

# Photochemical surface modification of poly(arylsulfone) ultrafiltration membrane and covalent immobilization of enzyme

SONG Hua, WU Guang-xia\*, LIU Kai

(Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China. E-mail: huahuasong\_01@163.com)

**Abstract:** The sensitivity of poly(arylsulfone) (PSf) for UV irradiation in different solvents (water and ethanol) was investigated. It is confirmed that acrylic acid (AA) and acrylamide (AAM) are grafted only onto the surface of the membrane instead of the interior by FTIR and scanning electron microscope (SEM). The membrane performance ( $\Delta J/J_0$  and contact angle  $\theta$ ) after photografting was studied. In the range of conditions used, the grafting yield increases with irradiation time and monomer concentration growing. After photografting and N-3-dimethyl-aminopropyl-N'-ethylcarbodiimide hydrochloride (EDC) activation, PSf membrane was immobilized with hydrogen peroxide oxidoreductase, and showed a higher activity than the control membrane.

**Keywords:** photografting; poly(arylsulfone) ultrafiltration membrane; immobilization

## Introduction

Poly(arylsulfone) (PSf), due to its mechanical, thermal and chemical stability as well as excellent film forming properties, is frequently used as materials for high performance ultrafiltration (UF) or microfiltration (MF) membranes. However, because of low surface energy, it adsorbs proteins strongly and hence shows large reduction of the permeation flux with time. It is generally acknowledged that hydrophilic surface is less susceptible to fouling (Pieracci, 1999). Thus there have been considerable interests in developing general strategies for introduction of hydrophilic groups onto the surface of ultrafiltration or microfiltration membranes as effective means for reducing the nonselective adsorption of proteins.

A variety of surface modification methods have been reported (Ulbricht, 1995). Among them, photoinduced grafting is known to be a useful technique for the modification and functionalization of polymer materials due to its significant advantages: low cost of operation, mild reaction conditions, selectivity to absorb UV light without affecting the bulk polymer, and permanent alteration of the membrane surface chemistry. Yamagishi *et al.* (Yamagishi, 1995a) developed a method of photografting vinyl monomers onto poly(arylsulfone) and poly(ethersulfone) UF membranes without the use of photoinitiators. A mechanism was proposed in which the sulfur-carbon bond in the poly(arylsulfone) chain backbone was cleaved under UV irradiation to generate radical sites at which graft polymerization took place. In a companion paper, Yamagishi *et al.* (Yamagishi, 1995b) described the grafting of a wide variety of hydrophilic vinyl monomers onto the surface of PSf and PES UF membranes to decrease protein fouling during the filtration of BSA. Photografting has often

resulted in production of significant amounts of homopolymer and cross-linked polymer if the initiator, benzophenone, abstracts hydrogen from monomer and reacted polymer. Ma *et al.* (Ma, 2000; 2001) designed a novel sequential photoinduced living polymerization and studied the principal factors affect it. In this method, the homopolymer can be reduced significantly in polymerization of monomers with abstractable hydrogens.

Polymeric materials functionalized via surface grafting provide substrates suitable for immobilizing biomacromolecules such as proteins, polysaccharides, and nucleic acids. Ulbricht *et al.* (Ulbricht, 1996) attached biomolecules onto the surface of PSf and PES membranes functionalized by acrylic acid. A protein (BSA), an enzyme (invertase), an antibody-enzyme conjugate (IgG-peroxidase) and a synthetic hexadecapeptide as a specific antigen of a murine monoclonal antibody were immobilized onto the membrane. The surface of conventional synthetic polymers interacts with biological systems only in a non-specific manner, whereas polymer surface modified with physiologically active biomolecules have an ability to undergo specific interactions that are the basis for the physiology of living organisms, and thus find a broad range of biotechnological and medical application.

In this paper, a heterogenous photografting polymerization modification approach (without photoinitiator) was used, which enabled the attachment of hydrophilic groups. The effect of different solvents (water and ethanol) on UV sensitivity of PSf membrane and photografting polymerization was studied. The grafted membrane was characterized by FTIR, SEM, water flux and contact angle. Hydrogen peroxide oxidoreductase was further immobilized onto g-PAA membrane surface.

## 1 Experimental

### 1.1 Materials

The specific viscosity of poly(arylsulfone) was 0.72. Acrylic acid was distilled in vacuum before use. Nitrogen was of high purity. Bovine serum albumin (BSA) was from Dongfang Instrument and Equipment Co., Chinese Academy of Sciences and stored in safe. N-3-dimethyl-aminopropyl-N'-ethycarbodiimide hydrochloride (EDC) was a product of Acros Orgnics (New Jersey, USA) and stored in frozen. Hydrogen peroxide oxidoreductase was from live catalase (Sigma C-9322) 2350 units/mg solid, 2970 units/mg prot.

### 1.2 Membrane formation

A solution of PSf (16%) in DMAC was prepared under stirring and degassed. UF membranes were prepared by casting the solution onto the polished glass plates which were immediately immersed into water of 10°C, then the water was changed several times and the membranes were kept wet until use.

### 1.3 Photochemical modification technique

A self-made photochemical reactor system was used to modify the membranes. It was equipped with a 1000 W high pressure mercury lamp. The reactor vessel had a quartz window which permitted the UV light penetration. Also the vessel had an inlet and an outlet for nitrogen purging, which was necessary to remove oxygen that could terminate the free radicals formed during photochemical modification.

A solution of monomer was prepared on weight basis. The membrane was placed in the vessel, and the vessel was filled with the monomer solution, sealed and placed in the photochemical reactor. The monomer solution was bubbled with N<sub>2</sub> (99.9999%) for at least 30 min, followed by UV irradiation under nitrogen atmosphere. Thereafter the sample was taken out, extracted with water in a Soxhlet apparatus for 24 h. Finally the membrane was dried to constant weight.

Photografting efficiency was calculated as follows:

$$\text{Percent graft of monomer} = \frac{W_1 - W_0}{W_0} \times 100\%$$

Where  $W_0$  is the weight of the blank membrane,  $W_1$  is the weight of the membrane after photografting polymerization.

### 1.4 Surface analysis

Attenuated total reflection-Fourier transform infrared (ATR/FTIR) spectra of the unmodified and modified PSf membrane were measured with a Nicolet Avatar 360 FTIR spectrometer (Thermo Nicolet Corporation). 32 scans were performed at a resolution of  $\pm 4 \text{ cm}^{-1}$ . A ZnSe crystal was used in the ATR accessory at an incident angle of 45° giving an IR penetration depth of 0–0.6  $\mu\text{m}$ . The morphology of the membrane surface and cross-sections was visualized by a scanning electron microscope (JSM-6301F). To see the cross-section, samples were first freeze fractured under liquid nitrogen. All the samples were glued to a sample support,

then coated with gold in vacuum.

### 1.5 Ultrafiltration

Stirred cell with 50 ml volume and 13.4 cm<sup>2</sup> effective membrane area was used. The membranes were pressurized with water at 0.1 MPa for at least 30 min, then water flux  $J_w$  was measured by timely collection until a stable value was reached. BSA UF was done almost in the same way. After 50% of the feed solution ( $c_0 = 1 \text{ wt}\%$  BSA/water) had been ultrafiltered, retentate and filtrate BSA concentrations were determined with a spectrophotometer, and the retention  $R$  was calculated.

### 1.6 Enzyme immobilizations

#### 1.6.1 Activation with carbodiimide

PSf membranes were immersed in a fresh solution of EDC (10 g/L) in water, which was adjusted to pH = 4.75 and stirred at 4°C for 30 min. Thereafter, the samples were taken out, rinsed quickly with ice water and immediately used for immobilization.

#### 1.6.2 Immobilization

Pre-activated membranes were tightly fixed in a vessel, leaving only the active layer surface open to solution, and enzyme solutions (10 ml,  $c_0 = 1 \text{ g/L}$  in 0.05 mol/L phosphate buffer, pH = 7.0) were applied at 25°C for 2 h. Then the membranes were taken out and rinsed with phosphate buffer.

#### 1.6.3 Enzyme assay

Catalase activity was determined spectrophotometrically, by direct measurement of the decrease of light absorption at 240 nm caused by the decomposition of hydrogen peroxide by the enzyme. Hydrogen peroxide solution (0.02 mol/L) as enzymatic substrate, in 0.05 mol/L phosphate buffer at pH 7.00 was used to determine activity of immobilized enzyme. For photografted membrane immobilizing the enzyme, batch-type operations were used. Measurements were carried out at 25°C in a thermostatted, vigorously stirred reactor in which the enzymatic membrane was placed and 10 ml substrate solution was applied. Enzyme activity was determined by analyzing the samples of the reacting mixture spectrometrically at the first minute of the reaction.

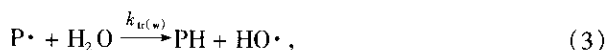
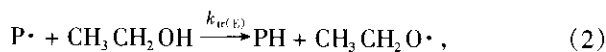
## 2 Results and discussion

### 2.1 Sensitivity of poly(arylsulfone) for UV irradiation

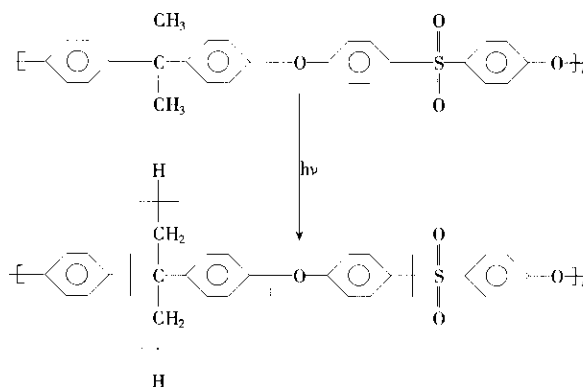
Fig. 1 shows the relative water flux and retention changes of irradiated PSf membranes as a function of irradiation time in two solvents (water and ethanol) without monomer compared with the unmodified membranes. The relative water flux increases sharply with irradiation time for PSf in ethanol but decreases slowly in water, and the retention decreases for PSf in ethanol but increases for PSf in water. This phenomenon results from a balance between pore size enlargement result from chain scission of PSf trunk polymer by the UV irradiation and pore size reduction result from photocrosslinking.

Scheme 1 shows the characteristic repeating units of PSf

and the primary bond scissions leading to the major degradation paths. The reactions in two solvents can be described as follows:



Because the chain transfer constant of ethanol is higher than that of water, so  $k_{tr(E)} > k_{tr(W)}$ , which results in that  $[\text{P}\cdot]$  is higher in water than in ethanol. As a result, velocity constant of crosslinking (4) is higher in water than in ethanol. PSf is easier to photocrosslinking in water than in ethanol. Accordingly in ethanol, the chain scission of PSf trunk polymer is most important, but in water, photocrosslinking is significant.



Scheme 1 Repeat units of poly(arylsulfone) and primary bond scissions of the major photoinduced degradation pathway

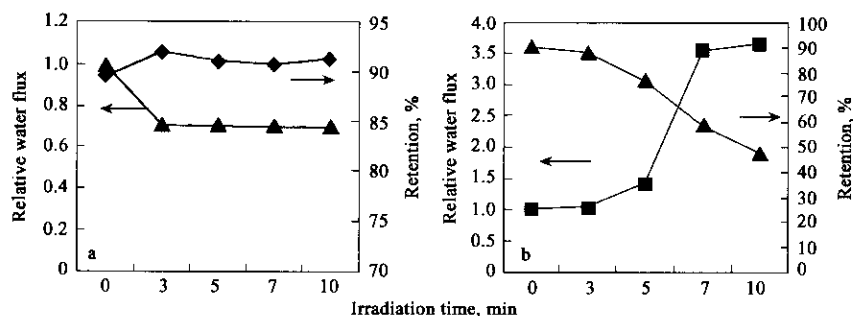


Fig. 1 Water flux and retention changes of membrane under UV irradiation in two solvents (a) water and (b) ethanol

## 2.2 Surface analysis of the modified membranes

Infrared spectroscopy (IR) was employed to provide information about the chemical structure of the modified membranes. ATR/FTIR spectra of original PSf and AA, AAm modified PSf membranes are shown in Fig. 2. The spectra of both membrane a and b are similar at wavelengths below  $1600\text{ cm}^{-1}$  since absorption originating from the grafted AAm are lost in the background noise from the poly(arylsulfone) support material. The  $1578$  and  $1486\text{ cm}^{-1}$  bands are due to the aromatic groups and the  $1323/1298\text{ cm}^{-1}$  doublet and the  $1151\text{ cm}^{-1}$  band are assigned to the aromatic sulfone chromophore. In b, a broad band near  $3100 - 3160\text{ cm}^{-1}$  represents N-H stretch, and the  $1666\text{ cm}^{-1}$  represents C=O stretch peak. c is the IR spectrum of g-PAA membrane. The g-PAA in the surface region of photofunctionalized PSf is identified; intense new IR absorption are detected at  $1699\text{ cm}^{-1}$  and assigned to C=O stretching. In c, there is a layer of PAA grafted on the surface of membrane, and the absorptions originating from PSf are lost in the background from the grafted PAA. d is the IR spectrum of pure PAA, it

is obvious that match of c and d is good, which can also prove that a layer of PAA is grafted onto the surface of PSf.

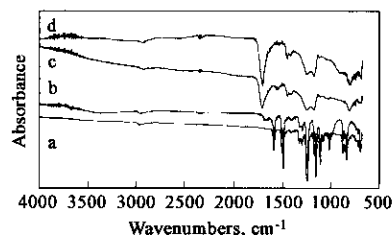


Fig. 2 FTIR spectra of original and modified PSf membrane  
a. original membrane; b. AAm modified membrane;  
c. AA modified membrane; d. PAA

SEM can be used to elucidate the structure of the membrane and the location of the grafted polymer. Fig. 3 shows the SEM picture of surface and cross section of the nascent or grafted membranes of different grafting degree. a and e are the SEM picture of surface and cross section of the nascent membrane. As shown in a, there are a lot of pores on the surface. b, c and d are the surfaces of membranes with

grafting yield of 1.34 % , 7.85 % and 10.25 % respectively. In b, part of pores are blocked up. With the grafting degree growing, the surface pores are become smaller, and finally a layer of PAA are formed on the surface of the nascent membrane, but in c, a lot of gaps are found. In d, the g-

PAA layer becomes denser. f is the cross section of the grafted membrane with a grafting yield of 7.85 % . Comparing f with e, it is found that nothing is added in the pore. Obviously, under this condition, PAA is only grafted on the surface of the membrane instead of its interior.

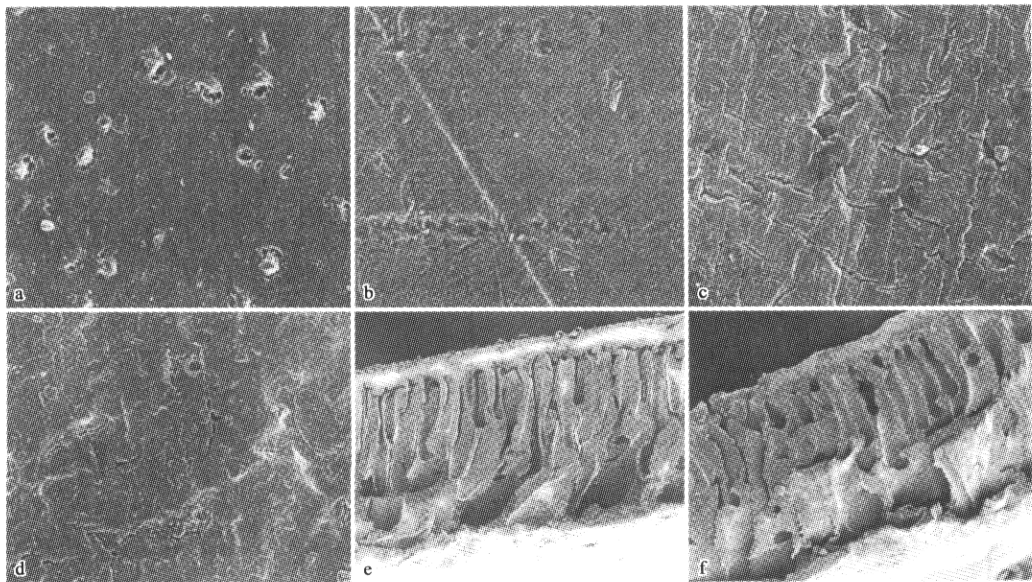
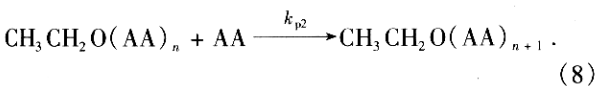
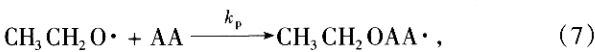
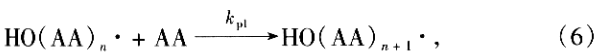
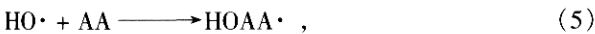


Fig.3 SEM picture of surface and cross section of unmodified and modified membrane  
a ,e. SEM picture of the surface and cross section of nascent memebbrane; b, c and d. surface of membrane with grafting yield of 1.34 % , 7.85 % and 10.25 % , respectively; f. cross section of grafted memebbrane with a grafiing yield of 7.85 %

2.3 Modified membrane performance

Table 1 shows the grafting degree, relative water flux, contact angle and reaction phenomenon at different reaction conditions . It can be seen that compared with photografting in ethanol, reactions in water have higher grafting degree, significant homopolymerization, and sharp water flux decrease. As for  $\Delta J/J_0$  , it results from a balance between enlargement of pores due to chain scission of the PSf trunk polymer by the UV irradiation and pore diameter reduction due to photografting and graft polymerization. From Fig. 1, it is known that pore size enlarge largely in ethanol, but decrease in water, so after grafting, the flux of grafted membrane in water decrease sharply to almost zero, but that of membrane in ethanol decrease not so obviously . Because g-PAA has hydrophilic functions, the grafted membranes both in water and in ethanol have significant contact angle reduction. In addition, reaction (2) and (3) create  $HO\cdot$  and  $CH_3CH_2O\cdot$  , which react with AA in the following reactions :



Due to the superconjugation effect of ethyl, the activity of  $HO\cdot$  is higher than that of  $CH_3CH_2O\cdot$  , so  $k_{p1} > k_{p2}$  , which accounts for the obvious homopolymerization in water.

Table 1 Performance of modified membrane

Experiment conditions <sup>a</sup>	Grafting yield, %	$\Delta J/J_0$ , % <sup>b</sup>	Contact angle, $\theta$	Phenomenna
5/W/5	13.17	100	34	Obvious homopolymerization
5/W/3	5.84	100	30	Obvious homopolymerization
3/W/5	5.50	100	20	Obvious homopolymerization
1/W/5	0.60	100	30	Obvious homopolymerization
5/W/1	0.32	39.13	41	Not obvious
0.5/W/5	Too little to weigh	100	20	Not obvious
0.1/W/3	Too little to weigh	41.20	30	Not obvious
20/E/10	10.25	35.71	45	Not obvious
10/E/10	7.85	55.26	30	Not obvious
5/E/10	1.63	40.48	30	Not obvious
1/E/10	Too little to weigh	56.25	50	Not obvious
0.5/E/10	Too little to weigh	50	45	Not obvious

Notes: <sup>a</sup>Monmer concentration(wt% of monomer)/solvent(W: water; E: ethanol)/irradiation time(min); <sup>b</sup> $\Delta J = J_0 - J$ ;  $J_0$ : flux of original membrane;  $J$ : flux of modified membrane

2.4 Effect of reaction conditions on grafting yield

2.4.1 Effect of irradiation time

The effect of irradiation time at different monomer concentrations on grafting yield is shown in Fig.4. The longer the irradiation time, the greater was the number of AA polymer segments attached to the surface.

2.4.2 Effect of monomer concentration

As can be seen from Fig.5, no matter in water or in ethanol, the grafting yield increases with the growing of

monomer concentration in the reaction conditions we used. But it has larger grafting yield in water than in ethanol. Because the chain transfer constant of ethanol is higher than that of water, so grafting polymerization reaction rate is higher in water than in ethanol, which results in higher grafting yield and longer grafted chain in the former than in the latter.

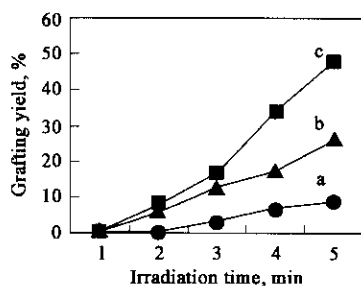


Fig.4 Effect of irradiation time on grafting yield in water at three monomer concentrations

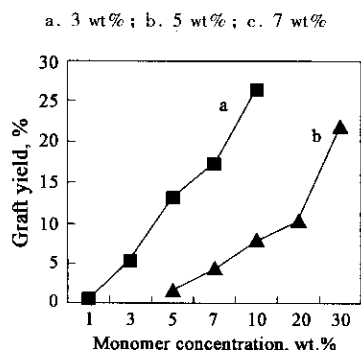


Fig.5 Effect of monomer concentration on grafting yield with a irradiation time 10 min in two solvents

a. in water ; b. in ethanol

### 2.5 Covalent immobilization of hydrogen peroxide oxidoreductase

In this section, the following control samples were used for evaluation of covalent immobilization efficiency: unmodified surface without EDC activation and g-PAA modified surface without EDC activation. Fig. 6 shows the relative activity as a function of operation time. Relative activity is the percentage of enzyme activity to the immediate activity of membrane prepared through grafting-activation-enzyme. With the day up, the relative activities of the three kinds of membrane decrease. But it is apparent that the activity follows the order: membrane prepared through grafting-activation-enzyme > membrane prepared through grafting-enzyme > membrane prepared by enzyme only. The activity of membranes without grafting and without activation which are assigned to physical absorption are very low. That of covalently immobilized enzyme is higher. As for membrane activity prepared through grafting-enzyme is larger than membrane prepared by enzyme only, it seems in conflict with the conclusion that the hydrophobic membrane is susceptible to absorb proteins. This phenomenon can be explained by two factors: first, compared with the unmodified membrane, the grafted membrane has the surface with higher heterogeneity and roughness (this was confirmed by M. Ulbricht *et al.*), which results in larger specific surface area, that accounts for bigger protein absorption. Second, the low activity of membrane prepared by enzyme only may attribute to protein

deactivation on the hydrophobic surface.

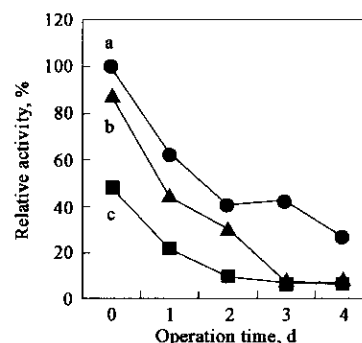


Fig.6 Operation activity of immobilized enzyme

a. membrane prepared through grafting-activation-enzyme; b. membrane prepared through grafting-enzyme; c. membrane prepared only through enzyme

### 3 Conclusions

Under UV irradiation in ethanol, the membrane pore enlargement markedly results in large water flux and small retention, but in water, the water flux of the irradiated membrane decreases and retention increases slowly. This may be attribute to the balance between pore enlargement from the chain scission of PSf trunk polymer and pore reduction from photocrosslinking.

By FTIR-ATR and SEM, it can be confirmed that PAA is grafted onto the surface of PSf UF membrane instead of its interior.

After surface modification, the water flux of UF membrane decreases because membrane pore is covered by the grafted layer of PAA, and contact angle reduces indicating higher hydrophilic activity.

Irradiation time and monomer concentration have great influence on grafting yield. Grafting yield increases with irradiation time and monomer concentration growing respectively under the reaction conditions used.

Hydrogen peroxide oxidoreductase is covalently immobilized onto the PAA grafted and carbodiimide-activated PSf membrane surface, and exhibits higher enzyme activity than control membrane.

### References:

- Ma H, Davis R H, Bowman C N, 2001. Principal factors affecting sequential photoinduced graft polymerization [J]. *Polymer*, 42: 8333—8338.
- Ma H, Davis R H, Bowman C N, 2000. A novel sequential photoinduced living graft polymerization [J]. *Macromolecules*, 33: 331—335.
- Pieracci J, Crivello J V, Belfort G, 1999. Photochemical modification of 10 kDa polyethersulfone ultrafiltration membranes for reduction of biofouling [J]. *J Membr Sci*, 156: 223—240.
- Ulbricht M, Belfort G, 1995. Surface modification of ultrafiltration membranes by low temperature plasma: II. Graft polymerization onto polyacrylonitrile and polysulfone [J]. *J Membr Sci*, 111: 193—213.
- Ulbricht M, Marx U, 1996. Novel photochemical surface functionalization of poly (sulfone) ultrafiltration membranes for covalent immobilization of biomolecules [J]. *J Membr Sci*, 120: 239—259.
- Yamagishi H, Crivello J, Belfort G, 1995. Development of a novel photochemical technique for modifying poly (sulfone) ultrafiltration membranes [J]. *J Membr Sci*, 105: 237—247.
- Yamagishi H, Crivello J, Belfort G, 1995. Evaluation of photochemically modified poly(sulfone) ultrafiltration membranes [J]. *J Membr Sci*, 105: 249—259.