis that in the EIOH and NaIOH pretreated membranes, swelling of pores occurs which is more sensitive to compression when water filtration experiments are performed. These results suggest that while pretreatment causes stable changes on the membrane structure, the membranes may also become more sensitive to the effects of operational variables like pressure.

4. Conclusion

Ethanol and NaOH pretreatments increased water permeability by 600% and 50%, respectively. The large increase in water permeability observed in EIOH treated membranes was ascribed to an enlargement of the pore size. Despite the increased water permeability, fouling was more severe in EIOH treated samples. Protein adsorption experiments indeed showed that EIOH pretreated membranes (11.12 and 13.02 μg/cm² of protein adsorption) was at least a 30% higher compared to the hot water and NaOH treated membranes. Changes induced by these pretreatments persisted after at least 7 days, so it is expected that the modified membranes can preserve their properties even longer. Such changes induced on the membranes by the pretreatments utilized in this study can have positive or negative effects on the performance of the membrane, depending on the application for which the membranes are used. NaOH pretreatment promoted an increase of flux during filtration without affecting retention. This result may be advantageous for those applications in which filtration needs to be accelerated without compromising the efficiency of separation. EIOH pretreatment promoted both an increase of flux and also a decrease of retention. However, such decrease of retention due to pore enlargement may result in more severe internal fouling if solutes of larger size are present in the feed mixture.

Table 6 summarizes the effect of the pretreatments proposed in this study on flux, retention, and flux recovery ratio. More systematic experiments should be performed to unravel the effects of the proportion of EIOH or NaOH, the use of other chemicals, and the effect of other variables, e.g. temperature during pretreatment, on the performance of the membrane. These types of pretreatments, where the chemicals are not in contact with the membrane for a long time, do not cause dramatic changes in the structure and performance of the membrane, but may help to slightly tailor membrane properties for particular applications in a quick, easy and stable way.

Acknowledgements

The authors gratefully acknowledge financial support from the China Scholarship Council.

References

Supporting information

S1. Zeta potential of pre-treated and virgin PSf membranes under different pH conditions
Charge exclusion as a strategy to control retention of small proteins in polyelectrolyte-modified ultrafiltration membranes

Mingbo Ji, Xianhui Li, Maryam Omidvarkordshouli, Sigyn Björk Sigurdardóttir, John M. Woodley, Anders Egede Daugaard, Jianquan Luo, Manuel Pinelo

Keywords:
- Lysosome
- Polyelectrolyte deposition
- Electrostatic repulsion

Abstract

While retention in commercial ultrafiltration membranes is commonly governed by size exclusion, addition of charged polyelectrolytes to the membrane surface has been proposed as a facile and inexpensive method to modulate retention during filtration of charged compounds, such as proteins. This study demonstrates that selected combinations of common polyelectrolytes can be efficiently used to control the retention of proteins. We show how the retention of positively charged lysosome increased from ~7% to ~30% when a negatively charged polyelectrolyte was deposited on a commercial polysulfone membrane, most probably due to a combination of associated pore narrowing effect and protein adsorption onto the membrane. By contrast, the retention decreased again from ~50% to ~30% upon further addition of a positively charged polyelectrolyte onto the membrane. The electrostatic repulsion between the positively charged surface and proteins prevented protein adsorption onto the membrane, while pore size was still large enough that size exclusion was limited. The retention decreased further to ~22% when increasing amounts of polyelectrolytes of the same charge as the protein were deposited on the membrane. In addition, variations in pH revealed that the negative effect of polyelectrolytes of the same charge as the protein on lysosome retention was reversed when the charge density of lysosome reached a certain level beyond which the positively charged electrolyte promoted dramatic increases of rejection. The results of this study suggest that use of polyelectrolyte-modified ultrafiltration membranes with a much larger pore size than the charged solute to be filtered could be a strategy to control retention of such solutes by minimizing the effect of size exclusion and fouling.

1. Introduction

Ultrafiltration (UF) is commonly used for purification and concentration in the biotechnology, pharmaceutical, and food industries [1]. Yet membranes used for these purposes face two main obstacles: high fouling tendency and low selectivity [2]. It is generally accepted that developing hydrophilic surfaces to prevent hydrophobic interactions between membrane and fouler is an effective approach for fouling mitigation [3,4]. However, enhancing the selectivity of UF membranes remains a major challenge.

Rejection of solutes in UF membranes is mainly based on size exclusion [5]. However, several studies have demonstrated that the selectivity of charged membranes against charged solutes is also affected by charge interactions [5,6]. It is reported that charged membranes have high transmission for solutes around the isoelectric point (IEP) of the membrane or the solute [7,8]. At the IEP of the solute, the solutes are neutral so transport through the membrane is governed by size exclusion rather than charge interactions. In contrast, at pH below or above the IEP, the solutes are positively or negatively charged, respectively, and charge interactions will play a vital role in the filtration process. Charge attraction can enhance the transport of solutes through the membrane (high transmission), whereas charge repulsion may prevent solutes from passing through the membrane (low transmission) [9]. For example, Kumar et al. demonstrated that the highest transmission of ovalbumin and lysozyme through positively charged membranes was achieved at the IEP of each solute (pH 5 and pH 11, respectively).
respectively) [10–12]. In addition, the dissociated charged groups of a membrane will improve hydrophilicity, which could contribute to improving the antifouling properties [13]. Therefore, to address the selectivity challenge, a unique UF membrane needs to be developed that simultaneously possesses a hydrophilic surface and a controllable surface charge.

Polyelectrolyte (PE) layers are commonly used for surface modification by alternating deposition of positively and negatively charged PEs onto a charged surface [14,15]. For instance, Zhu et al. [16] reported precise control of the surface zeta potential (ζ) of thin polymeric layers constructed by electrostatic Layer-by-Layer (LbL) assembly of hydrophilic negatively charged poly (acrylic acid) (PAA) and positively charged poly (diacllydilethylammoniumchloride) (PDADMAC). The protocol allowed surface ζ potential in the pH range of 6–10 to be achieved as well as an optimal antifouling performance of a given material (polymeric membrane) and fouler (different microorganisms). Deposition of multi-layers could help to tailor membrane pore size; the method has been widely used to obtain nanofiltration (NF) membranes but has not been commonly reported for the fabrication of UF membranes. In particular, there is still a lack of understanding about how PE layers work in UF membranes, especially regarding the mechanisms governing the separation of charged solutes. Hence, by controlling the type of PE as well as the conditions during deposition, along with evaluating the effect of pH and ionic strength, the present study proposes to define an easy-to-implement method that can enable to control the retention of proteins, while identifying the mechanisms responsible for such variations of retention.

The objective of the current work is to investigate the transport behavior of a charged low molecular weight protein (lysozyme), through PE-modified UF membranes and to elucidate the role of charge interactions between the protein and the membrane. For this purpose, via selection of specific PEs, namely PAA and polydopamine (PDA) with negative charges, and PDADMAC with positive charge, we used the LbL deposition method to modify a commercial polysulfone (PSf) membrane. To avoid severe pore size reduction, we fabricated UF membranes by simply coating a single layer of PAA and PDADMAC onto the commercial PSf membrane by a two-step deposition process [17]. Scheme 1 shows the workflow of our modification protocol. In order to construct a positively charged top surface, we first co-deposited dopamine and PAA on the membrane surface and then deposited PDADMAC. The modified UF membranes were characterized by water contact angle (WCA) and zeta potential measurements. Next, the performance of the modified UF membranes was studied by measuring the water permeability, solute flux, retention, and flux recovery ratio. Furthermore, we studied the performance of the membranes modified by controlling the ratio of PAA and dopamine during co-deposition. To the best of our knowledge, it is the first time that the mechanisms involved in the separation of small charged solutes in PE-modified UF membranes are studied in detail.

2. Materials and methods

2.1. Materials

Polysulfone membrane with a molecular weight cut off (MWCO) of 100 kDa (Alfa Laval, Sweden) was used as the porous substrate in this study. Polyacrylionic (PDADMAC, Mn, 150,000–200,000 g/mol, 20 wt% in water), polyanionic PAA, Mn, 10,000 g/mol, dopamine hydrochloride, bovine serum albumin (BSA, 66.7 kDa), lysozyme (Lys, 14.3 kDa), and dextran (7229, 229 kDa) were purchased from Sigma Aldrich, Denmark. Tris-HCl, NaCl, KCl, NaH2PO4/7H2O, and KH2PO4 were also obtained from Sigma Aldrich, Denmark. Water used in this study was deionized (DI) water.

2.2. Membrane preparation

2.2.1. Charged ultrafiltration membrane preparation

Virgin PSf membranes were cut into several round pieces and then pretreated overnight with deionized water to remove residual preservatives. Prior to membrane modification, the carboxyl groups (–COOH) in PAA and the amino groups (–NH2) in dopamine were measured by NaOH titration and were determined to be 0.014 and 0.0053 mol/g, respectively. Two different membrane modification schemes were tested. First, the PSf membranes were immersed in dopamine solution in 50 mM Tris-HCl solution and were shaken for 3 h at 25 °C [18]. The membranes were then washed and immersed in a PDADMAC solution (0.2 wt%, 0.01 M NaCl solution) for 6 h to deposit cationic groups on the membrane surface. The membranes so obtained were referred to as PAA + PDADMAC membrane. In the second scheme, the PSf membranes were immersed in a dopamine and PAA solution mixture (with a 1:1 M ratio of –NH2 –COOH in 50 mM Tris-HCl solution) and were shaken for 3 h at 25 °C to allow co-deposition of PDA and PAA on to the PSf membranes. The coated PSf membranes (PAA/PDA membrane) were washed using deionized water. The PDA/ PAA membranes were then immersed in a PDADMAC solution (0.2 wt%, 0.01 M NaCl solution) for 6 h to deposit cationic groups and were subsequently rinsed and stored in DI water. The resulting membranes were referred to as PAA/PDA + PDADMAC membrane.

To investigate the effect of charge on separation performance, we fabricated several PAA/PDA + PDADMAC membranes with different molar ratios of –COOH and –NH2 groups, namely 0.1, 0.5, 1.0, 2.0, and 3.0 (Table 1).

2.3. Membrane characterization

WCA is a direct characteristic for evaluating the hydrophilicity of membranes. The WCA of the membranes was measured using a contact angle goniometer (OCA20, Dataphysics Instruments, Germany) at ambient conditions. 1 μl of MilliQ water was carefully dropped on the top surface of each membrane and the contact angle between the water and membrane was recorded for 120 s. To minimize experimental error, the contact angle was measured on at least six random locations for each sample and the average was reported.

Membrane surface charge was characterized using a SunPASS™ 3 electrokinetic analyzer (Anton Paar, Austria). Before measurement, the membranes were equilibrated with 1 mM KCl for 2 h. The zeta potential measurement was then performed using 1 mM KCl as a background electrolyte. The pH of the solution was adjusted by an automatic titrator from pH 3.0 to pH 11.0. For each pH value, four data were recorded and the average was reported.

2.4. Membrane ultrafiltration performance

2.4.1. Pure water permeability measurement

Pure water permeability (Jw) was tested using a dead-end Amicon Stirred Cell Model 8050 with an effective membrane area of 13.4 cm2. The membrane was pre-pressured for 30 min at 2.0 bar by DI water filtration to compact the membrane and stabilize flux. Next, the time was recorded for each 2 mL of permeate until 12 mL of permeate was collected at 1.0 bar. The average flux J was obtained as follows Eq. (1):

$$ J = \frac{V}{S \times t} $$

(1)
of 6 wt% phenol solution in a colorimetric tube. The mixed solution was vortexed for 30 s to obtain a uniform mixture. Then the mixture was vortexed for 30 s with the addition of 5 mL of concentrated H2SO4. The final mixture was incubated in a fume hood for 30 min and its absorbance was determined at 490 nm using a Shimadzu UV-1280 spectrophotometer. The concentration of dextran could then be calculated using a calibration curve, and the retention (R) obtained as follows Eq. (3):

\[
R = \left(1 - \frac{c_{\text{permeate}}}{c_{\text{feed}}} \right) \times 100\% \tag{3}
\]

where \(c_{\text{permeate}}\) and \(c_{\text{feed}}\) represents the concentrations of the permeate and feed solutions, respectively.

For the protein filtration experiments, lysozyme and BSA solutions were prepared at a concentration of 1 g/L using PBS buffer (0.1 M, pH 7.4). The concentration of lysozyme was determined by measuring the solution absorbance at 280 nm. Similarly, \(I_{p0}\), FDR, and retention were measured. To investigate the pH effect on membrane filtration performance, pH 3.0 and pH 11.5 buffer solutions were prepared using acetic acid/sodium acetate and Tris-HCl, respectively.

The correlation between the solute retention (R) and the FDR was then characterized by dividing FDR by R (termed FDR/R and indicating the degree of flux lost per retention).

2.4.3. Static protein adsorption of modified membranes

The interaction between charged molecules and membranes was also studied by static protein adsorption measurements using BSA and lysozyme solutions. In each case, the test membrane was fixed at the bottom of the filtration cell and 20 mL of 1 g/L protein solution was added. The cell was kept in a fridge (4°C) and incubated for 24 h to reach adsorption equilibrium. Protein adsorption (µg/cm²) was determined by the concentration difference of protein solution before (\(c_{\text{before}}\)) and after the adsorption (\(c_{\text{after}}\)) experiment as follows Eq. (4):
Table 2
WCA of virgin PFS membrane and membranes modified by different ratios of PAA and dopamine followed by PDADMAC selected layer deposition. Means with different letters (a, b, and A, B, C) are significantly different (ANOVA, P < 0.05).

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Static water contact angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Init(a)</td>
</tr>
<tr>
<td>Virgin PFS</td>
<td>87.4 ± 2.3ª</td>
</tr>
<tr>
<td>PAA + PDA</td>
<td>79.1 ± 1.3ª</td>
</tr>
<tr>
<td>PDA + PDADMAC</td>
<td>71.2 ± 2.3ª</td>
</tr>
<tr>
<td>PAA/PDA + PDADMAC</td>
<td>75.1 ± 2.1ª</td>
</tr>
</tbody>
</table>

Adoption amount = \( \frac{\text{C}_{\text{mem}} - \text{C}_{\text{in}}}{S} \)  

where \( S \) is the effective membrane area (13.4 cm²). The adoption amount was determined by testing at least three samples for each type of membrane and was reported as an average.

2.4.4. Statistical analysis

All experiments were performed at least in triplicate, and the values given are the average of such replicates. Significant differences between values were determined by one-way ANOVA (R programming language, USA).

3. Results and discussion

3.1. Hydrophilicity and pure water permeability

Co-deposition of PAA/PDA on the membrane resulted in a lower contact angle (70.1°) than that of the virgin PFS membrane (87.4°) (Table 2). Indeed, the deposition of PDA on the membrane surface normally contributes to increased hydrophilicity, as has also been observed elsewhere [19–21]. Deposition of PDA along with PAA or PDADMAC also contributed to narrowing the pore size of the membrane, which was confirmed by the observed decrease in water permeability and increase in dextran retention in all the modified membranes (Fig. 1a and b). Previous studies have also reported the concomitant decrease in contact angle and water flux on PFS membranes when PDA and/or additional layers were added. For example, Shi et al. reported a decrease of WCA (70° to 56°) and pure water flux (680 L/m² h to 380 L/m² h) after co-deposition of PDA and polyethyleneimine (PEI) on PFS membranes [20].

3.2. Protein retention after deposition of selected polyelectrolytes

Since dextran is a neutral molecule and therefore not affected by membrane charge, the charge effect on membrane performance was investigated by using lysozyme as a model solute. Lysozyme has an isoelectric point of 11.35, which means that it has a positive charge at conditions near neutral pH (7.4). Additionally, lysozyme (14 kDa) has a much smaller molecular size than the virgin PFS membrane (100 kDa), so the potential charge effects become relatively more important compared to steric hindrance during transport through the membrane. The observed lysozyme retention by the virgin PFS membrane was 7% (Fig. 2a). As extra layers were incorporated on the membrane surface, retention of lysozyme increased compared to the virgin membrane, which was expected due to the impeding effect that the extra layers may exert on the passage of lysozyme. Lysozyme retention of the positively charged PDADMAC-deposited membranes was observed to be 40% for the PDA + PDADMAC membrane and 30% for the PAA/PDA + PDADMAC membrane, which was lower than that of the negatively charged PAA + PDA membrane (50%). The change in the membrane surface charge was confirmed by zeta potential measurements (Fig. 3). The virgin PFS membrane and PAA/PDA co-deposited membranes had similar IEF (3.7). After PDADMAC deposition, the IEF was increased to 6.8 and 7.5 for PDA + PDADMAC and PAA/PDA + PDADMAC membranes, respectively. At the working pH (7.4), the virgin and PAA + PDA membranes had a negative charge of -7.5 mV and -25 mV, respectively. The positively charged PDADMAC layer made the surfaces of the PDA + PDADMAC membrane (+1 mV) and the PAA/PDA + PDADMAC membrane (+2 mV) positive. In general, charged membranes exhibit high rejection against solutes of the same charge and low rejection against solutes with opposite charge.
Fig. 2. Protein ultrafiltration performance of virgin and modified membranes; (a) Lysozyme flux and retention, where — represents charge, —— represents strong negative charge, + + represents positive charge, + + + represents strong positive charge, (b) Flux decline ratio after lysozyme filtration and index of flux decline versus retention. Means with different letters (a, b) are significantly different (ANOVA, p < 0.05).

Fig. 3. Zeta potential of virgin PSF and membranes modified with different layers.

However, the opposite effect has been observed before for lysozyme in charged modified membranes. For example, Liu et al. [24] observed that membranes covered with polyamions (PSS + PEI + PAA), developed so-called "cation-exchange sites" and were able to bind to lysozyme, which resulted in adsorption of the protein onto the surface which contributed to increased retention. The higher retention by the negatively charged PAA/PDA membrane observed here could thus be explained by adsorption of lysozyme onto the membrane surface, while the addition of the positive layer of PDADMAC may have prevented such an effect, resulting in lower retention. Lysozyme molecules are considerably smaller than the membrane pores in size and can pass through the membrane relatively easily despite the existence of charge repulsion.

Adsorption of lysozyme to the PAA/PDA membrane surface normally results in partial pore blocking and fouling and is thus suggested to be the cause for the high FDR observed (80%) (Fig. 2b) [25,26]. By contrast, the PAA/PDA + PDADMAC membrane surface held a positive surface charge, which possibly prevented contact between lysozyme and the membrane walls, and resulted in lower fouling and in turn a higher flux (FDR of 35%) (Fig. 2b). In order to limit the effect of retention from the FDR, a fouling index was defined as the ratio between FDR and rejection, though it would naturally be more difficult to recover initial fluxes after high retention filtration. When considering the retention, no significant difference was observed amongst the three modified membranes. However, all these membranes showed better antifouling properties compared to the virgin membrane for which a considerable FDR was observed despite the very small values of retention of lysozyme. The fouling ratio index should be taken cautiously, though, because values of flux decline and retention cannot be directly compared in a quantitative manner.

3.3. Protein retention through layers with different charge balance

To further study the effect of charge on the membrane filtration performance, different concentrations of PAA were used during deposition of the PAA/PDA layer while maintaining the PDA concentration as well as the concentration of the subsequent PDADMAC layer constant. The PAA concentrations were selected so that the balance between carboxylic and amino groups ranged from 0.1 to 3 (M0.1-M3.0) (Table 1). Since PAA contains a large number of negatively charged carboxylic groups, the use of a high PAA concentration is expected to result in a higher amount of PDADMAC deposited on the membrane surface, which in turn would result in strong positively charged membranes. As expected, with the PAA/PDA ratio increasing, the IEP of the modified membranes increased to 5.1, 7.4, 7.5, 7.7, and 7.8 for M0.1, M0.5, M1.0, M2.0 and M3.0 membranes, respectively (Fig. 5). The modified membranes become positive at the working pH. The I_p decreased considerably from 150 L/m²h bar for M0.1 to 80 L/m²h bar for M3.0, which confirmed that an increasing PAA ratio had a negative impact on membrane water permeability. This decrease was ascribed to the fact that an increasingly negative charged PAA membrane could attract more PDADMAC, which would be expected to result in decreased pore size (Fig. 4a). Du Chanoin et al. [27] found that when PEs with opposite charge are deposited on charged membranes, the I_p decreases considerably and that such decrease can be correlated with the increase in thickness of the membrane (in the case of Du Chanoin et al. from 7 to 84 nm when the PE concentration increased from 0.032 mM to 20 mM), which confirms the hypothesis of higher PE attachment. This result is consistent with the decrease in I_p and dextran transmission when additional layers of PE were added to the membrane (Fig. 1). Interestingly, when increasing the amount of PDADMAC deposited on the membrane surface, the rejection of lysozyme decreased from 43% for M0.1 to 23% for M3.0 (Fig. 4b). This is in accordance with our previous hypothesis that a higher electrostatic repulsion could prevent contact and thus decrease adsorption of lysozyme to the membrane surface with the result that more lysozyme molecules are able to pass through the membrane. The decrease in retention is indeed consistent with the results shown in Fig 2, which shows that
rejection increased for negatively charged membranes but decreased for positively charged membranes. M2.0 and M3.0 membranes exhibited a similar lysozyme retention, which possibly indicates that the amount of PDADMAC able to bind the membrane reached a maximum beyond a certain ratio of PAA and PDA.

Furthermore, static protein adsorption measurements were performed in order to confirm the occurrence of increased positive charge density on the membrane with increasing PDADMAC deposition. When the modified PAA/PDA + PDADMAC membranes were exposed to BSA, considerably higher adsorption of BSA was observed at increasing PAA ratios (and in turn at increasing amounts of PDADMAC) (Fig. 4c). BSA is negatively charged at the working pH, and would thus be expected to bind to the positive PDADMAC surface. In contrast, the adsorbed amount of lysozyme (positively charged) decreased considerably after modification (Fig. 4c). Compared to virgin PSi membrane, the adsorbed amount of lysozyme decreased by almost 50% for all modified membranes, which confirms that lysozyme adsorbs with more difficulty to PDADMAC modified membranes.

Data on the FDR during lysozyme filtration using the modified membranes confirmed the positive effect of addition of PDADMAC on the antifouling properties. Indeed, whilst severe irreversible fouling occurred in the virgin PSi membrane after lysozyme filtration (61% flux decline), decline was reduced to 50% for the M0.1 membrane and to 28% for both the M2.0 and M3.0 membranes, which indicated that membrane modification could bring about significant reduction in irreversible fouling. The FDR/R index decreased dramatically from 8.5 for virgin PSi to approximately 1.2 for all PDADMAC modified membranes, which shows the improvement of antifouling properties of PDADMAC modified membranes.

3.4. Dextran and protein retention at different ionic strengths

Increasing the background ionic strength of the PDADMAC deposition solution during membrane modification with PAA/PDA + PDADMAC caused a dramatic drop in dextran solution flux when the concentration of NaCl was increased from 0.01 to 0.1 M (Fig. 6a). A significant increase in dextran retention was observed when
the concentration of NaCl of the PDADMAC deposition solution was increased from 0.01 to 0.5 M, while a progressive decrease was detected with additional increases in NaCl concentration. In the LbL deposition processes, two phases of charge compensation that affect the structure and hence the performance of the PE layer have been reported [25]. At low background ionic strengths, the charges of the PEs experience low screening from salt ions of the deposition solution, and the oppositely charged PEs deposited on the membrane dominate the charge balance (intrinsic charge compensation). In our study, intrinsic charge compensation seemed to occur until NaCl concentration reached 0.5 M; the result was a dense PE layer with enhanced resistance to water and solute transport (verified by low flux and high rejection of dextran in Fig. 6a). As the ionic strength was increased beyond 0.5 M, a so-called extrinsic charge compensation largely occurred, where salt counterions from the deposition solution dominated the charge balance [28]. As a consequence, the PE layer became thicker but less dense, because the charges of PAA and PDADMAC were screened by salt ions from the deposition solution, which limited PE adsorption and resulted in larger pores (lower retention and higher water permeability) [27]. This tendency became clearer during filtration of lysozyme, where lysozyme flux and transmission decreased as ionic strength increased from 0.01 M to 0.5 M, and then slightly increased at higher ionic strengths (Fig. 6b).

3.5. Protein retention and membrane fouling properties at different pH

In a further attempt to study the effect of charge balance on filtration performance, the pH of the solution to be filtered was modified. The pH of the solution is expected to affect the transport of proteins through the charged membranes because both the charge of the proteins and the membranes will vary. To test this idea, three values of pH were selected: 3.0 (at which lysozyme has a high positive charge density of 25 mV), 7.4 (3 mV), and 11.5 (at which the lysozyme is negatively charged) [29]. Similarly, the positive charge density of the PAA/PDA + PDADMAC membrane surface decreased from 26 mV to 2.4 mV as pH increased from 3.0 to 7.4 (Fig. 5). At pH 11.5, the PAA/PDA + PDADMAC membrane surface became negative (-15 mV). High retention of lysozyme was detected at the lowest and highest pH values (95.1 and 69.5% at pH 3.0 and 11.5, respectively), while a sharp decrease of retention was observed at pH 7.4 (30.1%) (Fig. 7a). While the high retention at high pH was explained by the adsorption of lysozyme to the oppositely charged membrane, the high retention at low pH seems to contradict our previous results that indicated a negative effect on the retention to membrane-solute systems of the same charge. Previous work, however, postulates that electrostatic interactions exert a key role in the retention of proteins only when the zeta potential exceeds 20 mV, whereas size exclusion is the dominant mechanism at lower protein charge densities [30]. If this hypothesis were correct, it would mean that the electrostatic interactions would indeed play a role in the high values of rejection when proteins have the same charge as the membrane, even when there is a notable difference between the size of the protein and the pore size of the membrane. However, as the charge of the protein moderates, a protein-membrane system of the same charge could contribute to a decrease in solute retention by hampering the possibility of adsorption of the protein to the membrane matrix. This can be inferred from the results showed in Fig. 7a; while lysozyme is poorly retained at pH 7.4 (moderately charged protein – same charged membrane), the retention increases again at high pH, probably due to adsorption to the charged membrane (polyelectrolytes have adsorption capacity for charged proteins). With regard to BSA, severe fouling was observed at all pH values tested. BSA is negatively charged at neutral and high pH (IEP 4.7), which makes it easily adsorbed on the positively charged modified membrane surface and leads to severe fouling. At pH 3.0, the high retention may also be explained by aggregation of BSA molecules. Being relatively near to the IEP, the charge may be weak enough to make aggregation unavoidable, and in this manner, the effective size of the solute is increased. Additionally, the BSA molecules may also be embedded in the membrane pores and cause pore blocking. However, the high recovery of permeate flux with simple flushing at pH 3.0 compared to higher pH (Fig. 7b) indicates that it is mostly reversible fouling that occurred at low pH, compared to more irreversible fouling when pH was increased.

4. Conclusions

Modification of UF membrane surface by deposition of PE layers was found to be a facile approach for controlling protein retention. PE-modification can be easily implemented in membranes with much larger pore size than the charged solute to minimize the size exclusion effect and fouling while retaining high retention and water flux. The retention of positively charged lysozyme increased up to 50% for the PE-modified membrane with the negative PEs (PAA/PDA) due to adsorption of the protein on the membrane surface and pore walls. Interestingly, lysozyme retention of positively charged PDADMAC-deposited membranes was observed to be less than that of PAA/PDA modified membranes. However, the lysozyme flux and retention of the PDADMAC-deposited membranes could be adjusted by changing the ionic strength during PAA/PDA + PDADMAC membrane modification and varying solution pH. Table 3 summarizes the obtained analytical merit/or obtained effects as a result of membrane modification process. The results reveal that adjusting solution pH changes the charge density of lysozyme and to a certain degree reverses the behavior of the positively charged electrolyte membrane, which dramatically enhances...
solvent rejection. Therefore, in comparison with the positively charged surface, the negatively charged surface showed high retention but severe fouling. On the other hand, the lysozyme flux decreased at low ionic strength, while it increased slightly at higher ionic strength. In this approach, size exclusion is not the main separation mechanism, and retention of charged proteins can be effectively controlled by membrane surface charges. This approach can be easily integrated into commercial polysulfone-based membranes for obtaining tailored-retention product streams containing protein, such as lactose separation from whey protein. Future studies may consider further manipulate the properties of the PE layer (e.g., post-modification via introduction of additional functional groups) to tune the rejection properties for protein species and other solutes.

Table 3
Summary of the obtained analytical merit/or obtained effects as a result of membrane modification process. + indicates enhanced, ++ indicates strongly enhanced, − indicates decreased, and −− indicates dramatically decreased.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Selected layer (Negative to positive)</th>
<th>Charge Balance (Increasing)</th>
<th>Ionic Strength (Low to high)</th>
<th>pH Balance (Low to high)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure Water Flux</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Charge (increasing)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>n/a</td>
</tr>
<tr>
<td>Hydrophilicity</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Pure Size</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Denaturants Flux</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Retention</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lysozyme Flux</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Flow</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>FDR</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
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<td>BSA Flux</td>
<td>−</td>
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<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Retention</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flux</td>
<td>+</td>
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<td>+</td>
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<td>Antifouling effect</td>
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</tbody>
</table>

Fig. 7. Ultrafiltration performance of modified membranes (PAA/PDA + P3ADMAC) using BSA and lysozyme at different pH. (a) Retention of solutes, (b) Flux decline ratio.

References


Grafting of charged small molecules on a commercial membrane surface to improve both permeability and selectivity

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In this study, we grafted charged, small molecules taurine (Tau), cysteine (Cys) and PEI 600 onto the surface of PSf membrane using a tannic acid (TA)-assisted coating method. FTIR and Zeta potential measurements verified the existence of charged small molecules on the surface of the modified membranes. For Tau and Cys grafted membranes, the negative charge on the membrane surface increased with solution pH. Presence of these small molecules not only improved the flux or permeability of modified membranes but also effectively inhibited membrane fouling. Charge interaction plays an important role in the protein filtration process. When pH is lower than the protein isoelectric point, charge attraction leads to pore blocking and severe membrane fouling. Under alkaline conditions, the more negative charges the protein and modified membrane carry, the less the membrane fouls, resulting in more protein passing through the membrane. However, the high selectivity of modified membrane to BSA and Hb was obtained at a pH slightly higher than isoelectric point of Hb, where membrane fouling and charge repulsion were in balance. We optimized the operating conditions of the Cys grafted PSf membrane, and the results showed that 6 h TA-coated Cys grafted PSf membrane can simultaneously achieve better selectivity and fouling resistance under low transmembrane pressure and appropriate stirring rate. Furthermore, Cys and Cys+PGA were grafted on the surface of PAN and US100 membranes.
Cys grafting was found to simultaneously enhance the permeability (pure water flux increase from 120 L/m² h bar to 210 L/m² h bar), selectivity (increase from 2 to 18) and fouling resistance (flux loss reduced from 22% to 9%) of the PAN membrane. In addition, polyelectrolytes grafting not only damages membrane permeability but also enhances membrane fouling.

1. Introduction

Ultrafiltration (UF) is widely used in food, medicine, water treatment, bio-separation and other fields [1] due to its advantages of low energy consumption, simple operation, mild conditions, and environmental friendliness etc [2]. However, UF also faces huge challenges such as low permeability, weak selectivity and severe membrane fouling [3]. These shortcomings are mainly related to the chemical properties of the membrane material and structure [4]. To counter these drawbacks researchers have made great efforts in membrane modification and have proposed many feasible methods, including blending modification, bulk modification and surface modification [5]. There are also many membrane modification schemes that are a combination of several modification methods. The performance of membranes has been significantly improved through these modification methods [6].

Selectivity of UF membranes is mainly based on size exclusion [7]. However, several studies have demonstrated that the selectivity of charged membranes against charged solutes is also affected by charge interactions [8]. It is reported that charged membranes have high transmission for solutes around the isoelectric point (IEP) of the solute [9]. At their IEP, solutes are neutral so transport through the membrane is governed by size exclusion rather than charge interactions. In contrast, at pH below or above the IEP, solutes are positively or negatively charge, respectively, and charge interactions will play a vital role in the filtration process. Charge attraction enhances the transport of solutes through the membrane (high transmission), whereas charge repulsion may prevent solutes from passing through the membrane (low transmission) [10]. For example, Kumar et al. demonstrated that the highest transmission of ovalbumin and lysozyme through positively charged membranes was achieved at the IEP of each solute (pH 5 and pH 11, respectively) [11]. In addition, the dissociated charged groups of a membrane will improve membrane hydrophilicity, which could contribute to improving the antifouling properties [12]. Therefore, to address the
selectivity and fouling challenge, a unique UF membrane needs to be developed that simultaneously possesses a hydrophilic surface and a controllable surface charge.

In a previous study, we found that although membrane coating with polyelectrolytes (PEs) could adjust substance rejection rate and improve membrane fouling resistance, the permeability of the membrane was greatly compromised [13]. This is because the coated macromolecular PEs block the membrane pores, making it difficult for substance and water molecules to penetrate [14]. We must therefore find other functional molecules for membrane surface modification. Hydrophilic, charged small molecules such as taurine (Tau), Cystine (Cys) and PEI 600 may be a better modifier because they have little effect on membrane structure [15]. To introduce these small molecules onto membranes, we need to introduce an active layer on the membrane surface.

Tannic acid (TA) is a type of plant polyphenol that can be directly extracted from a variety of plant-based materials, including tea, wood and galls. The cost of such TA is then much lower than that of dopamine [16]. As with dopamine coating, TA layers also can be formed spontaneously in a weak alkaline buffer aqueous solution at room temperature [17]. Because of the presence of large numbers of hydrophilic units (-OH), the TA layer will make the membrane surface more hydrophilic. Chen et al. [18] constructed an antifouling surface based on use of tannic acid and zwitterionic polymers. The prepared surface could effectively resist protein adsorption, bacterial attachment, and platelets adhesion.

Therefore we should be able to graft different types of charged small molecules onto the membrane surface using TA assisted coating. Scheme 1 shows the process of tannic acid coating assisted grafting of small molecules on a polymeric membrane surface. Characterization methods such as FTIR, water contact angle and zeta potential measurement were performed to verify that small molecules were successfully grafted onto the surface of PSf membranes. Next we conducted detailed study of the permeability, selectivity and fouling resistance of the modified PSf membranes. Furthermore, we optimized the selectivity of the ultrafiltration process by adjusting the pH of the solution, the operating pressure and the stirring rate. In addition, we modified PAN and US100 membranes and investigated their selectivity to BSA and Hb.
Scheme 1 Tannic acid coating assisted grafting of small molecules on a polymeric membrane surface

2. Materials and method

2.1 Materials and reagents

BSA (66.7 kDa, pI 4.8) and hemoglobin (64 kDa, pI 6.8) were purchased from Sigma-Aldrich. Tannic acid, PEI 600, cysteine, taurine and poly-γ-glutamic acid (PGA) were also obtained from Sigma-Aldrich. All reagents are analytical grade with no further purification. A polysulfone (PSf) membrane (100 kDa) was obtained from Ande membrane separation technology engineering (Beijing, China) Co. Ltd; a polyacrylonitrile (PAN) membrane (100 kDa) was purchased from Sterlitech Corporation (Kent, USA); a hydrophilic modified PSf membrane (100 kDa) was obtained from Lenntech Water Treatment Solutions (Delfgauw, Europe).

2.2 Membrane modification

Virgin PSf membranes were cut into several round pieces and then pretreated overnight with deionized water to remove residual preservatives. TA solution at a concentration of 2.0 g/L was obtained by dissolving tannic acid powder in Tris–HCl buffer solution (50 mM, pH 8.5). Next, pre-cleaned membranes were immersed in TA solution for several hours to coat with TA. TA coated membranes were then washed with deioined water to remove unstable
particles and named as PSf/TA membrane. Taurine, cysteine, PEI600 and cysteine+PGA solutions at a concentration of 2.0 g/L were prepared by dissolving these substrates in Tris–HCl buffer solution (10 mM, pH 8.5). TA coated membranes were immersed in the prepared small molecules solutions for 6 h at room temperature. Grafted membranes were washed with deionized water and then immersed in glutaraldehyde solution (2.5 wt%) for 2 h. The obtained membranes were named as PSf/TA-Cys, PSf/TA-Tau and PSf/TA-PEI membranes.

2.3 Membrane characterization

Membrane samples were cleaned and dried. ATR-FTIR was performed at 2 cm-2 resolution with a Thermo Nicolet IR 200 spectroscope (Thermo Nicolet Corporation, USA) in the range of 400 – 4000 cm-1. Typically, 64 scans were signal averaged to reduce spectral noise. At least three replicate spectra were obtained for each of the three membrane types. These spectra were subsequently corrected for wavelength-dependent penetration depth and background subtraction using OMNIC™ software.

Field emission scanning electron microscopy (FE-SEM, Hitachi S-4800, Japan) with an accelerating voltage of 20.0 kV was used to study the morphology of the membrane. Before introduction to the electron beam, each membrane sample was coated with a thin layer of gold by gold sputtering. SEM images were taken at a magnification of 5000 and 50,000 at 20 kV. At least six replicate SEM images were obtained for each membrane type.

Water contact angle (WCA) is a direct characteristic for evaluating the hydrophilicity of membranes. The water contact angle (WCA) of the membranes was measured using a contact angle goniometer (OCA20, DataPhysics Instruments, Germany) in the room environment. 1 µL of MiliQ water was carefully dropped on top surface of the membrane and the contact angle between the water and membrane was recorded on video for 120 s. To minimize experimental error, the contact angle was measured for at least six random locations for each sample and the average was reported.

Membrane surface charge was characterized using SurPASS™ 3 electrokinetic analyzer (Anton Parr, Austria). Before measurement, the membranes were equilibrated with 1 mM KCl for 2 h. Then the zeta potential measurement was performed using 1 mM KCl as electrolyte solution. The pH of the solution was adjusted by an automatic titrator from pH
3.0 to pH 11.0. For each pH value, four data records were taken and the average was reported.

2.4 Ultrafiltration of BSA/Hb binary solution

The main properties of UF membranes include permeability, rejection and fouling resistance. Binary solution was prepared at a concentration of 0.5 g/L BSA and 0.1 g/L Hb in 10 mM buffer solution (containing 15 mM NaCl). Ultrafiltration experiments of binary solutions were performed in a dead-end filtration device, and the effective area of the test membrane was 11.34 cm\(^2\). The main steps were as follows. (1) The membrane was fixed in the cell and the pressure was adjusted to 2 bar to pre-press the membrane for 30 minutes. The pressure was then adjusted to 1.0 bar for pure water permeability measurement. The entire experimental procedure was performed at room temperature. The pure water permeability, rejection and fouling resistance were calculated by the following formulas,

\[
J = \frac{V}{A \times t}
\]

(1)

\(J\) represents the pure water permeability, \(V\) represents the volume of pure water that passes through, \(A\) represents the effective area of the membrane, and \(t\) represents the filtration time.

(2) Next, the deionized water was replaced with the protein solution to be separated and the ultrafiltration experiment was performed at 1.0 bar. The filtration time of the permeate was recorded and the UV absorption of permeate was measured.

\[
R(\%) = \left(1 - \frac{c_p}{c_f}\right) \times 100
\]

(2)

\(R\) represents the rejection rate, \(c_p\) and \(c_f\) represent the protein concentration in the permeate and feed solution, respectively. (3) The fouled membrane was washed with deionized water several times, and the pure water permeability was measured again. The flux recovery rate (FRR) was used to characterize the fouling resistance of the membrane. \(FRR\) indicates the degree of membrane permeability recovery after fouling and cleaning.

\[
FRR(\%) = \left(\frac{J_{w,2}}{J_{w,1}}\right) \times 100
\]

(3)

\(J_{w,1}\) and \(J_{w,2}\) are the initial water permeability of the membrane and the water permeability of the membrane after the fouling-cleaning process, respectively.

A UV-1280 visible spectrophotometer (Shimadzu Company, Japan) was used to conduct
quantitative analysis of proteins in the feed solution and permeate solution under UV light (280 nm). Ultrapure water was used as the blank control sample. In addition, hemoglobin concentration could be measured at a wavelength of 406 nm.

Table 1. Comparison of BSA and Hb characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>BSA</th>
<th>Hemoglobin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size related parameters</td>
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<td></td>
</tr>
<tr>
<td>Molecular weight (kDa)</td>
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<td>64-67</td>
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<td>Equivalent ellipsoidal dimension (nm)</td>
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<td>Diffusion coefficient (10⁻⁷ cm² s⁻¹)</td>
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<td>7.5</td>
</tr>
<tr>
<td>Electrostatic parameters</td>
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<td></td>
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<tr>
<td>Net charge (at pH 7.4)</td>
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<td>-7.2</td>
</tr>
<tr>
<td>Isoelectric point</td>
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<td>Hydrophilic amino acids content (g/100 protein)</td>
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</table>

3 Results and discussion

3.1 Membrane surface composition and property

To determine change in membrane surface chemistry, FTIR spectroscopy was used to characterize the surface composition of the membranes. Although FTIR spectra of virgin PSf and modified membranes are similar, the appearance of some weaker new peaks verifies the existence of new functional groups. As shown in Figure 2, there is a new peak at 1720 cm⁻¹ for all modified membranes, which belongs to the carbonyl (C=O) stretching vibration [19]. The appearance of C=O indicates that TA is firmly coated on the PSf membrane surface. A new peak at 1040 cm⁻¹ belongs to the C-N stretching vibration. In general, TA will react with Tris (H₂N-C(CH₂OH)₃) during the oxidation process, which will introduce C-N groups into
the TA layer [20]. Therefore FTIR spectra of the PSf/TA membrane exhibits a peak at 1040 cm\(^{-1}\). There is no other significant characteristic peak for modified membranes because the peaks of virgin PSf membrane overlap with those of grafted molecules. To further confirm the existence of charged molecules, other characterization is necessary.

![FTIR spectra of PSf membranes](image)

Figure 1. FTIR spectra of virgin and modified PSf membranes based on TA coating and charged molecules grafting.

Zeta potential measurement was used to characterize the change in surface charge of modified PSf membranes. Since the grafted small molecules carry numerous charges, the surface of modified membranes can be expected to exhibit different charges. Figure 2 shows the charge on the surface of virgin and modified PSf membranes at different pH. It is clear that virgin PSf membrane possesses few negative charges in the test pH range of 3-10 and that the amount of charge does not change much with pH. For small molecule grafted membranes, the surface charge of membrane varies with increasing pH. For Tau and Cys grafted membranes in particular, the membrane surface charge increases sharply as the pH increases. The increased surface charge has an important influence on retention and fouling resistance of modified PSf membranes. As for the PEI grafted membrane, the modified membrane exhibits a strong positive charge when pH is low since PEI carries a lot of positive charges. When the pH is higher than 6, the surface of the modified membrane exhibits a
negative charge. The change in surface charge of modified membrane further confirmed that small molecules were successfully grafted onto the membrane surface. pH-induced changes in membrane surface charge can be used to adjust the separation performance of modified membranes.

Figure 2. Zeta potential of modified and virgin PSf membranes

We characterized the hydrophilicity/hydrophobicity of virgin and modified PSf membranes using dynamic water contact angle measurement. As shown in Figure 3, the virgin PSf membrane has a high water contact angle up to 87, which means that a lot of protein will be non-specifically adsorbed on the membrane during the separation process, and lead to membrane fouling. After TA coating, the water contact angle was reduced to 67.5 and the hydrophilicity of the membrane was enhanced, which is caused by abundant phenolic hydroxyl contained in the TA structure. Grafting of additional small molecules resulted in further changes in hydrophilicity of modified membranes. After Cys grafting, the contact angle decreased to 62 and the hydrophilicity further increased. The carboxyl and sulfhydryl groups in Cys structure greatly improve the hydrophilicity of modified membrane. The water contact angle of Tau grafted membrane was around 71, which is close to that of the TA coated membrane. After Tau grafting, only sulfonic acid group remained on membrane surface, so the contact angle did not change much. With regard to PEI grafted membrane, the contact
angle increased to 80.5 and the hydrophilicity decreased. This may be related to the weak hydrophilic ability of amino groups in the PEI structure [21]. The variation in hydrophilicity/hydrophobicity of the membrane surface thus has a significant impact on membrane permeability and fouling resistance.

Figure 3. Water contact angle of virgin and modified PSf membranes based on TA coating and charged molecules grafting

3.2 Permeability of PSf based membranes

Figure 4 shows the changes in membrane water permeability. Virgin PSf membrane had a low water permeability of 305 L/m² h bar. After TA coating, the water permeability increased to 400 L/m² h bar as the hydrophilicity of the membrane increases. With additional grafting of small molecules, the water permeability of Tau modified membrane increased to 480 L/m² h bar, while the more hydrophilic Cys modified membrane increased to 515 L/m² h bar, which was 1.71 times that of the virgin PSf membrane. However, the water permeability of PEI grafted membrane was not much different from that of TA grafted membrane, and was even reduced to a certain extent, which further confirms the reduction of the membrane hydrophilicity. The change of membrane water permeability is consistent with the change of water contact angle in Figure 3. The enhanced hydrophilicity greatly improves membrane permeability, which affects membrane separation performance.
3.3 Ultrafiltration performance of PSf based membranes

Next, we performed ultrafiltration experiments on virgin and modified PSf membranes and studied their separation behavior. BSA and Hb have similar molecular weights (66.7 kDa and 64.5 kDa, respectively) but different isoelectric points (4.8 and 6.8, respectively), so we can expect to separate their mixture by charge interaction. A BSA-Hb binary solution was used for an ultrafiltration experiment during which the solution flux, protein transmission, selectivity and fouling resistance of virgin and modified membranes were studied under different pH conditions.

Figure 5 a shows the binary solution flux of virgin and modified PSf membranes at different pH. The solution flux of virgin PSf membrane did not change much with pH, while modified membrane showed pH-related solution flux. Charge interaction played a key role during the separation process. BSA and Hb both exhibited positive charges at pH lower than 4.8 and were easily adsorbed on the surface of negatively charged Tau and Cys grafted membranes, which resulted in low solution flux. At pH between 4.8 and 6.8, although only positively charged Hb would be adsorbed on the surface of the modified membranes, the charge attraction between BSA and Hb molecules forms large agglomerates and blocks the membrane pores [22]. Therefore the Tau and Cys grafted membranes had low solution flux.
under acidic conditions. As the pH of solution further increased, the negative charges carried by protein and membrane increased simultaneously. The strong charge repulsion hinders the contact between protein molecules and membrane material, preventing protein adsorption and membrane fouling. Therefore the solution flux increased with pH. However, for PEI grafted membrane, the active -NH$_2$ groups might react with protein which severely damages the solution flux.

The flux of binary solution affected the transmission of BSA and Hb. As shown in Figure 5b, due to blockage of membrane surface and pores caused by fouling, the PEI grafted membrane retained almost 100% of the two proteins. Only trace amounts of BSA and Hb could pass through PEI grafted membrane, therefore we did not show transmission for PEI grafted membrane. The transmission of BSA and Hb by virgin PSf membrane hardly changed with pH, which is consistent with solution flux. The surface of virgin PSf membrane has little negative charge, and the amount of charge does not change with pH. Therefore there is virtually no effect of charge in the virgin. For Tau and Cys grafted membranes, membrane transmission of BSA and Hb increased with the increase of pH. At pH 6.8, the isoelectric point of Hb, the two modified PSf membranes showed the lowest transmission of BSA and Hb. Neutral Hb molecules with high concentration are prone to agglomerate to form macromolecules and block the membrane pores [23]. Therefore the modified membranes had a low protein transmission at this pH. However, BSA and Hb molecules become negatively charged with further pH increase. The protein molecules repel each other and dissolve completely, reducing the large aggregates in solution. Furthermore, charge repulsion hinders the contact between negatively charged protein and membrane material, which inhibit non-specific adsorption and membrane fouling. Therefore more protein can pass through the modified PSf membranes. In addition, strong charge repulsion is more conducive to protein transmission. At pH 9.0, the Tau modified membrane had the highest transmission of BSA and Hb, about 48%. This is consistent with the high surface charge of Tau modified PSf membrane in Zeta potential measurement results.

Although the transmission of protein by modified PSf membranes increases with increase in solution pH, the difference between transmission rates of BSA and Hb led to different selectivity. As shown in Figure 5c, virgin PSf and modified membranes all exhibited low
selectivity to BSA and Hb over the pH range 4.0 to 6.8. This is because Cys and Tau modified membranes had low transmission of BSA and Hb, and the difference in transmission was small. At pH 7.5, Cys grafted membrane showed the highest selectivity for BSA and Hb. Under these conditions, although some Hb molecules still blocks the membrane pores, the modified membrane allows more negatively charged Hb to pass through. However, the transmission of BSA molecules is low due to membrane fouling, thus the Cys grafted membrane obtained the highest selectivity at this pH. As pH increased further, fouling was suppressed while the transmission of BSA and Hb increased at the same time. The small difference in BSA and Hb transmission led to low selectivity. For the Tau grafted PSf membrane, since the large number of negative charges carried on its surface prevented membrane fouling, more BSA and Hb molecules were simultaneously passed through, resulting in poor selectivity.

Next, we analyzed the fouling resistance of virgin and modified PSf membranes, and the results are shown in Figure 5 d. There was no significant change in permeability loss for virgin PSf membrane when binary solution pH varied. However, Tau and Cys grafted membranes exhibited pH-related permeability loss. When pH was lower than 6.8, due to the agglomeration of protein molecules and charge attraction induced protein adsorption, the membrane suffered severe fouling. Therefore the two modified membranes exhibited high permeability loss. As pH increased, charge repulsion suppressed fouling and led to low permeability loss. At pH 9.0, Tau and Cys grafted membranes exhibited the lowest permeability loss of 38% and 42%, respectively. For the PEI modified membranes, membrane fouling is always serious due to the positive charges and reactive sites on membrane surface.

By studying the protein transmission, selectivity and fouling resistance of virgin and small molecules modified PSf membranes in different solution environments, it can be concluded that selectivity is determined by both charge interaction and membrane fouling. Charge attraction causes membrane pore blockage and fouling, which results in low selectivity. Strong charge repulsion inhibits membrane fouling and allows more proteins to pass through. However, the high protein transmission for both BSA and Hb reduces the membrane selectivity. Although there is some membrane fouling when pH is slightly higher than the
isoelectric point of Hb, differences between the transmission rates of BSA and Hb determine the membrane with the highest selectivity. Compared with the Tau grafted PSf membrane that carries more charge, the more hydrophilic Cys grafted PSf membrane has better selectivity for BSA and Hb.

Figure 5. BSA and hemoglobin binary solution filtration performance of modified and virgin PSf membranes at different pH. (a) binary solution flux, (b) observed transmission, (c) selectivity, (d) permeability loss. The binary solution is composed of 0.5 g/L BSA and 0.1 g/L Hb.

3.4 Influence of operating conditions on permeability and selectivity of PSf/TA-Cys membrane

We selected Cys grafted PSf membrane to study the influence of operating conditions on membrane permeability and selectivity. We investigated the permeability loss and selectivity of modified membranes under different transmembrane pressure, stirring rate and TA coating time. As shown in Figure 6 a, the permeability loss of Cys grafted PSf membrane did not change much with transmembrane pressure. However, its selectivity to BSA and Hb
increased as transmembrane pressure decreased. Membranes suffer severe concentration polarization under high transmembrane pressure, which weakens the charge interaction [24] and results in low selectivity. On the contrary, the concentration polarization effect is inhibited under low transmembrane pressure. However, charge interaction has a greater effect on transmission of BSA and Hb and therefore selectivity is improved.

Figure 6 b shows the permeability loss and selectivity of modified membranes at different stirring rates. We found that as stirring rate increased, membrane permeability loss decreased, suggesting that fouling is suppressed. When stirring rate was higher than 250 rpm, membrane permeability loss increased, indicating that more fouling occurs. At low stirring rates, mechanical force promotes the movement of protein molecules and membrane fouling is inhibited [25], so there is less permeability loss. When stirring rate is higher than a certain threshold, mechanical force may destroy protein structure, causing protein molecules to agglomerate and block membrane pores [26], which results in high loss of permeability. Selectivity of modified membrane to BSA and Hb is inversely related to membrane fouling. Selectivity to BSA and Hb was shown to be high when there is severe fouling. Membrane selectivity decreases while fouling is suppressed. This is consistent with previous conclusions. Selectivity of membrane to protein is determined by membrane fouling and charge interaction.

We further investigated the effects of different TA coating conditions on membrane fouling and selectivity. As shown in Figure 6 c, with the extension of TA coating time, membrane permeability loss increased, which means more fouling occurs. Membranes obtained the best selectivity to BSA and Hb when TA coating time was 6 h. A long TA coating time means that more TA molecules are coated on the membrane surface. However, Cys grafting cannot completely occupy the active site in the TA structure [27]. Therefore protein molecules may be reacting with TA molecules during the protein separation process, resulting in pore blockage and fouling. It is evident that permeability loss increased, and the selectivity decreased with the extension of TA coating time.

By studying the fouling resistance and selectivity of Cys grafted PSf membranes under different operating conditions, we can conclude that modified PSf membranes coated with TA for 6 hours and operating at a lower transmembrane pressure and appropriate stirring rate
can achieve good fouling resistance and selectivity for BSA and Hb.

Figure 6. Operation conditions optimized for better selectivity and fouling resistance. (a) transmembrane pressure, (b) stirring speed, (c) tannic acid coating time

3.5 The effect of grafting Cys and Cys+PGA on PAN and US100 membranes

Although Cys grafting improves the permeability, fouling resistance and selectivity of PSf membranes, the balance between selectivity and fouling resistance makes PSf low in selectivity at low fouling. This may be due to the hydrophobicity of the PSf material itself. Our modification focuses on the membrane surface and has little effect on the membrane pores/walls. Therefore we further modified two other membranes, PAN and US100, with a molecular weight cut-off of 100 kDa to investigate their ultrafiltration performance. Polyglutamic acid (PGA) is a polymer of glutamic acid which contains many carboxyl groups. Therefore grafting PGA onto a membrane surface can be considered to improve membrane surface charge and hydrophilicity. The surfaces of the PAN and US100 membranes were grafted with Cys and Cys+PGA.

As shown in Figure 7, after Cys grafting, water permeability of PAN and US100 membranes was greatly improved, which is consistent with the performance of modified PSf membrane.
The hydrophilic TA and Cys effectively enhanced the hydrophilicity and permeability of the membranes. However, when Cys and PGA were grafted at the same time, membrane permeability was lower than that of Cys grafted membrane. PGA molecules with high molecular weight may block membrane pores and damage permeability.

Figure 7. Water permeability of modified and virgin PAN and US100 membrane

We next investigated the ultrafiltration performance of virgin and modified membranes for BSA and Hb, which was characterized by solution flux, protein transmission, selectivity and permeability loss. As shown in Figure 8 a, Cys grafted PAN membrane exhibited the highest solution flux due to negatively charged Cys inhibiting membrane fouling. However, the solution flux of the PAN membrane dropped after simultaneous grafting of Cys and PGA. Macromolecular PGA grafting not only blocks membrane pores but, as a polyelectrolyte, the positive groups existing in the molecules may adsorb protein molecules during the filtration process [], resulting in a decrease in solution flux. For the US100 membrane, solution flux decreased after Cys and Cys+PGA were grafted, which may be the result of reduced membrane pores. As shown in Figure S1, the US100 membrane shows fewer pores on surface compared with PSf and PAN membranes. After modification, membrane pores became further reduced and therefore the protein solution flux is lower.

Figure 8 b shows the membrane transmission of BSA and Hb. We can see that virgin PAN
and US100 membranes have low transmission of BSA and Hb. Both membranes allow more proteins to pass through after grafting Cys. For BSA molecules in particular, the transmission rates reached 60% and 34%, respectively, for PAN and US100. Unlike the PSf/TA-Cys membrane, Cys grafted PAN and US100 exhibited low transmission of Hb. Membrane pore size limits the penetration of Hb molecules. Figure S1 shows that PAN and US100 membranes have fewer membrane pores on the surface compared to PSf membranes. In addition, as shown in Table 1, the equivalent ellipsoidal dimensions of BSA and Hb are 4×4×14 nm and 6.4×5.5×5, respectively, which means that Hb molecules have more difficulty passing through the membrane. Therefore PAN and US100 modified membranes have lower Hb transmission. After grafting Cys+PGA, the transmission of both membranes for BSA also decreased, which is related to the increased membrane fouling. It is also difficult for Hb molecules to pass the PAN and US100 membranes modified by Cys+PGA. The difference between the transmission of BSA and Hb improves the selectivity of membrane. As shown in Figure 8 c, virgin PAN and US100 membranes are basically not selective for BSA and Hb. After Cys grafting, the modified membrane allows more BSA to pass through within the limitations of membrane pore size, so the selectivity of modified membrane to BSA and Hb is greatly improved, and reaches 18 and 22, respectively. However, after grafting Cys+PGA, the selectivity of modified membrane to protein is reduced due to increased pore blockage and fouling.

The fouling resistance of membranes is shown in Figure 8 d. Cys grafted PAN membrane exhibited the smallest permeability loss, only about 9%, while the permeability loss of virgin PAN membrane was 22%. However, Cys+PGA grafted membrane had increased permeability loss compared to virgin membrane, reaching 48%, which means that many proteins were adsorbed on the membrane surface and pores. The US100 membrane grafted with Cys exhibited similar fouling resistance to virgin US100 membrane.

By investigating PAN and US100 membranes grafted with Cys and Cys+PGA, we found that the performance of PAN membranes was greatly improved by Cys grafting. With a permeability loss of only 9%, the Cys grafted PAN membrane retained high selectivity for BSA and Hb. In addition, grafting of macromolecular polyelectrolytes was found not only to damage the permeability of membranes but also caused serious membrane fouling due to
charge attraction. Therefore the polyelectrolytes grafting modification method may be not suitable for the modification of membranes to be used for protein solution separation.

Figure 8. BSA and hemoglobin (Hb) binary solution filtration performance of modified and virgin PAN and US100 membranes at pH 9.0. (a) binary solution flux, (b) observed transmission, (c) selectivity, (d) flux loss. The binary solution contained 0.5 g/L BSA and 0.1 g/L Hb.

3.6 Hypothesis of charge mechanism in protein filtration process

Based on the discussion of above experimental results, we propose a hypothesis of the charge mechanism operating during filtration of high-concentration protein. As shown in Figure 9, when pH is lower than the isoelectric point of the protein, positively charged protein molecules adsorb and accumulate on the membrane surface and pores, which causes blockage of membrane pore and high rejection and results in severe fouling and low selectivity. When pH is higher than the isoelectric point, charge repulsion not only promotes the dissolution of protein (more dispersed) but also inhibits the contact between protein and
the membrane material, thus reducing membrane fouling and allowing more proteins to pass through. In addition, the selectivity to protein is the result of a combined effect of membrane pore size, charge strength and membrane fouling. Anti-fouling membranes with high permeability and charge do not have good selectivity. Appropriate membrane pore size and the presence of fouling can improve membrane selectivity. Therefore, for greater selectivity, it is more practical to select a negatively charged hydrophilic membrane and operate under alkaline conditions for protein solution filtration process.

Figure 9. A hypothesis on movement of protein through a charged ultrafiltration membrane

4 Conclusions

We successfully grafted small molecules, Tau, Cys and PEI, on the surface of PSf membranes using the TA coating method and investigated the influence of these molecules on filtration of BSA/Hb binary solution. Experimental results showed that grafting of Tau and Cys could greatly enhance the hydrophilicity of PSf membranes and improve their permeability. Under alkaline conditions, Tau and Cys grafted membranes exhibited better fouling resistance and allowed more proteins to pass through due to charge repulsion. However, high permeability does not mean high selectivity. Cys grafted membranes exhibited the highest selectivity at pH 7.5. By optimizing the operating conditions, we found that high fouling resistance and high selectivity could be achieved under low transmembrane pressure and an appropriate stirring rate using a membrane that had been coated with TA for 6 h. In addition, we grafted Cys and Cys+PGA on the surface of PAN and US100 membranes and found that Cys grafted PAN membrane had a selectivity for BSA and Hb as high as 18. Further grafting of
polyelectrolyte PGA not only damaged membrane pores and permeability but also caused serious membrane fouling. Our research provides a simple membrane surface modification method that can simultaneously enhance membrane permeability, selectivity and fouling resistance. Due to the non-specific adhesion of tannic acid, this method can be used on almost all membrane materials.

**Acknowledgements**

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Supporting information

S1. SEM image of modified and virgin PSf, PAN and US100 membranes under 50000 × magnification