Appl Biochem Biotechnol (2009) 158:11–19
DOI 10.1007/s12010-009-8641-9

# Separation and purification of aloe polysaccharides by a combination of membrane ultrafiltration and aqueous two-phase extraction

**Jian-min Xing · Fen-fang Li**

Received: 23 March 2008 / Accepted: 6 April 2009 /
Published online: 5 May 2009


**Abstract** A two-step process was developed for the purification of polysaccharides from the pulp of Aloe varavia using aqueous two-phase system (ATPS) extraction and a novel copolymer ultrafiltration membrane. The first step was ATPS under optimal separations conditions using a total composition of 18% PEG2000, 25% ammonium sulfate, pH 3.0, and 0.3 M NaCl. To form the copolymer membrane, poly(acrylonitrile-acrylamide-styrene) was prepared by solution polycondensation using azoisobutyronitrile as initiator. Then, membranes were formed from the dissolved copolymer by the phase inversion method. Copolymer structure was investigated by infrared spectrum and thermogravimetric analysis (TGA). The copolymer membrane surface and cross section were observed by scanning electron microscopy. The water flux of this membrane was 26.33 mL/($cm^2$ h), and retention was 96% for bovine serum albumin and 34% for dextran T40000. The separation and purification of aloe polysaccharide were carried using this copolymer membrane following ATPS. The TGA of aloe polysaccharide demonstrated a high purity of the polysaccharide. By gas chromatographic analysis, it was shown that mannose is the main monosaccharide in the aloe polysaccharide, and only a few glucose residues are present.

**Keywords** Ultrafiltration membrane · Water flux · Aqueous two phase · Aloe polysaccharide · Purification

## Introduction

Aloe is a member of liliaceae family. A total of 360 aloe species are growing in the dry regions of North America, Europe, and Asia. Aloe is most widely accepted and used for various medical, cosmetic, and nutraceutical purposes. The aloe plant is composed of elongated and pointed leaves. Each leaf consists of two parts, an outer green rind and an inner clear pulp. The pulp (consisting principally of polysaccharide) has been utilized since ancient times to treat burns and other wounds. It is thought to be able to accelerate healing

J.-m. Xing · F.-f. Li (✉)
School of Chemistry and Chemical Engineering, Central South University, Changsha 410083, China
e-mail: lfflqq@mail.csu.edu.cn

rate and to reduce infection risk. Research results have shown that the polysaccharides have some specifically biological activities such as anti-inflammation, anti-cancer, anti-diabetes, and macrophage activation [1–5]. In addition, it has been shown to inhibit AIDS virus replication in vitro. There has been growing research interest in the separation and purification of aloe polysaccharides by various separation methods such as ethanol precipitation, gel permeation chromatography, and membrane separation [6, 7]. Ethanol precipitation seems to be predominant in the production of aloe polysaccharides recently. Gel permeation chromatography has been proven feasible and effective, but high investments for building equipment and complex operations limit its large scale application. Compared with the above-mentioned methods, membrane separation methods have an edge over others: They do not usually require the addition of chemicals, are less energy intensive, and are easy to operate. Thus, membrane separation is a possible candidate for the separation of aloe polysaccharide, but the viscosity of aloe gel is very high, and it will cause membrane fouling, so it is necessary to decrease the viscosity of aloe gel juice for membrane separation. At present, aqueous two-phase extraction systems (ATPS) are not an extensively used separation and purification technology. Because ATPS can be made highly selective and can be suited for continuous operation at large scale, it has been recently popularized in the separation and purification of proteins, natural products, enzymes, amino acids, and so on [8–12]. In this paper, a specific aqueous two-phase system was chosen as a pretreatment method based on our preliminary research work. The poly(acrylonitrile-acrylamide-styrene) (poly(AN-AM-St)) membrane was prepared by the phase inversion method, and the polysaccharide was separated by a combination of aqueous two-phase extraction and membrane separation. The flux through the membrane was greatly improved by first using the ATPS, and the high purity polysaccharide was obtained.

## Experimental

### Experimental Materials

Styrene, acrylonitrile, acrylamide, azoisobutyronitrile, and *N*,*N*-dimethylacetamide were of analytical grade from Changsha chemical reagent factory in Changsha, and dextran T40000 and PEG2000 of analytical grade and bovine serum albumin (BSA) were the biochemical reagent from Sinopharm Chemical Reagent in Shanghai. Fresh whole aloe leaves (A. varavia) were obtained from the Hainan province in China.

### Preparation of Poly(AN-AM-St)

The reaction was carried out in a three-necked flask equipped with stirrer, condenser, and nitrogen gas inlet. Styrene 4.1664 g, acrylonitrile 7.4474 g, acrylamide 1.4216 g, and azoisobutyronitrile 0.1303 g were dissolved in 95 mL of *N*,*N*-dimethylacetamide at 60 °C. After 6 h, the solution was cooled rapidly in the water bath at 25 °C. The polymer was dried in the vacuum oven at 60 °C.

### Preparation of Poly(AN-AM-St) Membrane

Copolymers17.00 g and PEG 1.00 g were dissolved in 100 g *N*,*N*-dimethylacetamide with mechanical stirring and heating about 24 h at 45 °C. The solution was filtered and poured onto a glass plate without the application of mechanical spreading. Then, the solvent was

allowed to evaporate about 2 min at 50 °C in a vacuum oven. The plate was dipped into a water bath to separate the poly(AN-AM-St) membranes from the glass plate. The membranes were washed overnight in flowing water, rinsed several times in deionized water, and kept at 10 °C until used.

## Characterization of Poly(AN-AM-St) Membranes

The FTIR of copolymer was determined using a Nicolet AVATAR 360 spectrometer from USA. Membrane structure was examined by scanning electron microscopy (SEM), for which membrane samples were prepared by soaking in isopropanol, then hexane, and subsequent vacuum drying at 50 °C for 24 h. Thermal properties of membranes were determined by differential thermal analysis (Labsys1600, SETARAM, France). The test was carried out at a heating rate of 5 °C/min in a nitrogen gas atmosphere.

The permeation flux of the membrane was determined by measuring the weight of the fluid permeating the membrane during a certain period of time and calculated using the following equation:

$$F = W/(A \times t)$$

where $F$ is the permeation flux, $A$ is the effective area of the membrane, $t$ is the time for permeation, and $W$ is the weight of the permeation fluid passing through the membrane. Rejection was calculated with the following equation:

$$R = 1 - C_{\mathrm{P}}/C_{\mathrm{M}}$$

where $C_{\mathrm{P}}$ is the permeation concentration and $C_{\mathrm{M}}$ is the feed concentration. The data presented are the averages of three of these measurements. The pressure drop used in all permeation experiments was 0.1 MPa in a dead-end filtration apparatus. Experiments began with solution only on the feed side of the membrane and zero permeate volume.

## Separation and Purification of Polysaccharides by ATP and Membrane Technology

Aloe leaves were first rinsed and drained to remove the yellow sap from the rind and then cleaned with deionized water. The rind was removed with a sharp blade. The clear pulp was homogenized using FSH-2 homogenizer (Hainan zhonghe instrument, Haikou) at 8,000–18,000 rpm. The homogenized pulp was centrifuged (800B centrifuge from Changsha Yintai instrument) at 4,000 rpm for 15 min. The aqueous two-phase systems were prepared from stock PEG2000 and salt solutions by adding the $(NH4)_2SO_4$ and PEG2000 to 2,000 g aloe gel juice to give a predetermined final total composition consisting of 18% PEG2000, 25% $(NH4)_2SO_4$, 0.3 M NaCl, pH 3.0.After phase separation, samples were removed carefully from top and bottom phases separately. The bottom phase, which had previously been shown to concentrate aloe polysaccharide, was selected for further purification by membrane separation.

## Polysaccharide Analysis

The polysaccharide concentrations were measured in bottom-phase, feed, and permeate solutions by the method of phenol-sulfuric acid. The monosaccharide composition of aloe polysaccharide was determined as described earlier [1] Briefly, aloe polysaccharide was hydrolyzed with 2 M trifluoroacetic acid (3 mL) at 110 °C. The subsequent treatment of

the resultant dry hydrolysate with acetic anhydride and pyridine produced the corresponding alditol acetate, which was analyzed by gas chromatography from Shimadzu, Japan. Chromatogram conditions: A 30 m×0.32 mm×0.25 μm OV-1 capillary column was used. Temperature increasing procedure started at 40 °C, increased to 160 °C at 4 °C/min, then to 250 °C at 5 °C/min, and maintained for 20 min. $N_2$ carrier gas was employed at a constant flow rate of 20 mL/min. The inlet temperature and the interfacial temperature were 280 °C.

## Result and Discussion

### FTIR and Thermogravimetric Analysis of Poly(AN-AM-St)

FTIR spectrum of poly(AN-AM-St) is shown in Fig. 1. According to the FTIR, the nitrile group gave the absorbance band at 2,230 $cm^{-1}$; the $–CONH_2$ was characterized by the pair of N–H absorbances (at 3,340 $cm^{-1}$, 3,140 $cm^{-1}$), and C═O stretching bands were detected (at 1,680 $cm^{-1}$, 1,650 $cm^{-1}$); characteristic peaks of the benzene ring were found at 1,603 $cm^{-1}$, 1,494 $cm^{-1}$. The copolymer also was analyzed by thermogravimetric analysis (TGA). The TGA curves of poly(AN-AM) and poly(AN-AM-St) of two copolymer compositions were investigated as shown in Fig. 2. It was found that the thermal decomposition temperature of poly(AN-AM-St) did not change compared to that of poly(AN-AM), but rate of mass loss increased with increased content of St. These data provide evidence that a monomer unit consisting of AN, AM, and St had been polymerized.

### Performance and Structure of the Copolymer Membrane

Figure 3 shows the water flux vs. time for the copolymer membrane. After 3 h at a pressure drop of 0.1 MPa, the flux of pure water stabilized at 26.33 mL/($cm^2$ h). Rejections were

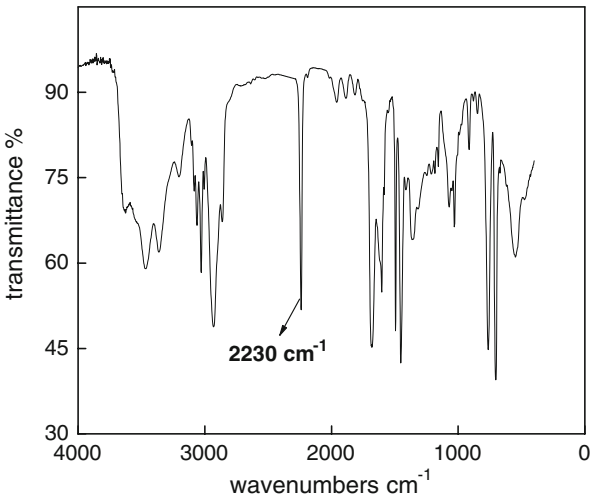


**Fig. 1** FTIR spectrum of membrane copolymers

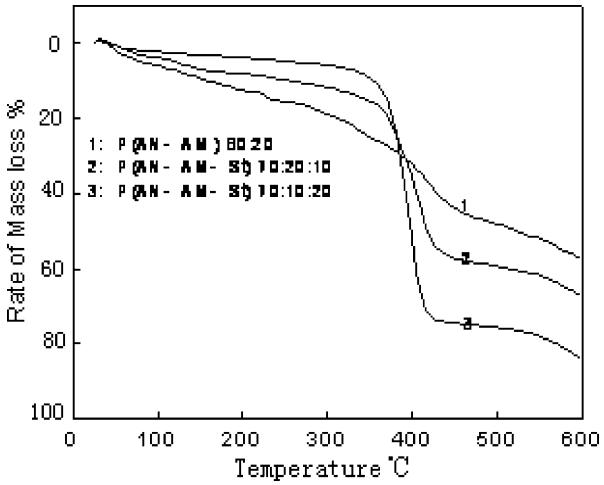


**Fig. 2** TGA curves of membrane copolymers having 0%, 10%, and 20% styrene

measured with 0.1% aqueous solution of BSA (68,000D) and 0.1% aqueous solution of dextran T40000, and retention was 96% for BSA and was only 34% for dextran T40000. The result shows that the membranes have a good rejection for BSA, so the molecular-weight cutoff of the polymer membrane is at or near 68000D.

The membrane structure was investigated by SEM. SEM of surfaces and cross sections of poly(AN-AM-St) membranes are shown in Figs. 4 and 5, respectively. As can be seen from Fig. 4, the membrane clearly shows the presence of pores on membrane surface, and Fig. 5 shows an asymmetric porous structure of the cross section with a dense "skin" layer on top of the top (right) surface and a supporting layer formed with many void and macrovoid pores in the cross section.

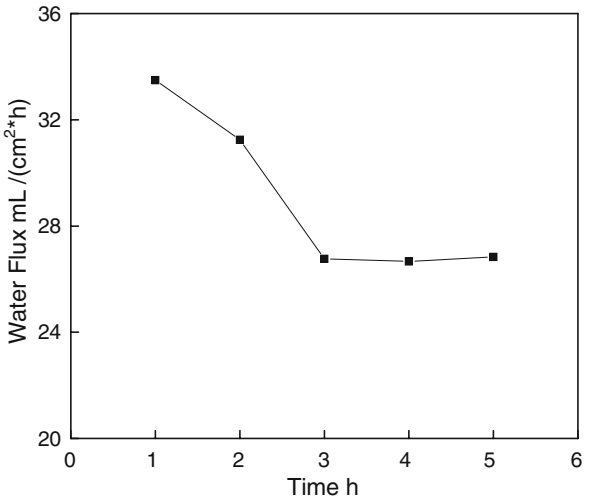


**Fig. 3** Pure water flux vs. time for copolymer ultrafiltration membrane

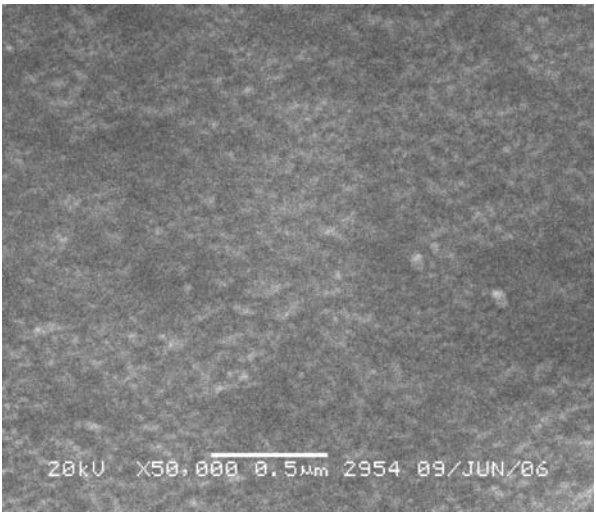


**Fig. 4** SEM of membrane surface

Separation and Purification of Aloe Polysaccharide by ATPS and Membrane Ultrafiltration

Preliminary work had shown that aloe polysaccharide concentrates in the bottom phase of the PEG2000/$(NH4)_2SO_4$ ATPS. After the aloe gel was separated by using the aqueous two-phase system at the optimal condition given above, the bottom polysaccharide-rich phase was filtered by using the poly(AN-AM-St) membrane. Figure 6 shows the effect of permeation time on saccharide content of the permeate solution. Saccharide concentration in the permeate was measured by the method of phenol-sulfuric acid. The results show that the oligosaccharides and monosaccharide decreased as permeation time increased in the

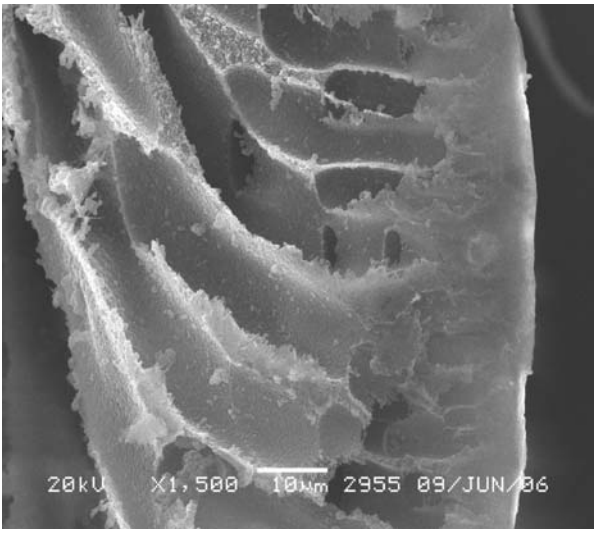


**Fig. 5** SEM of cross section

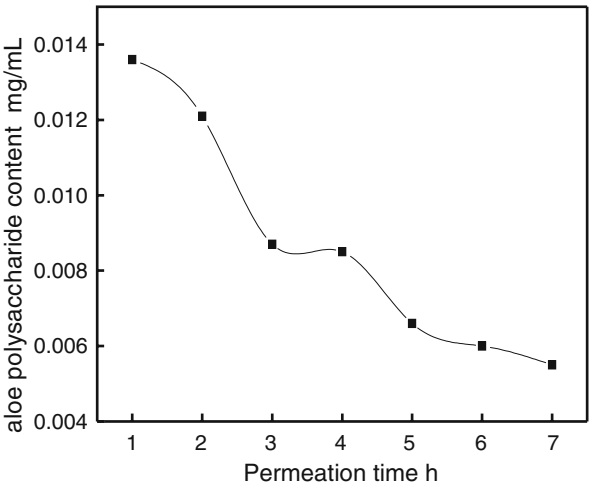


**Fig. 6** Effect of permeation time on saccharide concentration in accumulated permeate measured by the phenol-sulfuric method during dead-end filtration

permeation solution, indicating that after an initial breakthrough, accumulated saccharide content was diluted as permeate volume increased during dead-end filtration. The permeate concentration fell by a factor of 3 in 7 h, at which time the separation was terminated. Volumetric flux decline also was investigated. The comparison between the flux of the bottom-phase solution of the aqueous two-phase system and that of the aloe gel juice through the poly(AN-AM-St) membrane are shown in Fig. 7. Curves 1 and 2 represent the flux of the bottom-phase solution and of aloe gel juice, respectively. The rate of flux decline was much greater for the aloe gel juice than that of the bottom-phase polysaccharide solution.

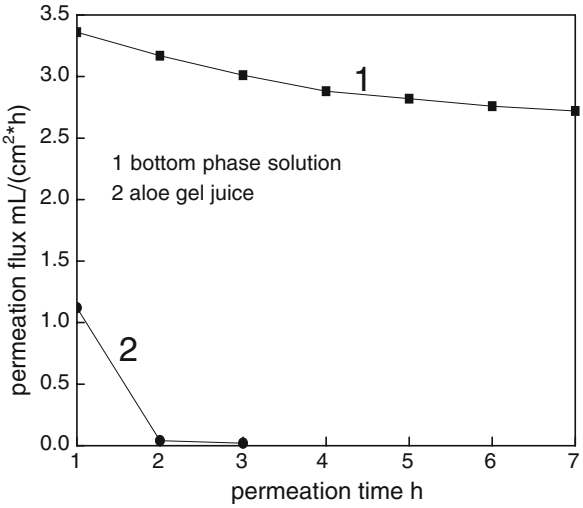


**Fig. 7** Flux decline during filtration of aloe juice (*curve 2*) and bottom-phase polysaccharide solution (*curve 2*)

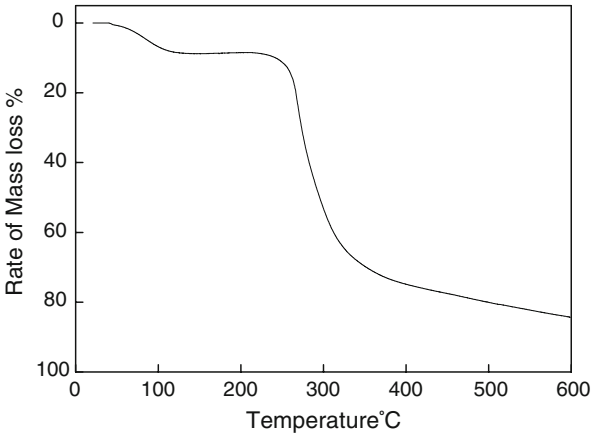


**Fig. 8** TGA curves of dry aloe polysaccharide lyophilized from retentate

Aloe Polysaccharide Analysis

The aloe polysaccharide powder was obtained by lyophilization of the retentate after membrane concentration. The dried material was 95.28% polysaccharide by weight. The thermal stability of lyophilized aloe polysaccharide was investigated, and the result is shown Fig. 8. It was found that there is a single temperature of thermal decomposition demonstrating high polysaccharide purity. The aloe polysaccharide composition was analyzed by gas chromatography. The retention times of standard monosaccharides and of aloe polysaccharide hydrolysis products are compared in Table 1. By comparing retention times, the results show that the hydrolyzed monosaccharides have retention times equal to or less than that of mannose. Retention times as long as those of glucose and mannose were not observed. Also, by comparing peak heights, it may be inferred that mannose is the main monosaccharide in the polysaccharides, and very low peak heights above 22.61-min retention time indicate only a few glucose residues.

## Conclusion

The separation and purification of aloe polysaccharides was carried out by using membrane ultrafiltration preceded by aqueous two-phase extraction. For the novel membrane used, poly(AN-AM-St) was prepared by solution polycondensation, and the properties of this

**Table 1** Retention times of standard monosaccharides and hydrolyzed aloe polysaccharide monosaccharides (30 m×0.32 mm×0.25 μm OV-1 capillary column, $N_2$ carrier gas).

| Monosaccharide | Retention time of standard monosaccharide (min) | Retention time of polysaccharides (min) |
|---|---|---|
| Xylose | 17.413 | – |
| Mannose | 22.649 | 22.500 |
| Glucose | 22.787 | 22.610 |
| Galactose | 23.144 | – |

copolymer were investigated by IR and TGA. The ultrafiltration membrane film was prepared by the phase inversion method, and the pure water flux (26 mL/cm$^2$ h) and molecular-weight cutoff (68,000 Da) were determined. Aqueous two-phase extraction utilized a PEG/$(NH_4)_2SO_4$ system and concentrated aloe polysaccharide in the salt-rich bottom phase. After ultrafiltration, retentate polysaccharide was analyzed and found to consist of 95.28% of a high-mannose polymer with a single decomposition temperature as determined by TGA.

## Reference


1. Ni, Y., Turner, D., & Yates, K. M. (2004). *International Immunopharmacology, 4*, 1743–1755. doi:10.1016/j.intimp. 2004.07.006.
2. Womble, D., & Heldermanj, H. (1992). *Immunopharmacology and Immunotoxicology, 14*, 63–77. doi:10.3109/08923979209009213.
3. Ramamoorthyl, L., Kemp, M. C., & Tizard, I. R. (1996). *Molecular Pharmacology, 50*, 878–884.
4. Ramamoorthyl, L. (1998). *Molecular Pharmacology, 53*, 415–421.
5. Wu, J. H., Chen, X., & Cheng, Y. (2006). *Life Sciences, 78*, 622–630. doi:10.1016/j.lfs.2005.05.097.
6. Waldron, K. W., Robertson, J. A., & Selvendran, R. R. (1999). *Carbohydrate Polymers, 39*, 101–108. doi:10.1016/S0144-8617(99)00004-1.
7. Jimmy, T. C., David, A. W., & Kenneth, M. Y. (2005). *Carbohydrate Research, 340*, 1131–1142. doi:10.1016/j.carres.2005.02.016.
8. Farshad, R., Gashaw, M., & Farzaneh, F. (2007). *Journal of Chromatography. A, 1141*, 32–40. doi:10.1016/j.chroma.2006.11.053.
9. Zhang, C. M., Fabricio, M. B., & Scott, B. (2005). *Journal of Biotechnology, 117*, 39–48. doi:10.1016/j.jbiotec.2004.12.015.
10. Mian, L., & Peeples, T. L. (2004). *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences, 807*, 69–74. doi:10.1016/j.jchromb.2004.03.030.
11. Zhai, S. L., Luo, G. S., & Liu, J. G. (2001). *Separation and Purification Technology, 21*, 197–203. doi:10.1016/S1383-5866(00)00193-3.
12. Bora, M. M., Borthakur, S., Rao, P. C., & Dutta, N. N. (2005). *Separation and Purification Technology, 45*, 153–156. doi:10.1016/j.seppur.2004.08.006.