JOURNAL OF Applied Polymer SCIENCE

Preparation and Characterization of Glutaraldehyde Crosslinked Chitosan Nanofiltration Membrane

Xi Zhang, Xiaoyi Jin, Chenyan Xu, Xinyuan Shen
State Key Laboratory for Modification of Chemical Fibers and Polymer Materials, DongHua University, Shanghai 201620, People's Republic of China
Correspondence to: X. Shen (E-mail: shenxy@dhu.edu.cn)

ABSTRACT: In this article, we prepare chitosan (CS) membrane on polyacrylonitrile (PAN) ultrafiltration membrane, and utilize the protonated amine group ($\text{—NH}_3{}^+$) on the CS to retain γ-aminobutyric acid (GABA) in a solution with a pH condition below the amino acid isoelectric point, so as to separate the amino acid from a mixture with sodium acetate that simulates the amino acid fermentation broth. To improve the acid resistance of the composite membrane, we chelate the amine groups on the CS by copper sulfate first, then crosslink the hydroxyl groups in glutaraldehyde solution, and remove the copper ion in hydrochloric acid finally to release the amine groups. This crosslinked CS/PAN composite membrane achieves 95% GABA rejection in pH 4.69 solution under the operation pressure of 0.2 MPa, while over 90% of the sodium acetate permeates the membrane. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 128: 3665–3671, 2013

KEYWORDS: crosslinking; membranes; separation techniques

Received 24 June 2012; accepted 4 September 2012; published online 8 October 2012
DOI: 10.1002/app.38580

INTRODUCTION

Chitin is a kind of natural polymer materials with plentiful resource.[1] The chitin and its deacetylated modification chitosan (CS) membranes exhibit excellent adsorption, permeation, and mechanical properties, as well as unique bio-affinity and decomposition. They can take further modification as esterification, etherification, N-derivation, oxidization, and crosslinking.[2]

Chitin and CS nanofiltration (NF) membranes are attractive subjects for separation of ionic particles. Sun et al.[3] prepared a glycol-chitin/polyacrylonitrile (PAN) composite membrane crosslinked by epichlorohydrin. The negatively charged NF membrane showed 95.2% rejection to divalent anion SO_4^{2-} and 79.9% permeation to divalent cation Mg^{2+}. Zhao et al.[4] introduced carboxymethyl group onto CS O- and N- sites to form amphoteric CS/polyethersulfone composite microfiltration membrane which possessed a higher adsorption of bovine serum albumin (BSA) at low pH 3.0–4.7, but stronger repulsion to BSA at pH 6.0–8.0 than the CS composite membrane. Matsuyama et al.[5] produced an *N*-succinyl CS and polyvinyl alcohol amphoteric gel membrane with apparent isoelectric point (pI) at pH 6.0. In a mixture solution with three kinds of solutes of the same size, the composite membrane is permeable to the anionic solute benzene sulfonic acid while the cationic solute theophylline is rejected at pH 3.0, permeability of the two reversed at pH 11.0. The permeability of a neutral solute styrene glycol was only influenced by mesh size. Huang et al. used diisocyanate[6] and 1,4-butanediyl diglycidyl ether[7] as crosslinking agents to modify 2-hydroxypropyltrimethyl ammonium chloride quaternized CS/PAN composite membrane. The positively charged membranes exhibit excellent capability of removing multi-valent metal ions from wastewater. Dong et al.[8] crosslinked a negatively charged chitin xanthate/PAN composite membrane by H_2O_2, and improved its rejection to multivalent anions, such as PO_4^{3-}, SO_4^{2-}, by optimized membrane preparation conditions.

So far, all those reported positive charge or negative charge modifications on the membranes were grafting foreign groups on O- or N-sites of chitin or CS. Commonly, these groups' reactivity is in the order of $\text{—NH}_2 > \text{—OH} > \text{—NH—COCH}_3$.

In this project, we chelated the amine groups on the CS by copper sulfate first, then crosslinked the hydroxyl groups in glutaraldehyde solution, and finally removed the copper ion in hydrochloric acid. So we kept as many amine groups as possible for protonation in acid solution, and improved CS membrane's acid resistance as well.

In the conventional production of γ-aminobutyric acid (GABA), the final product is isolated from neutralized solution by ion exchange process. However, it costs high and always generates a great amount of wastewater. Feng and Wang[9] tried to purify this amino acid fermentation broth by polyamide NF membrane.

The following work marks the first trial in preparing CS-based positively charged membrane by its native amine groups' protonation, as well as the purification of GABA acid from mixture by positively charged NF membrane.

EXPERIMENT

Materials

CS, MW600,000, degree of deacetylation (DDA) 77%, was provided by Shanghai Tianqing Biochemical Co.; PAN powder, MW70,000, was provided by Nitrile Industrial Department of Shanghai Petrochemical Co.; GABA, B.R., was purchased from Shanghai Yuanji Chemical Co.; polyethylene glycol (PEG) 200–1000, dimethyl sulfoxide (DMSO), 40% formaldehyde solution, 25% glutaraldehyde (GA) solution, and dehydrate sodium acetate were of analytical grade.

Neutralized formaldehyde was prepared by adding 2 mL 1% phenolphthalein ethanol solution into 100 mL 40% formaldehyde solution, and the blending solution was titrated by 0.1 $molL^{-1}$ NaOH till it turns reddish.

Preparation and Characterization of CS/PAN Composite Membrane

Preparation and Characterization of PAN Ultrafiltration Membrane. PAN membrane was prepared[10] with 11 g PAN powder, 1 g PEG200, and 88 g DMSO. The membrane was characterized[11] for porosity and average pore radius, etc.

Preparation of CS and CS/PAN Composite Membrane. CS powder was added into 3% acetic acid aqueous solution with agitation, and a uniform transparent solution was obtained.

Cast 10 mL CS solution onto PAN membrane which had been fixed in a culture dish, evaporated solvents, and dried the membrane in ambient atmosphere, then immersed the membrane in sodium hydroxide (NaOH) aqueous solution in order to convert the $-NH_3^+$ into $-NH_2$ form on the CS surface, as well as to promote ionic bonds formed between part of CS's amine groups and PAN's carboxylic acid groups.[12] The completed composite membrane was washed with and kept in de-ionized water.

The CS membrane for FTIR analysis was prepared by the same process on glass plate instead of PAN.

Preparation of Crosslinked CS and CS/PAN Membrane. Step by step, we immersed the membranes in 1% copper sulfate ($CuSO_4$) aqueous solution for 2 h, 1% GA aqueous solution for 1 h, 0.01 $molL^{-1}$ hydrochloric acid (HCl) for 2 h, as well as 0.001 $molL^{-1}$ NaOH solution for 90 min, and washed the membranes after every step of immersion.

Fourier Transform Infrared Spectroscopy. FTIR spectra were obtained with Perkin Elmer FTIR System BX in a spectral range of 400–4000 cm^{-1}, at a scan speed of 0.2 cms^{-1} and 12 scans per sample.

Contact Angle Analysis. Contact angle measurement was carried out with Micro Optical Angle Measurement OCA40. We injected 3.0 μL water onto membrane surface in a rate of 0.52 $\mu L\ s^{-1}$, took images and measured the drop angles.

Scanning Electron Microscopy. Scanning electron microscopy (SEM) was conducted on the surface and the cross-section of PAN membrane, unmodified and crosslinked CS/PAN composite membranes with Jeol Scanning Electron microscopy JSM-5600LV.

Scanning Probe Microscopy. Scanning probe microscopy (SPM) was carried out with NanoScope IV from Veeco, at scanning rate 1.00 Hz and scanning size 10 $\mu m \times 10\ \mu m$, in ambient atmosphere.

Molecular Weight Cut Off. The molecular weight cut off (MWCO) is defined as 90% retention of a neutral solute. A set of PEGs 200, 400, 600, 800, and 1000 at 1% aqueous solution were filtered under pressure 0.5 MPa. The solutes concentrations in feed and filtrate solutions were detected by total organic carbon analysis (TOC).

Nanofiltration

NF was carried out with a plate membrane evaluation apparatus from Membrane Science & Engineering Research Institute of Eastern China Science & Technology University, at ambient environment (15–20°C), under the pressure of 0.2–0.6 MPa, with varied pH conditions, in GABA as well as its blending aqueous solution with sodium acetate. The permeate solutions were sampled after 10 min of pre-pressure treatment for flux and rejection measurement.

Where,

- J is the permeation flux ($L\ m^{-2}\ h^{-1}$)
- V is the volume of permeate solution (L)
- A is effective area of the membrane (here, $A = 19.625\ cm^2$)
- t is sampling time (h)
- R is the rejection ratio of solutes (%)
- C_p is solute concentration in permeate solution
- C_m is solute concentration in feed solution

$$\text{Solutionflux } J = V/(A \times t) \tag{1}$$

$$\text{Soluterejection } R = \left(1 - C_p/C_m\right) \times 100\% \tag{2}$$

The GABA concentrations in the feed and permeate solutions were titrated with NaOH solution as the amine group of GABA reacted with neutralized formaldehyde to form bis hydroxylmethyl amino acid.[13]

Table I. The FTIR Absorption Band of Chitosan[2]

Vibration	υ_{as} (C–H)	υ_{sym} (C–H)	$\upsilon_{(C=O)}$ Amide I	$\upsilon_{(C-N)} + \delta_{(N-H)}$ Amide II	$\delta_{(=CH2)} + \delta_{(-CH3)}$	$\delta_{(C-H)} + \delta_{sym(CH3)}$	$\upsilon_{(C-N)} + \delta_{(N-H)}$ Amide III	υ_{as} (C–O–C)	$\upsilon_{(C-O)}$ Secondary –OH	$\upsilon_{(C-O)}$ Primary –OH
υ (cm^{-1})	2919	2878	1658	1588	1420	1379	1318	1153	1094	1037

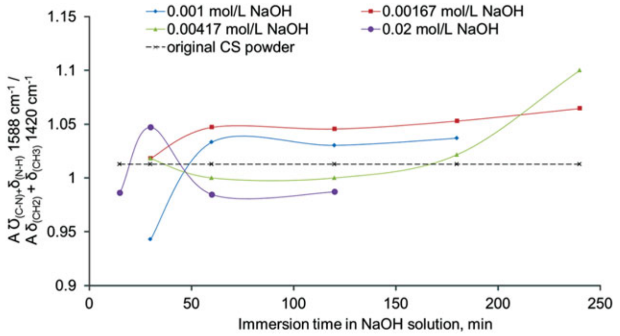

Figure 1. IR absorbance of amine groups $\upsilon_{(C-N)} + \delta_{(N-H)}$ at 1588 cm^{-1}/$\delta_{(CH2)} + \delta_{(CH3)}$ at 1420 cm^{-1} of CS membrane vs. immersion conditions in NaOH solution. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

$$H_2N - CH_2-CH_2-CH_2-COOH \xrightarrow{HCOOH} HOCH_2NH - CH_2-CH_2-CH_2-COOH \xrightarrow{HCOH} (HOCH_2)_2 N - CH_2-CH_2-CH_2-COOH \qquad (3)$$

Sodium acetate concentration was measured by DWS-51 Sodium ion meter, and the solution pH was valued by PXSJ-216 PH meter from Shanghai Precision Science Instrument Co.

RESULTS AND DISCUSSION

GABA ($C_4H_9NO_2$, MW103.12, CAS 56-12-2) Isoelectric Point

The amino acid is a zwitterionic compound, when feed solution takes a pH condition close to amino acid's pI, the transmission of neutral amino acid depends on size exclusion; in case of charged amino acids, both size exclusion and electrical repulsions influence its behavior of transmission during filtration.

$$^{+}H_3N - CH_2-CH_2-CH_2-COOH \overset{K_{a1}}{\longleftrightarrow} {}^{+}H_3N - CH_2-CH_2-CH_2-COO^{-} \overset{K_{a2}}{\longleftrightarrow} H_2N - CH_2-CH_2-CH_2-COO^{-} \qquad (4)$$

As we all known,

$$\begin{aligned} K_{a1} &= [H^{+}]\cdot[A^{+-}]/[A^{+}] = 3.7\times10^{-11} \\ K_{a2} &= [H^{+}]\cdot[A^{-}]/[A^{+-}] = 1.7\times10^{-10} \\ [H^{+}] &= K_{a1}\cdot[A^{+}]/[A^{+-}] = K_{a2}\cdot[A^{+-}]/]A^{-}] \\ [H^{+}]\cdot[H^{+}] &= K_{a1}\cdot K_{a2}\cdot[A^{+}]/[A^{-}] \end{aligned}$$

When,

$[A^{+}] = [A^{-}]$ So,

$$\begin{aligned} pI &= (pK_{a1} + pK_{a2})/2 \\ &= (-\log 3.7 + 11 + \log 1.7 - 10 + 14)/2 = 7.3311 \end{aligned}$$

Characterization of Membranes' Modification by FTIR Spectroscopy

Table I lists the characteristic FTIR absorption bands of CS.

In acid solution, the amine group $-NH_2$ on CS shifts to $-NH_3{}^{+}$ form, but it does reverse in NaOH solution after CS film formation.

Immersed in NaOH solution for however long, the methyl group ($-CH_3$) status on CS membrane surface as well as its FTIR vibrational absorption band remains intact.

To minimize the influence of membrane thickness on amine groups' IR absorbance, we express the recovery of the amine groups as the ratio of IR absorbance at 1588 cm^{-1} pertaining to amide II band to that at 1420 cm^{-1} responding to methylene and methyl.[14]

Figure 1 presents that the dilute NaOH solution (e.g., 0.001 $molL^{-1}$) effectively deprotonates the amine groups when the membranes are immersed for 60–150 min, but higher concentration (e.g., 0.00417–0.02 $molL^{-1}$) NaOH solutions result in alkaline CS.

So we fix all the following CS membranes to 90-min treatment in 0.001 $molL^{-1}$ NaOH.

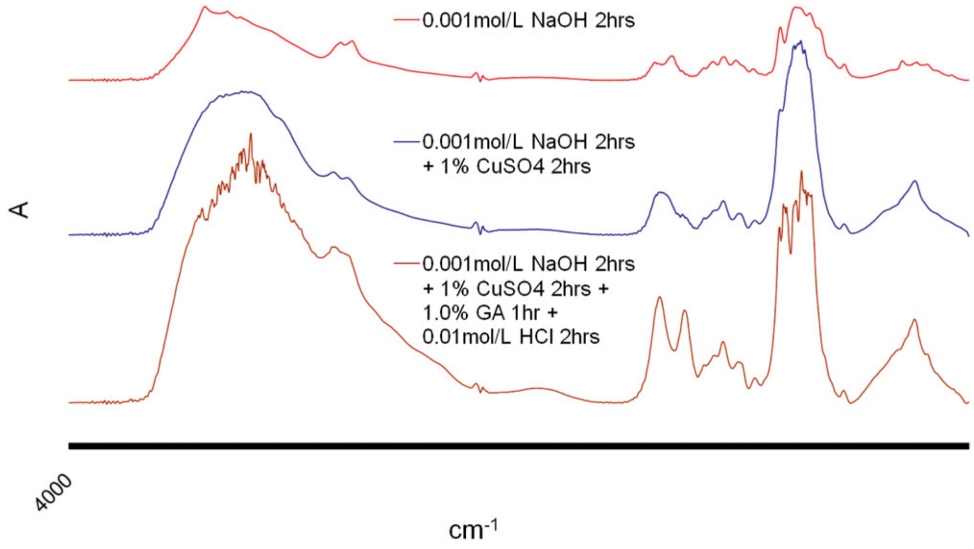

Figure 2. FTIR absorption vs. CS membrane modification. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

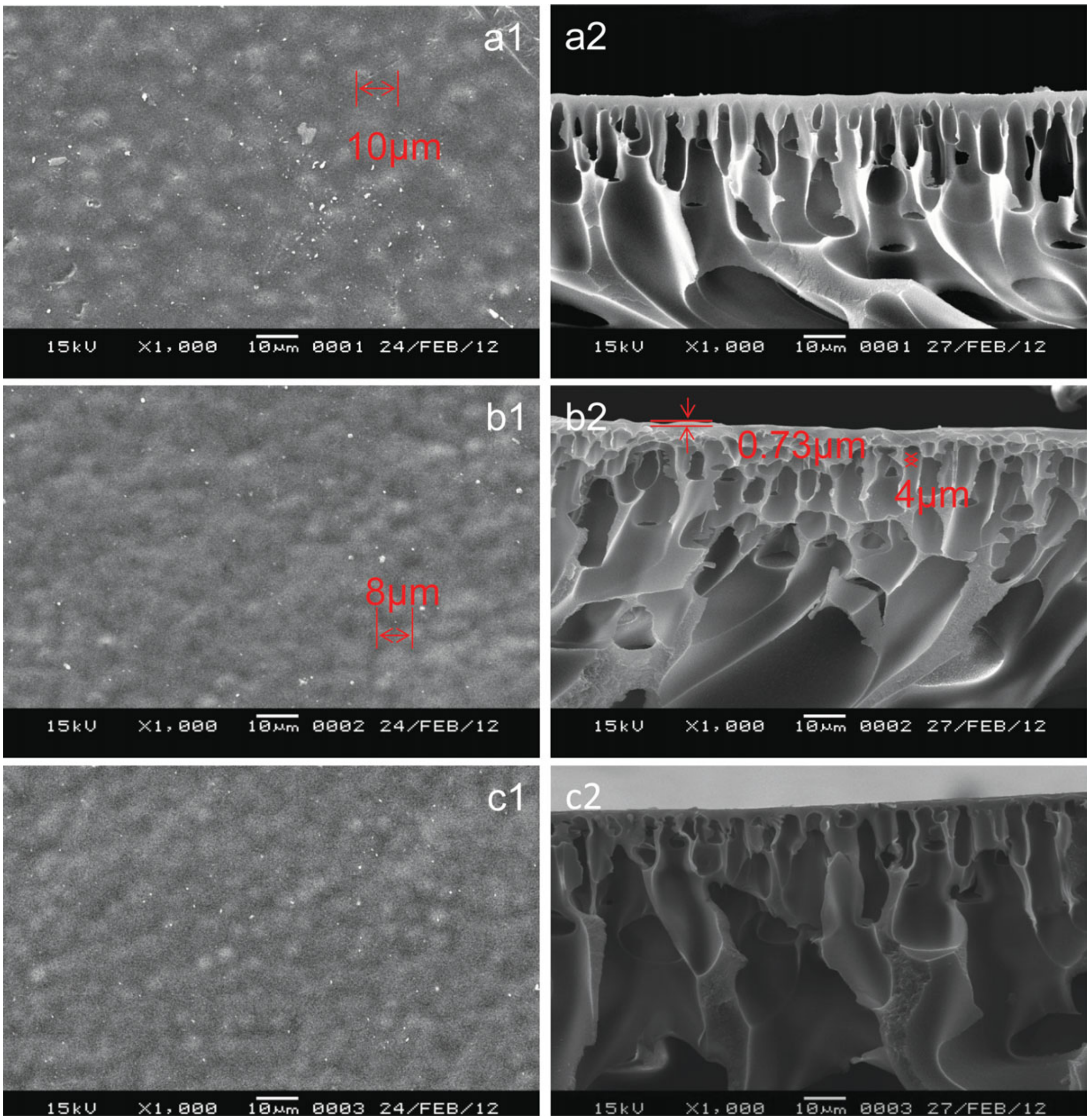

Figure 3. SEM images of membrane surface and cross-section: (a) PAN membrane, (b) CS/PAN composite membrane, and (c) crosslinked CS/PAN composite membrane. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 2 traces the variation of FTIR absorption bands relating to CS membrane modification:

- After a 2-h immersion in 1% $CuSO_4$ aqueous solution, the absorption band at 1588 cm^{-1} corresponding to N—H bending of amide II disappeared. At the same time, the secondary hydroxyl groups get more space for vibration and show stronger absorption band at 1094 cm^{-1}.
- Following GA crosslinking and Cu^{2+} being removed, this N—H bending absorption band shows up at 1544 cm^{-1} remarkably, the deviation toward low frequency

Table II. Membranes' Features

Membranes	MWCO (Da)	Porosity (%)	Average pore radius (m)	Pure water flux (L m^{-2} h^{-1}) (pressure 0.4 MPa)	Contact angle (°)	SPM	
						R_a (nm)	R_{RMS} (nm)
PAN membrane	–	70	1.47×10^{-8}	125	80	71.82	87.42
CS/PAN composite membrane	1050	–	–	12	102	50.49	63.84
Crosslinked CS/PAN composite membrane	850	–	–	8.5	86	35.44	43.41

Table III. Effect of Different Conditions in Chitosan Membrane Preparation

	Cast membrane drying condition		NaOH aq. Solution		Filtration			
							1% amino acid aq. solution	
Chitosan concentration in 3% HAC aq. Solution (%)	Temp. (°C)	Time (h)	Concentration (mol L^{-1})	CS membrane immersion time (min)	Pressure (MPa)	Pure water flux (L m^{-2} h^{-1})	Flux (L m^{-2} h^{-1})	Rejection (%)
1	20	72	0.001	30	0.3	269		
				60		275		
				90		269		
1.33	20	72	0.001	30	0.5	12.84	12.84	0
				60		15.9	15.9	2.63
				90		16.51	15.73	8.93
				120		7.95	6.11	7.9
				180		0		
1.33	20	72	0.006	30	0.5	15.29	25	5.26
				60		27.51	44	2.63
				90		4.59	7.95	-
1.33	20	72	0.01	30	0.5			0
				60			3.87	
				90			1.02	
				120			4.07	
1.33	40	20	0.001	30	0.5		0	
				60		0		
				90		0		
2	20	72	0.001	30	0.5		0	
				60		0		
				90		0		

comes from environmental change caused by —OH crosslinking.

- GA crosslinking makes C—O—C—O—C stretch vibration absorption at 1153 cm^{-1} much stronger, which results from the acetalization between GA's carbonyl and CS' hydroxyl groups.[15]

Observation of Membranes Features by Contact Angle, SEM, and SPM

To our surprise, in contact angle images, the CS membrane becomes more hydrophilic after its hydroxyl groups crosslinking with GA's carbonyl groups. We infer that the crosslinking reduces hydrogen bonding and weakens the interaction between CS chains, so it makes water molecular much easier to permeate CS membrane.

PAN substrate exhibits an orange peel-like surface [Figure 3(a)], and digitiform pores 3–6 μm in diameter are beneath the surface. The pore expands toward the membrane bottom side.

The pore diameter of PAN substrate in SEM images is much larger than 14.7 nm as previously calculated in section "Preparation and characterization of PAN ultrafiltration membrane" (Table II), because the pore sizes obtained from solution flux is restricted by the narrowest part of the pore, and the membrane is a bit distorted during the operation.

CS coat condenses and smoothens the surface of PAN substrate, and the crosslinking further flattens the CS surface.

From Figure 3(b2,c2) cross-sections, we read CS coat thickness as about 0.73 μm.

SPM roughness analysis is in line with what we find from SEM images of membrane surface (Table II).

Filtration

Effect of CS Membrane Preparation Condition. Table III indicates the effect of CS/PAN membrane preparation conditions on the membrane filtration performance:

- 1% CS solution results in a very thin functional layer with high solution flux and no amino acid rejection, while 2% CS solution offers a thick layer with zero flux; 1.3% CS solution casts a functional layer with reasonable flux and amino acid rejection.
- CS membrane, dried at 40°C for 20 h, forms a very tight and well crystallized functional layer with very low flux. So

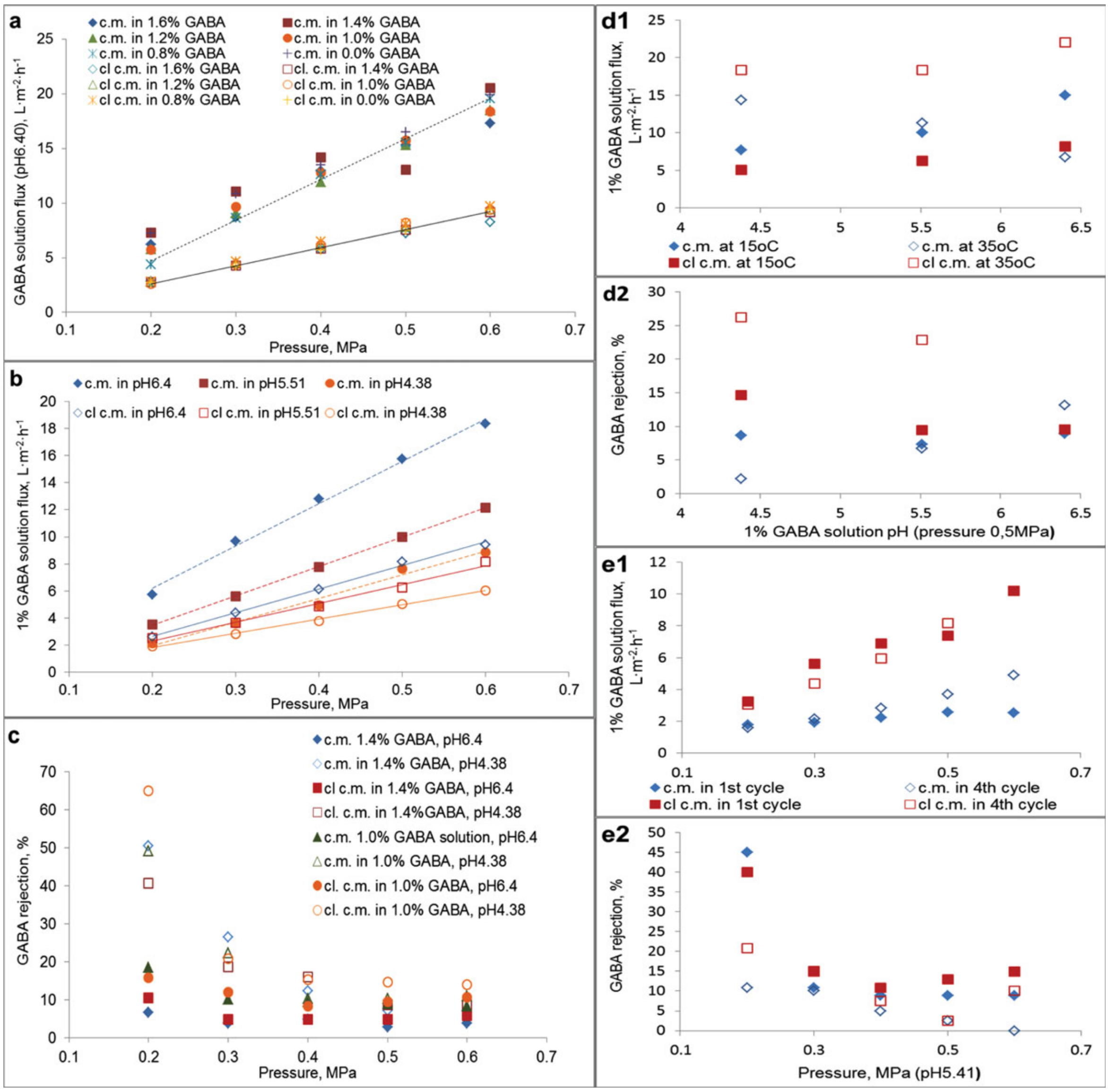

Figure 4. GABA solution flux and GABA rejection in various operation conditions (c.m. is CS/PAN composite membrane and cl c.m. is crosslinked CS/PAN composite membrane, operation temperature is 15–20°C): (a) solution flux vs. GABA concentration, (b) 1% GABA solution flux vs. solution pH, (c) GABA rejection vs. GABA concentration and solution pH, (d) solution flux and GABA rejection vs. operation temperature and solution pH, (e) solution flux and GABA rejection vs. operation cycles. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

we prefer to dry the CS membrane at 20°C for 72 h. The product keeps constant performance after 20 cycles of 5 h operation.

- Concerning the restoration of —NH_2 from —NH_3^+, 0.01 molL^{-1} NaOH solution would cause too small a flux in the CS membrane, and 0.006 molL^{-1} NaOH results in a great fluctuation in CS membrane filtration performance versus immersion time. With 0.001 molL^{-1} NaOH solution, both CS membrane flux and amino acid rejection increase with immersion time extending in the range of 30–90 min, but drop upon 120 min immersion. We can conclude that high NaOH concentration or long immersion time may damage the pore structure in CS membrane and consequently reduce flux.

So we decide to prepare the CS membrane with 1.3% cast solution, followed by 72 h drying at 20°C and 90 min immersion in 0.001 molL^{-1} NaOH solution. The completed membrane is washed with and kept in deionized water.

Effect of CS Membrane Crosslinking. FTIR analysis (Figure 2) shows that after 60–120 min of immersion in 1% $CuSO_4$ aq. solution, absorption band at 1588 cm^{-1} reflecting N—H bending of CS amide II disappeared.

Crosslinking in 1% GA solution for 60 min optimizes the CS membrane acid resistance, as well as filtration performance, while 2% GA solution causes CS membrane to brittle in a short time, and 0.5% GA cannot give CS membrane adequate acid resistance by 1 h immersion.

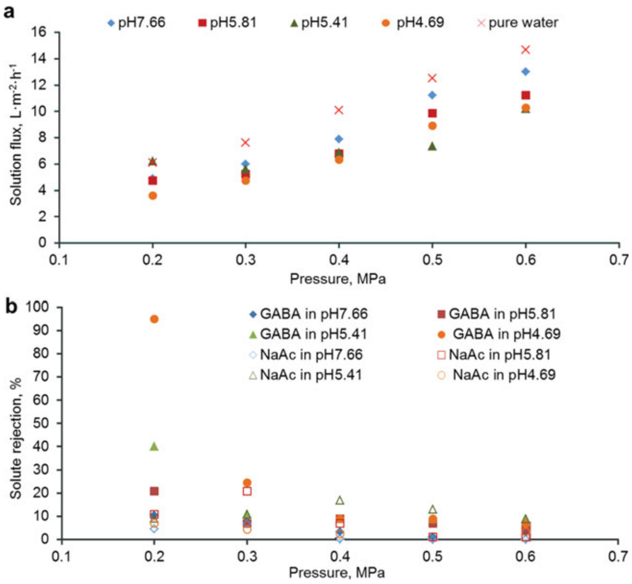

Figure 5. 1% GABA and 0.5% NaAc blending solution filtration by crosslinked CS/PAN composite membrane. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

To remove Cu^{2+} from amine groups chelating after the CS hydroxyl groups crosslinking, immersion in 0.01 molL^{-1} HCl for 2 h is selected according to degree of amine groups restoration and tensile strength retention of crosslinked CS membrane.

Effect of Operation Conditions. The flux of the GABA solution depends mainly on the membrane pore size and the operation pressure, but very little on GABA concentration. In 1% GABA solution, compared to pH 6.40 status, CS/PAN composite membrane retains 63% flux in pH 5.51 and 44% in pH 4.38, while crosslinked CS/PAN composite membrane can keep 83% and 64%, respectively. The flux decline may result from increased membrane swelling, contamination, or repulsion from aggregating positive charge on the membrane surface.

GABA rejection increases as the solution pH decreases, and it drops against the rise in the operation pressure and the solution flux.

Compared to a mild operation condition at 15–20°C, CS/PAN composite membrane exhibits a higher flux and a lower solute rejection at 35°C and pH 4.38 solution because of swelling or even dissolving, but crosslinked CS/PAN composite membrane upgrades both solution flux and solute rejection in such a harsh environment.

Having worked in acidic solution (pH 5.41) for longer than 15 h, CS/PAN composite membrane gradually rises the solution flux and declines the solute rejection, it cannot be recovered anyhow. Crosslinked CS/PAN composite membrane always keeps stable solution flux but losses solute rejection significantly after 15 h of operation in the same condition, the lost solute rejection can be recovered by washing with deionized water.

Seperation of GABA from Sodium Acetate. Under the operation pressure of 0.2 MPa, the crosslinked CS/PAN composite membrane shows 23% flux drop when the solution pH decreases from 7.66 to 4.69, but it promotes GABA rejection from 10.5% to 95% due to developing positive charge density on the membrane surface, when NaAc rejection remains in a range of 0–20% in all conditions.

CONCLUSIONS

A CS NF membrane was prepared with support of PAN ultrafiltration membrane. Its acid resistance was dramatically improved by GA crosslink between hydroxyl groups, when its amine groups were protected by copper ion chelation in advance. The crosslinked CS/PAN composite membrane, with its aggregating protonated amine groups in pH 4.69 solution, achieved good separation of GABA from NaAc in mixture with rejections 95% and 7%, respectively.

ACKNOWLEDGMENTS

This research was financially supported by State Key Laboratory for Modification of Chemical Fibers and Polymer Materials (No. LZ0902).

REFERENCES

1. Jiang, T. D. Chitin, 1st ed.; Chemical Industry Press: Beijing, **2005**; Chapter 1, p 4.
2. Jiang, T. D. Chitosan, 1st ed.; Chemical Industry Press: Beijing, **2001**; Chapter 1, p 18.
3. Sun, H. W.; Chen, G. H.; Huang, R. H.; Gao, C. J. *J. Membr. Sci.* **2007**, *297*, 51.
4. Zhao, Z. P.; Wang, Z.; Ye, N.; Wang, S. C. *Desalination* **2002**, *144*, 35.
5. Matsuyama, H.; Tamura, T.; Kitamura, Y. *Sep. Purif. Technol.* **1999**, *16*, 181.
6. Huang, R. H.; Chen, G. H.; Sun, M. K.; Hu, Y. M.; Gao, C. J. *J. Membr. Sci.* **2006**, *286*, 237.
7. Huang, R. H.; Chen, G. H.; Yang, B. C.; Gao, C. J. *J. Appl. Polym. Sci.* **2010**, *118*, 2358.
8. Dong, T. T.; Chen, G. H.; Gao, C. J. *J. Membr. Sci.* **2007**, *304*, 33.
9. Feng, B.; Wang, W. *J. Food Sci. Biotechnol.* **2006**, *25*, 5.
10. Shen, X. Y.; Wang, Q. R.; Chen, X. Y.; Yu, C. Z. *Membr. Sci. Technol.* **1995**, *15*, 34.
11. State Oceanic Administration. HY/T039 Measurement of Microfiltration Membrane Pores Performance; State Oceanic Administration Press: Beijing, **1995**.
12. Nanda, D.; Tung, K. L.; Hsiung, C. C.; Chuang, C. J.; Ruaan, R. C.; Chiang, Y. C.; Chen, C. S.; Wu, T. H. *Desalination* **2008**, *234*, 344.
13. Shakiestone. The chemical reaction of the amino acid. http://zh.wikipedia.org/wiki(accessed June 17, **2012**).
14. Brugnerottoa, J.; Lizardib, J.; Goycooleab, F. M.; ArguÈelles-Monalc, W.; DesbrieÁresa, J.; Rinaudo, M. *Polymer* **2001**, *42*, 3569.
15. Yang, Q.; Liang, R. B.; Dou, F. D.; Shen, X. Y.; Tan, Z. Q. *Cellulose Sci. Technol.* **2005**, *13*, 13.