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Purification of *Ginkgo biloba* flavonoids by UF membrane technology

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ABSTRACT

In order to enhance the recovery rate and reduce energy consumption, an ultrafiltration process was applied to purify the crude product of *Ginkgo biloba* extract as an alternative to conventional methods. The effect of membrane type, operation temperature, the transmembrane pressure, feed concentration on the permeate flux profile, solute rejection, and membrane fouling were investigated. By single factor experiments, the results showed that (1) flux increased obviously with enhancing the feed temperature or pressure; (2) flux decreased as the concentration of feed rose or runtime extended; (3) change regularity of the rejection (*R*) was different to that of the flux; (4) temperature was the most important factor; (5) poly (ether sulfone) membrane (MWCO, 10,000 Da) was better than the other membranes and improved the flavonoids content from 24 to 68%. The membrane fouling was analyzed by scanning electron microscopy and Fourier transform infrared spectroscopy.

Keywords: Ultrafiltration; *Ginkgo biloba* flavonoids; Purify; Membrane fouling

1. Introduction

Ginkgo biloba, a special economic tree species from China, contains a large number of amazing active components that are widely used in medicine and health products. The flavonoid is one of the most important components among those active ingredients [1]. It has recently been proved that the flavonoids can prevent ischemia-induced oxidation, improve cerebral blood flow and antagonize the action of platelet-activating factor [2]. For a long time, the degree of flavonoids purity always is the goal of researchers because the higher purity, the better the medicinal efficiency and higher price.

Methods such as organic solvent extraction, macroporous resin adsorption, super critical fluid extraction, and microwave treatment are utilized to enhance the flavonoids purity from the crude products of *Ginkgo biloba* extract (GBE). The organic solvent extraction is complex and unable to obtain the high-purity product. The porous resin method is often used by many manufacturers. However, this method still has many problems such as high solvent consumption, serious environmental pollution, depressing the activity of the active component and low purity [3]. The super critical fluid extraction and the microwave treatment are still in laboratory-scale experiment stage. Nowadays, commercially standardized GBE products have normally a flavonoids

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content of 24 wt.% and a terpene content of 6 wt.% by the porous resin method.

Recently, membrane separation by ultrafiltration (UF) membrane was applied to separate different components from plant extracts. For example, the modified polyethylene vinylidene fluoride (PVDF)-polyvinylpyrrolidone UF membrane was used to further purify the flavonoids from the GBE crude products. The flavonoids content in the final product could be enriched to 34.8 wt.% from 21.3 wt.% in the GBE crude product, and the mass transfer of flavonoids decreased with increasing pH value of GBE solution. The flux of GBE solution increased linearly at low pressure and approached a steady state at high pressure because of the concentration polarization [4]. As a pressure-driven process to separate macromolecules from the smaller species in the feed solution through an UF membrane, UF process has been broadly applied in waste water treatment, drinking water production, separation, refinement and concentration in the pharmacy and environmental protection. As an important method to replace the traditional separation methods in the modern medicine purification industry, UF technology has some advantages, such as simple work-up procedure, milder conditions and environmental friendliness. However, the membrane fouling is a limiting factor. Nowadays some researchers have studied the further purify from the GBE crude products by UF technology [5–8].

In this study, effects of different UF membranes, operating parameters on flavonoids purification productivity and membrane fouling were investigated. Scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR) were used to analyze membrane fouling.

2. Experimental

GBE crude products and Rutin standard substance were provided by Shanxi Jintai Biological Engineering Co. Ltd. Absolute ethyl alcohol was purchased from Beijing Chemical Works. Sodium nitrite, aluminum nitrate, and sodium hydrate were purchased from Beijing Chemical Reagent. The membranes were prepared by our group and their parameters were listed in Table 1.

The flat UF membranes were fixed to the stainless steel module, and the effective membrane area was 0.0026 m^2. Ethanol aqueous solution (30 vol.%) was used to dissolve GBE crude product. GBE solution was subsequently prefiltered with the qualitative filter paper.

Table 1
Membrane parameters

No.	Material	MWCO (kDa)
PVDF 500KD	PVDF	500
PVDF 100KD	PVDF	100
PSf 20KD	PSf	20
PES 10KD	PES	10
PES 6KD	PES	6

About 800 mL feed liquid was transferred to the feed bottle. Then open the constant temperature water bath and set the temperature. Open the feed pump and set the transmembrane pressure (TMP) when the difference between the temperature of feed liquid and the temperature of constant temperature water bath was less than 1℃. The flux was measured every 20 min. At the end of the experiment, the absorbance of the filtered fluid and concentrated solution was measured. The flux is expressed as

$$\text{Flux} = Q/(A \times t)$$

where Q denotes the permeate volume, A is the membrane effective area, t presents the runtime. The rejection for a given solute is calculated by

$$\text{Rejection} = (1 - C_p/C_f) \times 100\%$$

where C_p and C_f are the concentration of the given component in the permeate and the feed, respectively. The content of flavonoids was determined with aluminum nitrate spectrophotometry at the wavelength of 500 nm by an UV-2000 ultraviolet spectrophotometer (UNICO, China). The relation between the flavonoids concentration and absorbency (A) was $A = 0.00762C - 0.00448$, $R^2 = 0.9984$, where A presents absorbency, C denotes the concentration of flavonoids, R is the linear correlation coefficient of the equation.

The powder of the flavonoids samples were completely dried at room temperature in a desiccator prior to FTIR analysis. All FTIR spectra were obtained using a Nicolet M760 FTIR spectrometer in transmission mode (resolution 4 cm^{-1} and averaging over 2,000 scans). The viscosity of the feed was measured by NDJ-1 rotation viscosimeter (Shanghai Changji Geological Instruments Co. Ltd.). SEM was used to observe the morphology of membrane cross-section. The membrane samples for SEM imaging were firstly cryogenically immersed in liquid nitrogen, fractured,

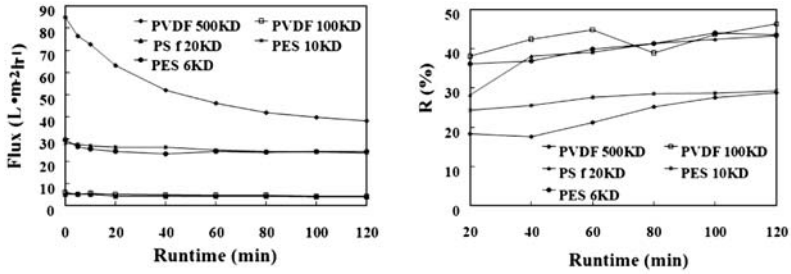

Fig. 1. Effects of membrane type on flux and rejection.

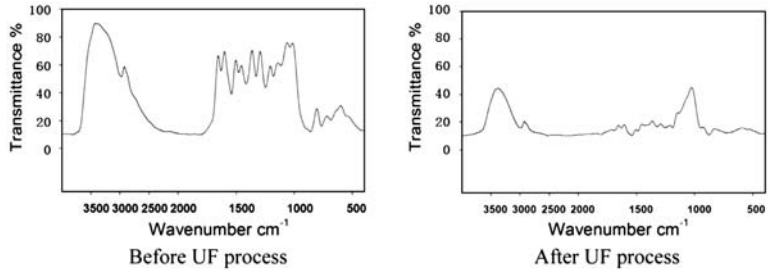

Fig. 2. FITR curves of unfiltered and filtered *Ginkgo biloba* flavonoids.

and then sputtered with metallic gold. A QUANTA 200 field emission scanning electron microscope (FEI Company, Holland) was used to observe the membrane morphology.

3. Results and discussions

3.1. Effects of membrane type

For the membranes in the experiment of the effect of membrane type, the initial concentration of flavonoids was 275 mg L^{-1}. TMP of the membranes was 0.30 MPa. The operating temperature was 35℃. It was shown in Fig. 1 that: (1) the flux of PVDF 500KD membrane was far larger than the flux of other membranes; the flux decreased significantly as runtime extended, for the first 20 min, the flux decreased sharply; then followed by a slow decreases from 20 to 100 min; the flux eventually stabilized until 120 min, the flux eventually stabilized at 38 L m^{-2} h^{-1}; the rejection of PVDF 500KD was far lower than the rejection of other membrane; the rejection increased as the experiment went on; (2) the flux regulation of polyether sulfones (PES) 10KD was similar to that of PES 6KD; the flux eventually stabilized at 24 L m^{-2} h^{-1}. The rejection of PES 10KD is higher than the rejection of PVDF 500KD, but it is lower than the rejection of other membranes; (3) the flux regulation of PVDF 100KD was similar to that of PSf 20KD; the flux eventually stabilized at 4 L m^{-2} h^{-1}. Among the five membranes, the rejection of PVDF 100KD is highest. FITR curves of unfiltered and filtered *Ginkgo biloba* flavonoids were shown in Fig. 2. It was obvious that the light transmittance of the permeate was higher than that of the feed. For the following experiments, the each value of flux was gotten at the 120th min.

The mass fraction of flavonoids in the filtrate was measured. The result was shown in Fig. 3. The mass fraction of flavonoids in the filtrate of the PVDF 500KD membrane was 41%. The PES 10KD membrane was better on flavonoids purification and could improve the flavonoids content from 24 to 68%. The dates provide the gist for the application of the UF membranes on the medicine purify.

3.2. Effects of operating temperature

The initial concentration of flavonoids was 263 mg L^{-1}, TMP of PVDF 500KD, and PES 10KD was 0.20 and 0.30 MPa, respectively. Fig. 4 showed that: (1)

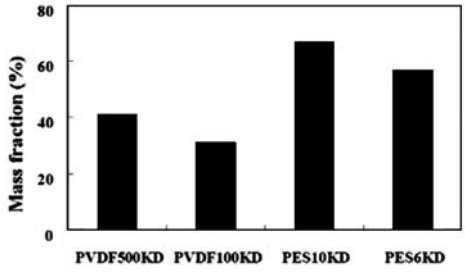

Fig. 3. Effects of membrane type on mass fraction.

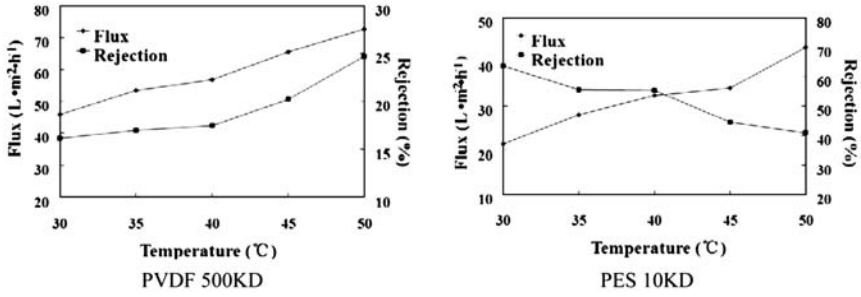

Fig. 4. Effects of temperature on flux and rejection.

for PVDF 500KD, the flux and the rejection increased significantly with the increasing feed temperature; (2) for PES 10KD, the flux increased and the rejection decreased as feed temperature lifted. The effect of temperature on membrane separation performance was mainly reflected in two aspects: on the one hand, with the temperature increasing, the viscosity of the feed decreased (Fig. 5), while the mass transfer coefficient and flux increased; On the other hand, due to the increased temperature, the probability of protein denaturation increased, while the hydrogen bonds between flavonoids and polysaccharides increased and induced to form much bigger molecular micelle which could increase flavonoids rejection. The average molecular weight of flavonoids was 300 Da. When the temperature enhanced, the mass transfer coefficient increased; therefore, the flux improved. However, the change regularity of the rejection was different for different membrane. The aperture of PVDF 500KD membrane was far larger than the diameter of flavonoids; all of flavonoids could almost permeate the PVDF 500KD membrane. With the temperature increasing, the hydrogen bonds between flavonoids and polysaccharides became greater, which induced that many flavonoids were absorbed by polysaccharides. When polysaccharides were removed, flavonoids were removed too. It was to say that the concentration of permeate became smaller, and the rejection grew. For PES 10KD, the mass transfer of flavonoids held a leading post; more flavonoids would seep through the PES 10KD with the temperature increasing. This meant that the concentration of permeate became larger, and the rejection decreased. In selecting operating temperature, the efficiency of membrane separation, stable flux and the tolerance of solute to temperature should be considered. The flux of PES 10KD was significantly lower than that of PVDF 500KD. But the flavonoids content in the final product could be purified to 68 wt.% for PES 10KD and to 41 wt.% for PVDF 500KD from GBE crude product (24 wt.%).

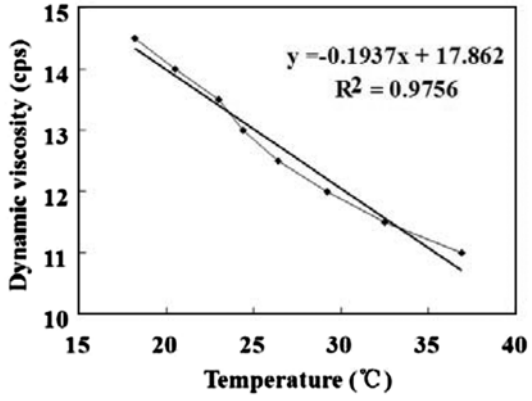

Fig. 5. Effects of temperature on dynamic viscosity of the feed.

3.3. Effects of TMP

The initial concentration of flavonoids was 289 mg L^{-1}, and the temperature of the feed for PVDF 500KD and PES 10KD was 35 and 40℃, respectively. With increased TMP, the flux and the rejection had an increased trend (Fig. 6). UF was a pressure-driven membrane separation process, and the separation efficiency was directly linked the pressure. With the pressure increasing, the flow rate of the feed increased, which induced that the shear force produced by the fluid raced up and concentration boundary layer decreased, so the flux increased. In addition, the solute accumulated on the surface of membrane and formed a concentration polarization layer because of the solvent through the membrane. Thus, the rejection increased.

3.4. Effects of feed concentration

The operating temperature and TMP for PVDF 500KD were 35℃ and 0.20 MPa, and those for PES 10KD were 40℃ and 0.30 MPa. From Fig. 7, it was

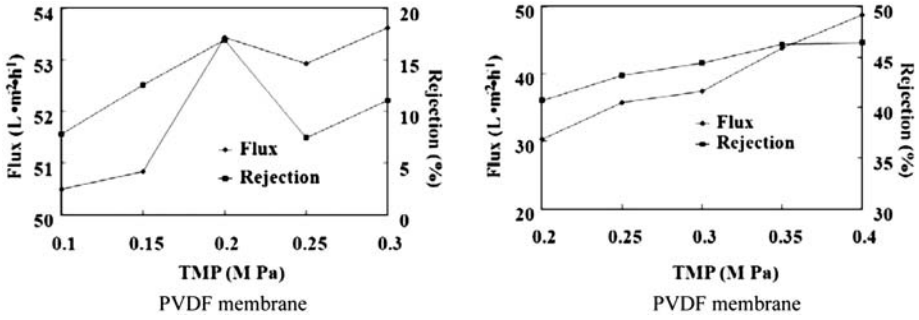

Fig. 6. Effects of TMP on flux and rejection.

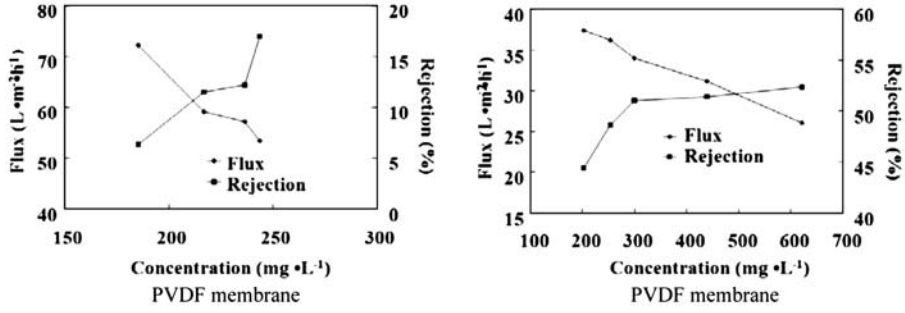

Fig. 7. Effects of concentration of the feed on flux and rejection.

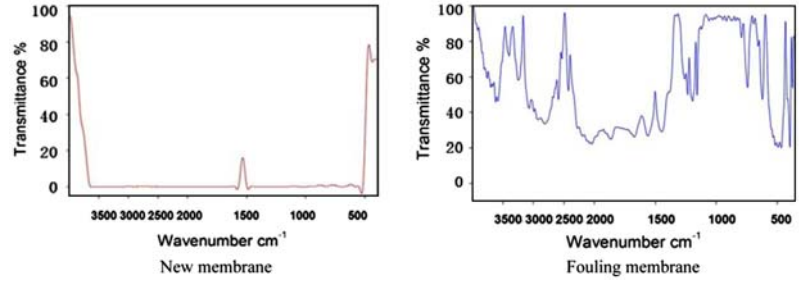

Fig. 8. FITR curve of PVDF 500KD.

observed that the flux decreased gradually and rejection increased gradually with flavonoids concentration increased. The flux and the rejection stabilized at high concentration. With increased feed concentration, the concentration at membrane surface also increased, which led to higher transfer resistance, so the flux decreased and the rejection increased. The impurities of the feed were removed and formed a gel layer on the membrane surface when the concentration of the membrane surface exceeded the solubility of impurities. The gel layer caused the decrease of the flux and the increase of the rejection.

3.5. Membrane fouling

It could be seen from Figs. 8 and 9 that before and after being fouled, different spectra were observed via FTIR. The transmittance of new membrane was above 95%, which was significantly higher than the fouled one. Compared with the new membrane, fouled membrane has much more absorption peaks. Fouled membrane had C=O stretching vibration absorption peak (1,877 cm^{-1}) in the infrared spectra, which revealed that there was precipitation of *Ginkgo* flavonoids on the membrane surface, and the characteristic absorption of aromatic system (1,650–1,450 cm^{-1}) was also

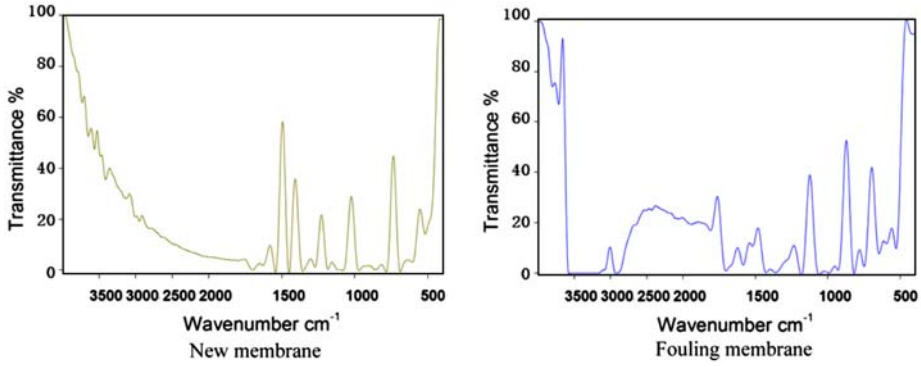

Fig. 9. FITR curve of PES 10KD.

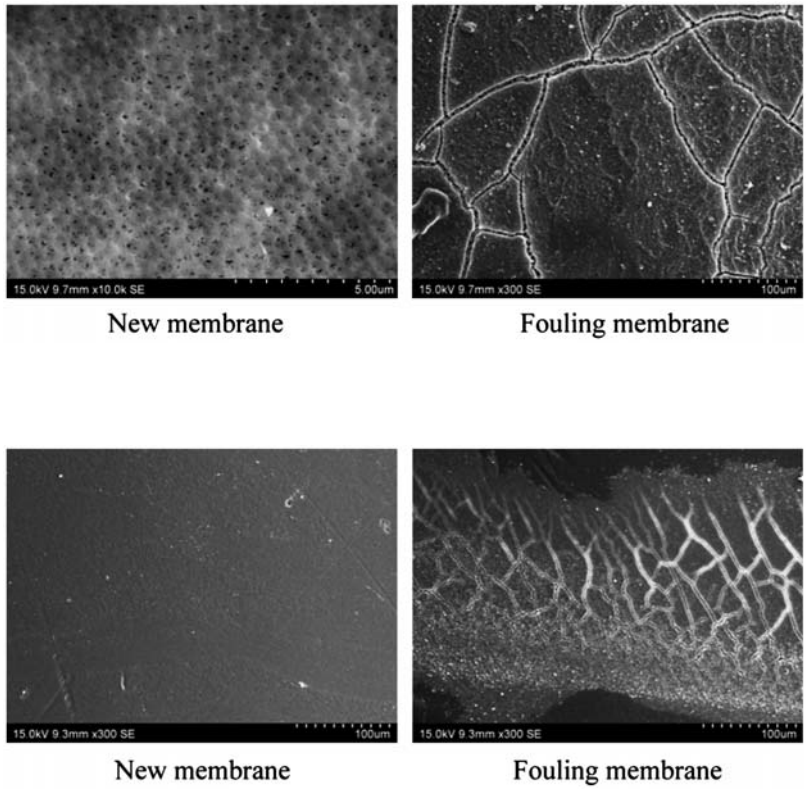

Fig. 11. SEM images of PES 10KD.

found. Strong absorption peaks appeared at 3,723, 3,272, 2,696, 621 cm^{-1} for the fouled membrane, which indicated that membrane surface has been covered by pollutants. Figs. 10 and 11 further revealed that on the surface of the new membrane, there was homogeneous structure. However, for the fouled membrane, after long-time operation, particles, colloid-ion and solute in the feed solution were adsorbed or deposited inside the membrane pore or on the membrane surface. Some membrane pores were plugged, and a cake layer formed on the membrane surface. The texture of the fouled materials can be displayed clearly by SEM.

4. Conclusions

The crude product of GBE was purified by the UF system. Comparing to TMP, feed concentration and runtime, the operating temperature was the most important operating parameter. The flux increased significantly with enhanced the feed temperature or TMP. The flux decreased as the concentration of feed stream rose or runtime extended. However, the change regularity of the rejection was different to that of the flux. PES membrane (MWCO, 10,000 Da) was better to purify flavonoids and could

improve the flavonoids content from 24 to 68%. By SEM and FTIR analysis, the cake layer was formed on the surface of the membrane because of membrane fouling.

Acknowledgments

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