

Synthesis of Membrane Adsorbers via Surface Initiated ATRP of 2-Dimethylaminoethyl Methacrylate from Microporous PVDF Membranes*

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Abstract Surface-initiated atom transfer radical polymerization (SI-ATRP) was used to tether poly(2-dimethylaminoethyl methacrylate) (PDMAEMA) onto microporous PVDF membranes in order to synthesize membrane adsorbers for protein adsorption. The alkaline treatment and bromine addition reaction were used to anchor ATRP initiators on membrane surface. Then PDMAEMA was grafted from the membrane surface via SI-ATRP. Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS) and scanning electron microscopy (SEM) revealed the chemical composition and surface topography of the PVDF-g-PDMAEMA membrane surfaces. These results showed that PDMAEMA was grafted from the membrane surface successfully and a grafting yield as high as 1500 $\mu\text{g}/\text{cm}^2$ was achieved. The effects of the grafting time and the density of initiators on the static and dynamic binding capacity of bovine serum albumin (BSA) were systematically investigated. Both the static and dynamic binding capacities increase with the bromination and polymerization time. However, the benefits of the initiator density on binding capacities are limited by the graft density of PDMAEMA chains.

Keywords: ATRP; PVDF membrane; Protein adsorption; Polyelectrolytes; Graft polymerization.

INTRODUCTION

Protein substances with biological activities play a vital role in human health, in medical and food field. There were more than 2500 biotech-based drugs in the discovery phase^[1, 2]. Biomacromolecules, oftentimes proteins, need to be purified before they can be used. This is due to the requirement of high purity, concentration enrichment, prevention of catalysis poisoning, reduction of protein denaturation *etc*^[3]. Unfortunately, the protein separation is a difficult process resulted from the complexity of proteins themselves and their biological environments^[4]. The cost of the downstream recovery and purification accounts for over 60% of the cost of productive process^[5, 6]. Therefore, it is indispensable to create a rapid, safe and efficient method to isolate and purify protein-products in large volumes in the biotechnology industry.

Historically, the adsorptive chromatography has been widely used for high-resolution protein bioseparation. But the traditional packed-bed chromatography has some obvious drawbacks, such as high pressure drop, internal diffusion limitation, as well as the complicated transport phenomena in packed beds which make the scale-up very difficult^[7–11]. A radically different approach to overcome the limitations

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associated with packed beds is to use synthetic microporous or macroporous membranes as chromatographic media, which is referred to as membrane affinity chromatography (MAC). The MAC is achieved by attaching specific ligands onto the pore surface in membranes, and the target proteins were adsorbed on these ligands with the solute transporting through the membrane predominantly by convection, which significantly decreases pressure drop, process time and recovery liquid volume^[3, 10]. In addition, the easy and cheap scale-up makes the MAC very attractive. Therefore, the MAC has been intensively investigated and industrially applied as an alternative to the conventional resin-based chromatography column^[10, 12–15].

Efficient affinity membrane adsorbers can be fabricated by spinning/casting bioactive polymers into membranes^[16–18] or attaching the ligands onto pre-formed micro/macroporous membranes *via* surface modification. The surface modification methods include photografting^[19–21], radiation grafting^[22–24] as well as surface-initiated atom transfer radical polymerization (ATRP)^[2, 25–32]. ATRP is a “living”/controlled radical polymerization, which can be used to prepare well-defined polymer brushes from the solid matrix^[2, 25–34]. Husson and co-workers^[27] used ATRP to modify PVDF membranes with ion-exchange groups. They also described the use of SI-ATRP to prepare weak cation-exchange membrane from the regenerated cellulose membranes with high protein binding capacity^[2, 28, 32].

Ideally affinity membrane substrates are mechanically resilient, chemically and thermally resistant. As one of the most important membrane materials, PVDF has all the above characteristics and is widely used for microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and ion exchange (IE) in the fields of water treatment^[35,36]. However, PVDF has been rarely used as the affinity membrane substrate. This is due to the significant hydrophobicity of the PVDF membrane, which can prompt nonspecific adsorption of proteins. Hydrophilic modification of the PVDF membrane surface was conducted *via* blending, coating and grafting with a variety of hydrophilic polymers^[35]. Poly(2-dimethylamino ethyl methacrylate)(PDMAEMA) is a weak polyelectrolyte and a stimuli-responsive polymer, which can have electrostatic interactions with some proteins^[2, 32, 37, 38]. The surface anchored PDMAEMA chains demonstrated the adsorption of BSA at acidic condition and desorption of BSA when the electrostatic attractions were eliminated^[39]. It is believed that the grafting of PDMAEMA on PVDF membrane with a sufficiently high grafting yield can depress the nonspecific protein adsorption and impart the PVDF membrane specific protein binding properties.

The C-F initiated ATRP directly from the membrane surface provided a facile method for PVDF membrane modification and functionalization. But it still remains a challenge to obtain high grafting yield and good

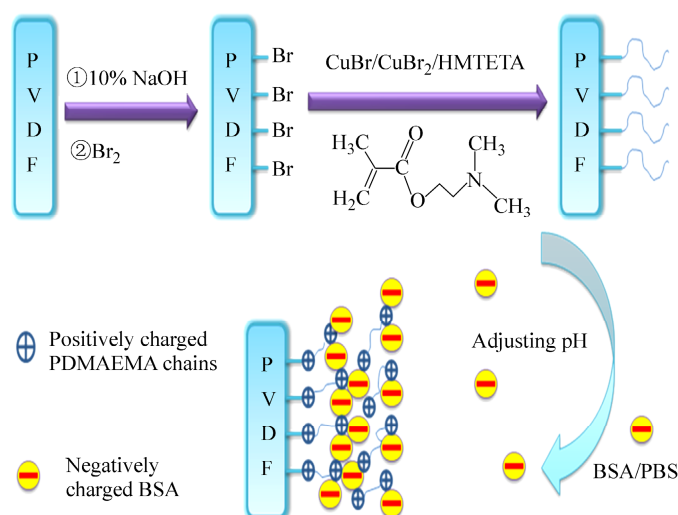


Fig. 1 Schematic representation of the SI-ATRP of the PDMAEMA from PVDF membrane surface towards a membrane adsorber

control on both the grafting density and chain length of the grafted polymers^[40]. In this article, SI-ATRP method was used to modulate the protein adsorption properties of the microporous PVDF membrane by the grafting of DMAEMA. The C-Br ATRP initiator was first anchored onto the membrane *via* alkaline treatment and addition reaction. Then, the PDMAEMA chains were grafted from the PVDF membrane surface *via* SI-ATRP at room temperature. The reaction process is shown in Fig. 1. The reaction time of the bromine addition and the polymerization time of SI-ATRP were varied independently to manipulate the grafting density and the length of the PDMAEMA chains. Their effects on BSA adsorption properties of the PVDF membrane were systematically investigated.

EXPERIMENTAL

Materials

Hydrophobic poly(vinylidene fluoride) (PVDF) microporous membrane (0.22 μm , 26.5 cm $\times$ 3.75 cm, America) was purchased from Millipore Co. Ltd. The membrane was sliced into rectangular pieces (*ca.* 3 cm $\times$ 3 cm), and sequentially cleaned by ethanol, deionized (DI) water, ethanol at 40 °C to remove the additives. Then the films were dried under vacuum at 50 °C before use. 2-(Dimethylamino) ethylmethacrylate (DMAEMA, 99.5%), 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA, 98%), cuprous bromide (CuBr, 99.999%) and cupric bromide (CuBr₂, 99.5%) were purchased from Aladdin Co. Ltd, China. Bovine serum albumin (BSA) from bovine blood was purchased from Sigma-Aldrich. Buffers were prepared with analytical-grade chemicals and DI water. Buffer B (10 mmol/L potassium phosphate buffer, pH = 6.0), Buffer E (1 mol/L NaCl in Buffer B, pH = 4.0) were used for binding capacity measurements. All of the reagents were commercially analytical grade and used as received without any further purification. Deionized (DI) water with resistivity higher than 4 M Ω /cm was provided by the water treatment system composed of reverse osmosis and ion-exchange system (Tianjin Motian Membrane Engineering Technology Co. Ltd., China).

Immobilization of ATRP Initiators on the PVDF Membranes

The immobilization of ATRP initiators on the PVDF membranes included two steps. At first, The PVDF membranes were treated with alkaline to produce the carbon double bond (C=C)^[41–47]. For the second step, bromine was immobilized to the surfaces of PVDF membranes through the addition reaction between bromine and C=C. A typical procedure was as follows: the PVDF membranes were treated for 10 min by the 10 wt% of NaOH solution which was preheated at 60 °C, then the treated membranes were washed with a large amount of DI water and dried at 50 °C. For the anchoring reaction of C-Br initiators, the alkalized PVDF membranes were added to 40 mL of CCl₄, followed by the addition of 3 mL of Br₂. The reaction system was gently stirred at ambient temperature. After a predetermined reaction time, the membranes were taken out and washed by a lot of ethanol. After being dried in a vacuum oven at 50 °C to constant weight, the membrane was weighed (W_0) with an analytical balance to a precision of 0.1 mg, then stored in ethanol/DI water (1/1, *V/V*) for use.

Surface Initiated Atom Transfer Radical Polymerization of DMAEMA

The PVDF-g-PDMAEMA membranes were fabricated by grafting linear PDMAEMA chains onto the surfaces of PVDF-Br membranes with the ATRP method at ambient temperature. A typical procedure was as follows: the PVDF-Br membrane, DMAEMA (10.6 mL, 63 mmol), CuBr₂ (0.010 g, 0.044 mmol) and HMTETA (0.35 mL, 1.35 mmol) were mixed with the solvent (methanol/DI water, 1/3, *V/V*, 44.7 mL). The solution was gently stirred and degassed with nitrogen for 30 min in a water bath at 30 °C. CuBr (0.032 g, 0.22 mmol) was added into the reaction system to start the polymerization. The reaction system was sealed after being degassed with nitrogen for another 10 min. After the predetermined polymerization time, the PVDF-g-PDMAEMA membrane was removed from the mixture and washed sequentially with hydrochloric acid (0.1 mol/L), ethanol and DI water at 40 °C. After being dried in a vacuum oven at 50 °C to constant weight, the PVDF-g-PDMAEMA membrane was weighed (W_1). The grafting yield of PDMAEMA (GY) was calculated by Eq. (1).

$$GY = \frac{W_1 - W_0}{A} \quad (1)$$

where A is the membrane area.

Surface Characterization

Fourier-transform infrared spectroscopy (FTIR) (Bruker, Vector-22, Germany) was used to evaluate the surface chemical composition of the modified and unmodified membranes. X-ray photoelectron spectroscopy (XPS) (Thermo Scientific, USA) was used to investigate the surface element composition of the membrane at each step. The membrane surface and cross-sectional morphologies were observed by field emission scanning electron microscopy (FESEM, Quanta 200, FEI, USA) under high vacuum condition. Representative samples of the membranes were cut into 0.5 cm² pieces, attached with double-side tape to steel stabs, and shadowed with gold prior to SEM measurements^[40].

Static Nitrogen Adsorption Measurements

The pore size, pore volumes and surface areas of the membranes were measured using a Micromeritics ASAP-2020 (Micromeritics, USA). All samples were degassed at 105 °C for 4 h prior to each adsorption experiment to remove adsorbed contaminant. After the pretreatment, the nitrogen adsorption isotherms was measured at liquid nitrogen condition (−196 °C) between pressures of 0.195 kPa and 106 kPa. The same instrument was used to measure adsorption and desorption isotherms of each sample.

Permeation Flux Measurements

The pure water flux was measured using a lab-scale dead end filtration unit. The membrane was cut into suitable pieces and put into the unit. The feed flow is forced through the membrane by N₂. The membrane was soaked in ethanol for 5 min to wet out the membrane. The permeation flux data were collected at 0.1 MPa after the membrane was pressurized at 0.2 MPa for 10 min.

Protein Static Binding Capacity

BSA was used as a reference protein to measure static protein adsorption capacities of modified membranes (PVDF-g-PDMAEMA). Each membrane (3 cm × 3 cm) was placed in a conical flask (50 mL) and incubated in 10 mL of BSA solution (1 mg/mL in buffer B) for 20 h to reach equilibrium in a shaker bath at 22 °C. The static binding capacity is defined as per unit volume of membrane and calculated by mass balance using initial and equilibrium concentrations of protein solution. The concentration of protein was measured by UV detection (VIS-7220, Beijing Ruili analysis Co. Ltd., China) at 595 nm *via* Coomassie blue staining^[48].

Protein Dynamic Binding Capacity

BSA was used as the reference protein to measure dynamic protein adsorption capacities of modified membranes (PVDF-g-PDMAEMA). The membrane was cut into discs (3 cm in diameter) and was placed in a glass column. A typical injection cycle operation is as follows: the effluent from the membrane module was monitored continuously with UV detection at 595 nm *via* Coomassie blue staining. 20 mL of buffer B was passed through the column to equilibrate the membrane. Then 10 mL of BSA solution (1 mg/mL in buffer B) was passed through the column with the flow rate at 1 mL/min. Then, 20 mL of buffer B was passed through the column to rinse off the nonspecific BSA adsorption. Finally, 10 mL of buffer E was passed through the column to elute the specific BSA binding.

RESULTS AND DISCUSSION

Surface Modification of the PVDF Membrane

The direct ATRP from the secondary halogenated sites of PVDF membranes had been demonstrated^[39]. However, the high C—F bond energy may limit the grafting yield and exclude this method as a technique to fabricate membrane adsorbers with high binding capacities^[40]. In order to obtain high grafting yields, we tried to immobilize the C—Br ATRP initiators onto the PVDF membrane surface *via* alkaline treatment and bromine

addition reaction. The alkaline treatment of PVDF can introduce more active groups, which mainly include C=C bonds, carbonyl groups (C=O), hydroxyl groups (C—O—H) and ether bonds (C—O—C) *etc*^[41–47]. Two methods were attempted to introduce the C—Br initiators. One is the reaction of the hydroxyl groups with 2-bromoisobutyryl bromide (2-BIB) to form bromoisobutyrate groups^[28], the other is the nucleophilic addition reaction of C=C with bromine. In our case, the latter method was used because it was found to introduce the ATRP initiators more effectively than the former and no damage was observed on the membrane from the bromination reaction. Following the bromination, the PVDF-Br membrane was used as the macroinitiator to initiate the ATRP of DMAEMA. CuBr/HMTETA was used as the catalyst complex and CuBr₂ was added in order to achieve a better control on the polymerization.

Figure 2 presents typical FTIR spectra for a pristine PVDF membrane (spectrum a), an alkalized membrane (spectrum b), a PVDF-Br membrane (spectrum c) and a PVDF-g-PDMAEMA membrane (spectrum d) following 2 h of ATRP. Compared with the pristine PVDF membrane, there was no obvious change for the FTIR spectrum of the alkalized membrane. For the spectrum of the alkalized membrane, there supposed to be an absorption band at 1650 cm⁻¹ attributed to the C=C stretching. But it is not obvious due to the small amount of C=C formed and the inherent weak absorption of C=C stretching. For the spectrum of PVDF-Br, there is not either obvious change which is due to the overlapping of the C-Br absorption with the spectrum of PVDF. For the spectrum of PVDF-g-PDMAEMA, the absorption band at 1732 cm⁻¹ is attributable to the stretching of the ester carbonyl group. Control experiments were performed at the same condition without adding CuBr. There were no signals of carbonyl band at 1732 cm⁻¹. Therefore, it is demonstrated that the chains of PDMAEMA were successfully grafted onto the PVDF membrane.

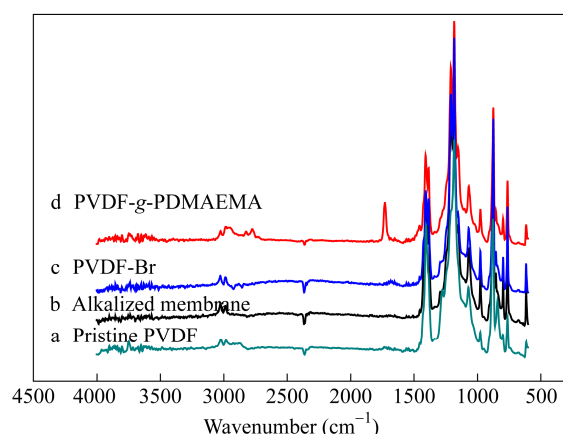
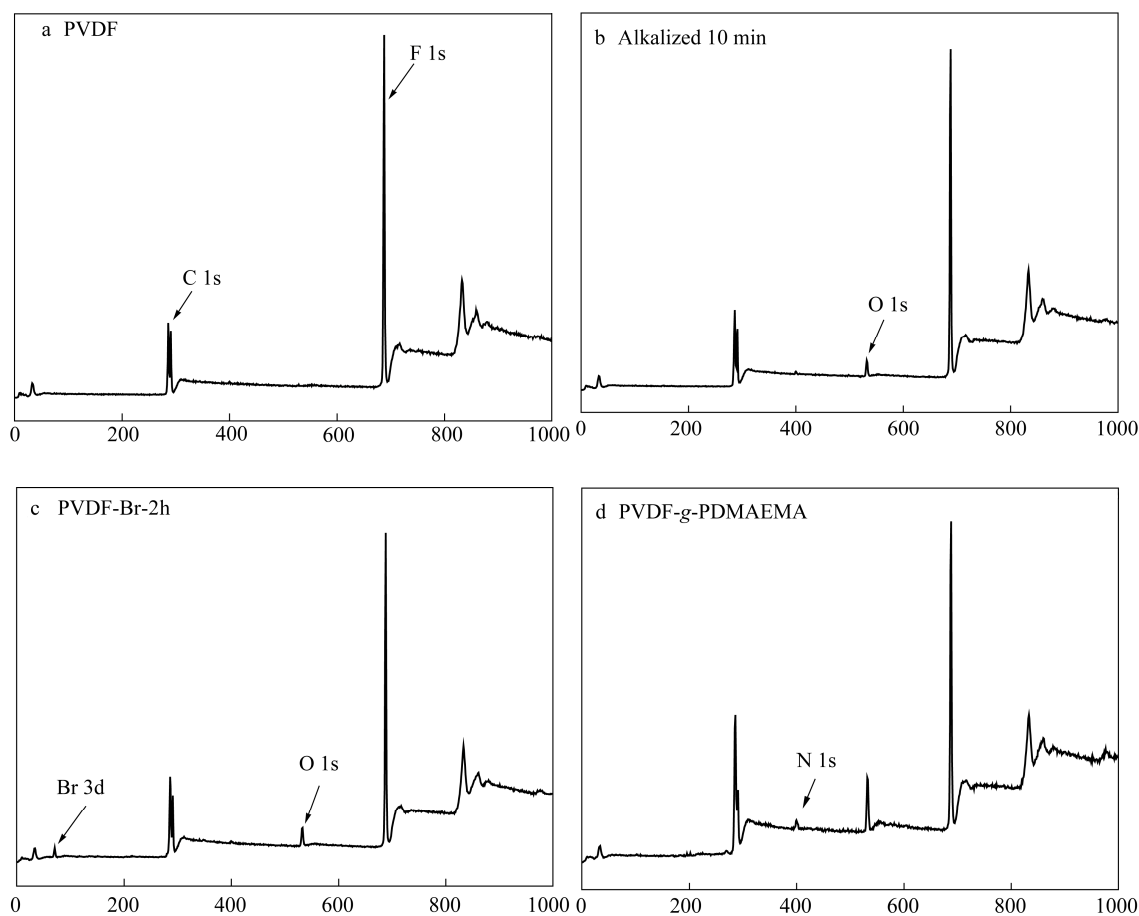


Fig. 2 FTIR spectra of the pristine PVDF membrane (a), the alkalized membrane (b), the PVDF-Br membrane (c) and the PVDF-g-PDMAEMA membrane (d)

In order to obtain detailed information on the evolution of the surface chemical composition with the chemical modifications, including alkalization, bromination and the ATRP, the membrane surface chemical composition was further investigated by XPS. The XPS spectra of the treated and untreated membranes are shown in Fig. 3. For the untreated PVDF membrane, only two elements, the carbon and the fluorine were detected in the wide-scan spectrum (Fig. 3a). The C 1s core-level spectrum of the PVDF membrane can be curve-fitted with two peak components of about equal areas with the binding energy (BE) at 286.2 eV for the —CH₂— species and 290.9 eV for the —CF₂— species (Fig. 3e). The molar ratio of the two peak component values is in good agreement with the theoretical value of 1:1. A new peak at 588 eV appears in Fig. 3(b), which is O 1s signal generated in the alkaline treatment. The C 1s core-level spectrum of the alkalized membrane (Fig. 3f) can be curve-fitted with five peak components. The new peak components with BEs at about 287.1 and 288.9 eV are attributed to the C—O and C=O species, respectively, which were generated in the alkaline

treatment. The peak component with BE at about 284.7 eV arises from the combined contributions of the CH— species and the CH= species. Figures 3(c), 3(g) and 3(g') show the respective XPS wide-scan, C 1s and Br 3d core-level spectra of the PVDF-Br membrane, following the 2 h addition reaction of Br₂. For the comparison of C 1s core-level spectrum of alkalinized and PVDF-Br membranes, it is clear that peak intensity of C—H and CH= species decreases substantially, while the peak intensity of C—O/C—Br species increases. These results demonstrate that double carbon bond (C=C) has reacted with bromine. The addition reaction time was varied to tune the C—Br ATRP initiator density. The element contents of the PVDF-Br membrane were determined by XPS, and the results are shown in Table 1. It is clear that the molar ratio of bromine increased with the addition reaction time, indicating the increase of the C—Br ATRP initiator density. This agrees well with our expectation. For the PVDF-*g*-PDMAEMA membrane, the decrease of the fluoride molar ratio and the booming of oxygen and nitrogen contents confirmed the successful tethering of PDMAEMA chains. Figures 3(d) and 3(h) show the XPS wide-scan and C 1s core-level spectra of the PVDF-*g*-PDMAEMA membrane, which was prepared by 2 h of bromination followed by 8 h of the grafting polymerization of DMAEMA. The peak component with BE at about 286.8 eV is assigned to the C—N species of the grafted polymers. Compared to the C 1s core-level spectrum of the PVDF-Br membrane, it is obvious that the intensity of the —CH— species associated with the PDMAEMA chains increases while that of C—Br species and —CF₂— species decrease. This is believed to be due to the loss of chain end bromine and the surface being covered by the grafted polymer. The above results demonstrated that the PDMAEMA chains were successfully grafted from the PVDF membranes.



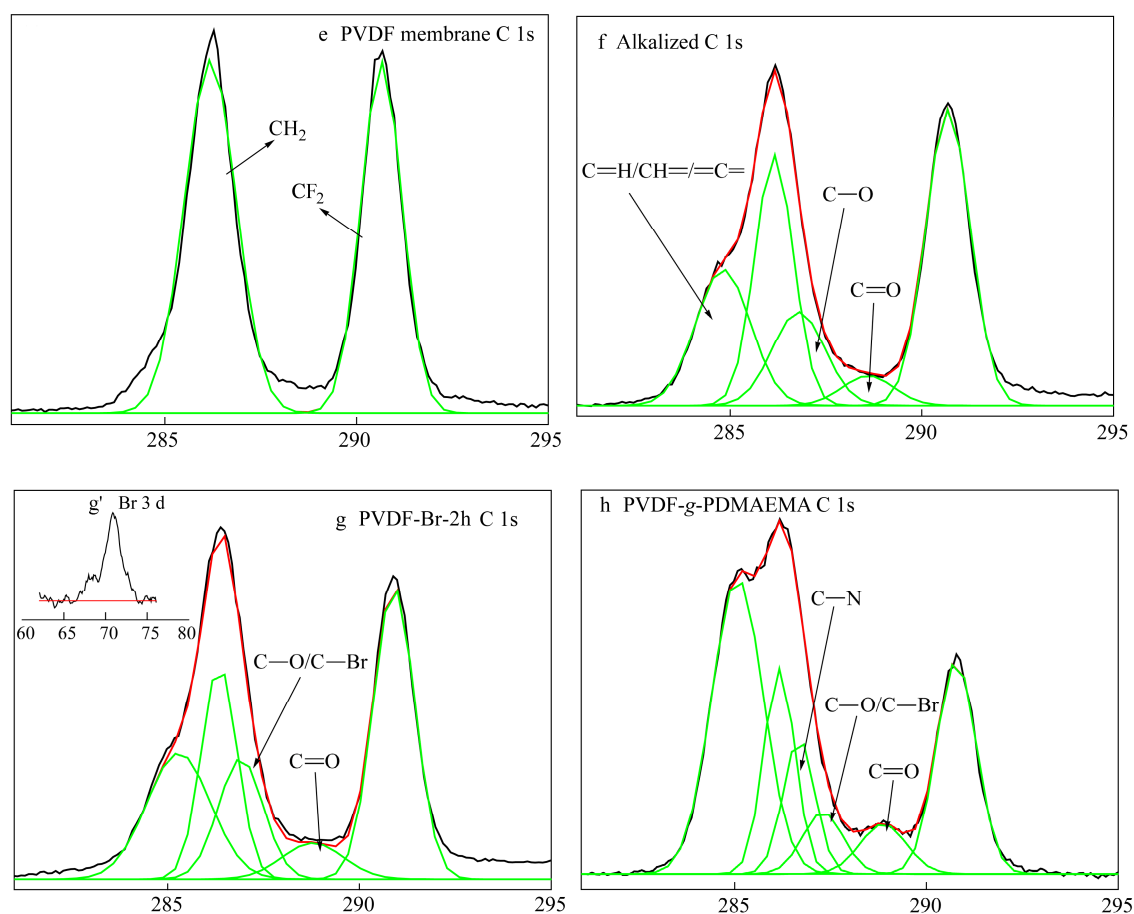


Fig. 3 Wide scan and C 1s core-level spectra of the pristine PVDF membrane (a, e), alkalized PVDF membrane (b, f), PVDF-Br membrane (c, g), PVDF-g-PDMAEMA membrane (d, h) as well as Br 3d core-level spectrum of PVDF-Br (g')

Table 1. The element contents of membrane surface at each modification step

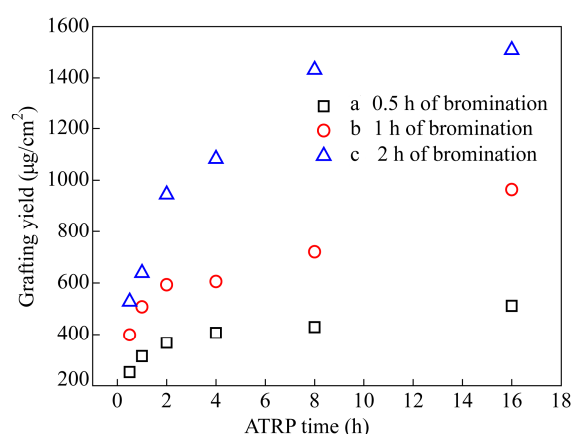
Sample	Atom content (%)				
	F	C	O	Br	N
Pristine PVDF	46.8	53.2	—	—	—
Alkalized PVDF	40.91	55.78	3.31	—	—
PVDF-Br-0.5h	40.18	55.94	3.40	0.48	—
PVDF-Br-1h	39.04	55.65	4.68	0.63	—
PVDF-Br-2h	37.43	56.62	4.09	1.46	—
PVDF-g-PDMAEMA	11.26	63.99	14.19	—	10.56

The effects of the PDMAEMA grafting on the chemical composition of the pore wall were also investigated by XPS analysis of the very central area of the cross-section of the membranes. The elemental contents are shown in Table 2. It is interesting to see the oxygen element in the cross section of PVDF section, which might be aroused by the additives added by the manufacturers. For the sample PVDF-g-PDMAEMA, it can be seen that the cross-section has much lower nitrogen content than the membrane surface. This indicates that the ATRP grafting of PDMAEMA on the pore wall was severely hindered.

Table 2. The element contents of the central area of the PVDF-g-PDMAEMA membrane cross-sections

Sample	Atom content (%)			
	F	C	O	N
Pristine PVDF	43.96	54.01	0.39	—
PVDF-g-PDMAEMA	31.83	55.82	2.43	2.89

As the successful tethering of PDMAEMA chains onto the PVDF membrane surface was confirmed by FTIR and XPS, the ATRP method used in this article was demonstrated to be an effective surface modification method. Furthermore, as a controlled chain growth technique, ATRP should allow manipulation of the structure of the grafted polymer brushes. Herein the bromination time was varied to manipulate the density of the anchored initiators and then the grafting density of PDMAEMA chains; the ATRP time was varied to manipulate the chain length of PDMAEMA chains. The kinetic plot of the grafting yield (GY) with respect to the polymerization time is shown in Fig. 4. As an example, the plot of the GY of the ATRP from PVDF-Br membrane of 1 h of bromination can be divided into two stages, the first 2 h and the next 14 h. It can be seen that the polymerization rate was high in the first stage and then is apparently lower in the second stage. Similar kinetic behavior has been observed for other surface-initiated ATRP in aqueous media^[49]. It should be mentioned that similar method has been used to graft PDMAEMA onto the poly(ethylene terephthalate) membrane surface but almost linear increase of GY in first four hours was achieved^[50]. Finally, the GY reached to a plateau of 960 $\mu\text{g}/\text{cm}^2$. For the PVDF-g-Br membrane of 2 h (Fig. 4c) and 0.5 h (Fig. 4a), the ATRP of DMAEMA shows the similar polymerization behavior and the GY reached to a plateau of 1500 $\mu\text{g}/\text{cm}^2$ and 510 $\mu\text{g}/\text{cm}^2$, respectively. By comparing the plots of three PVDF-Br membranes having different initiator densities, it can be seen that the higher initiator density results in higher reaction rate and higher GY, which is consistent with the literature reports^[51, 52]. In general, a good control over the length and density of grafted polymer chains were achieved by changing the grafting time and the initiator density. In addition, the highest GY value achieved by this method is 1500 $\mu\text{g}/\text{cm}^2$, which is three times that reported in our previous work^[40], indicating improved efficiency of this method for the synthesis of high capacity membrane adsorbers.

**Fig. 4** Variation of the grafting yield (GY) on the ATRP time initiated by PVDF-Br membranes of three different initiator densities

Surface Morphologies of the Modified PVDF Membrane

The scanning electron microscopy (SEM) was used to investigate the morphologies of membranes. Figure 5 shows SEM images of the top surfaces of the pristine membrane and the PVDF-g-PDMAEMA membranes. The PVDF-g-PDMAEMA membranes (b, c, d) were prepared by 8 h ATRP of DMAEMA initiated by PVDF-Br membranes having different initiator densities (bromination times of 0.5 h, 1 h, 2 h). Because the ATRP time and conditions were the same for the preparation of these membranes, it was assumed that the average M_w of PDMAEMA chains were similar for these membranes and the morphology change is reflecting the effects of the

grafting densities. It can be seen that with the grafting density increasing, the pore diameter shrunk and the porosity decreased. A new layer of grafted polymer appeared building new membrane surfaces. The cross section of the membranes was also observed by SEM, and the images are shown in Fig. 6. It can be seen that the alteration of the cross section by the ATRP was not as obvious as the membrane surface. Only the sample having high GY (Fig. 6d) has thicker fabric structure caused by ATRP of DEMAMA. This corresponds well with the cross-section XPS results.

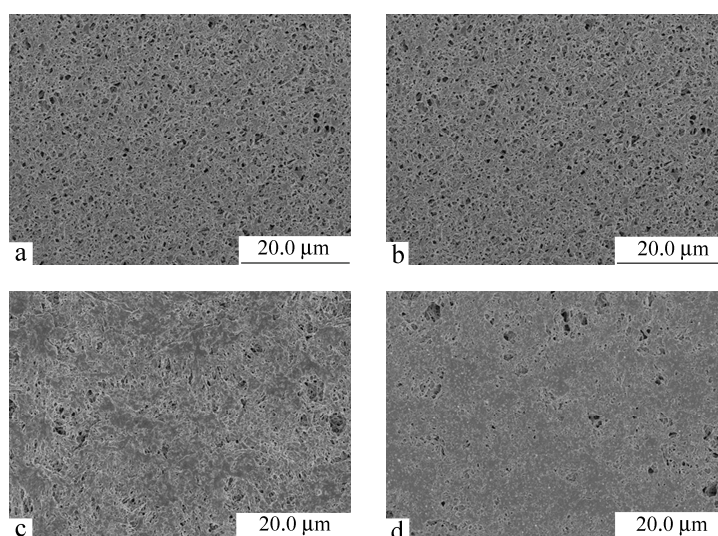


Fig. 5 The SEM micrographs of the top surfaces of the pristine membrane (a) and PVDF-g-PDMAEMA membranes prepared by the bromination (b: 0.5 h, c: 1 h, d: 2 h) followed by 8 h of ATRP

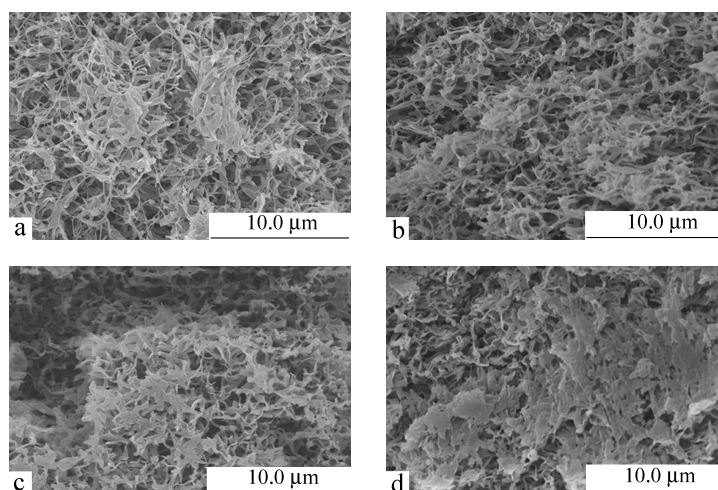


Fig. 6 The SEM micrographs of the cross-section of the pristine membrane (a) and PVDF-g-PDMAEMA membranes prepared by the bromination (b: 0.5 h, c: 1 h, d: 2 h) followed by 8 h of ATRP

Specific Surface Areas and Pore Characteristics

The method of Brunauer-Emmett-Teller (BET) was employed to determine surface area. A plot of N_2 volume adsorbed versus pressure was built to calculate the volume of N_2 required to form a monolayer on the surface and pores lumen of the membrane. An area of 0.162 nm^2 covered by each N_2 molecule was used to calculate the special surface area. The Barrett-Joyner-Halenda (BJH) method was used to measure the pore sizes and pore-size distribution with the Kelvin model of pore filling^[28]. Table 3 summarizes the BET surface area and pore

characteristics of the PVDF-g-PDMAEMA membranes prepared *via* SI-ATRP from the membrane PVDF-Br-1h. It can be seen that PVDF-g-PDMAEMA membranes have much lower BET surface area than the PVDF membrane. This is believed to be due to the pore filling by the grafted polymer chains. Similarly, the average pore diameter decreases with the polymerization time. The pore filling effect can also be seen from the drop of the DI water flux with the PDMAEMA grafting.

The grafting yields (GYs) were also normalized by the BET surface area, and the resulted GY_{BET} was shown in Table 3. It can be seen the GY_{BET} values are much smaller than the GY values normalized by the membrane area. By incorporating the XPS and SEM results of the cross-section, it is believed that the SI-ATRP mostly occurs at the membrane surface, which is also the same location for protein adsorption. Obviously there is appreciable space for the PVDF-g-PDMAEMA membrane to improve its adsorption capacity. There remains a challenge to improve the efficiency on pore wall grafting and further work is needed to address that.

Table 3. BET surface areas and BJH adsorption average pore width of the unmodified and the PVDF-g-PDMAEMA membranes at different polymerization times

Polymerization time	BET surface area (m ² /g)	BJH desorption average pore width (nm)	DI water flux (L/m ² h)	GY_{BET} (μg/cm ²)
Pristine PVDF	32.6	11.15	531.2	
30 min	19.1	7.29	99.6	0.39
1 h	20.2	7.59	96.1	0.52
2 h	26.9	7.15	91.5	0.63
4 h	23.3	6.18	73.7	0.75

The Protein Adsorption Properties

Effects of the grafting time and the initiator density on the static and dynamic protein (BSA) adsorption are systematically investigated. The isoelectric point (pI) of BSA is 4.8 and the pK_a of PDMAEMA is 7.5 in aqueous solution. The amino groups of PDMAEMA can be protonized at acidic conditions^[39]. In this study, the BSA solution pH was 6.0 and the PDMAEMA has a protonation degree of 96.9%. The PDMAEMA was positively charged and BSA was negatively charged. BSA can bind to the grafted PDMAEMA brushes *via* the electrostatic interaction. In addition, the intramolecular electrostatic repulsion renders the PDMAEMA an extended conformation favoring the electrostatic interaction between BSA and the grafted polymer chains.

Figure 7 shows the evolution of the static BSA binding capacity of the modified membranes with the polymerization time. It can be seen that the BSA binding capacity increases with the ATRP time at all the three initiator densities. This is due to the increased chain length and increased ion exchange group densities with the

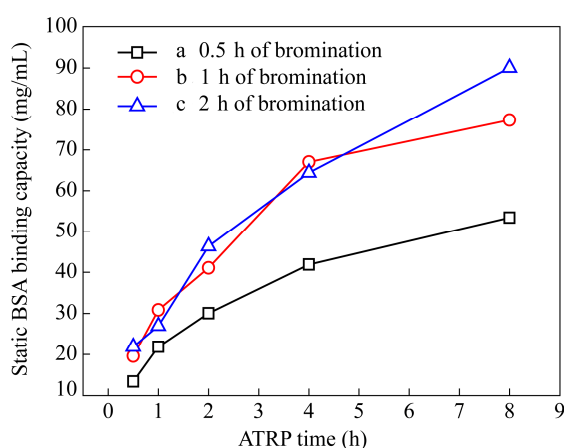


Fig. 7 Variation of the static binding capacity of membranes with the ATRP time initiated by PVDF-Br membranes of three different initiator densities

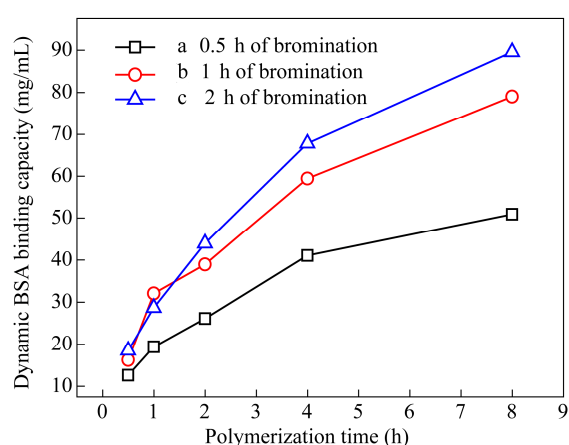


Fig. 8 Variation of the dynamic binding capacity of membranes with the ATRP time initiated by PVDF-Br membranes of three different initiator densities

ATRP time. By comparing the BSA binding capacity of membranes from different bromination time, it can be seen that the membranes of 1 h bromination have higher BSA binding capacity than those of 0.5 h bromination but have similar binding capacity to those of 2 h bromination time. This is because that the benefits of the PDMAEMA density on the binding capacity are limited by the too high graft density of PDMAEMA excluding protein binding. This also corresponds with the pore filling effects concluded from Table 2. In spite of this, for the membrane, the static BSA binding capacity is as high as 90 mg/mL, showing excellent protein binding properties of this type of membrane. Figure 8 shows the evolution of the dynamic BSA binding capacity of the modified membranes with the polymerization time. It can be seen that all the values of dynamic binding capacities are slightly lower than those of static binding capacities while the effects of ATRP time and bromination time are the same.

CONCLUSIONS

A reproducible and valid methodology has been successfully used to convert commercially available, microporous PVDF membranes to membrane adsorbers using SI-ATRP. Both FTIR and XPS results confirmed that the PDMAEMA chains were successful grafted from PVDF membrane surfaces. Increasing the polymerization time and initiator density can increase the grafting yield of the PVDF-g-PDMAEMA membranes, respectively. The static and dynamic BSA binding capacity increases with the polymerization time. More research efforts are required to tailor the microstructure and topology of the grafted polymers for better protein adsorptive performance.

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