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Desalting of Aqueous Extract of *Acanthus ebracteatus* Vahl. by Nanofiltration

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Abstract: Nanofiltration was applied for desalting of aqueous extract of *Acanthus ebracteatus* Vahl. The optimal condition for the desalting was found to be at flow velocity of 0.875 m/min with applied pressure of 10 kg/cm^2 to obtain the average NaCl rejection of 31%. The diafiltration process comprised of 1.5 concentration followed by the diafiltration ratio of 2 allowed 80% salt removal. The mathematical model developed for the diafiltration process agreed very well with the experimental results. In addition, the aqueous extract of *A. ebracteatus* Vahl. after the removal of salts exhibited better cytotoxicity against both human epidermoid carcinoma and human cervical carcinoma.

Keywords: *Acanthus ebracteatus* Vahl., nanofiltration, diafiltration, desalting

INTRODUCTION

Acanthus ebracteatus Vahl. has been widely used as a traditional medicine for years. In Malaysia, its leaves and the boiled seeds are commonly used for making a cough medicine, while in China, the plant is prescribed against hepatosplenomegaly, hepatitis, lymphoma, and asthma. As Thai traditional

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medicine, the plant is widely used as a purgative and an anti-inflammatory. It has been also reported that its leaves dispensed with pepper (*Piper nigrum L.*) can be used as tonic pills for longevity (1).

A. ebracteatus Vahl. has high constituents of alkaloids, flavonoids, polysaccharides, fatty acids, tritepenoids, and saponins. The extract from *Acanthus sp.* has been tested for anti-tumor and anti-cancer potentials (2, 3). The inhibition effect of *A. ebracteatus* Vahl. extract on hepatic foci in rats was reported (3). However, the kidney damage to tested rats during the treatments with *A. ebracteatus* Vahl. extract was also reported (4). This toxicity was related to a high salt level of the aqueous extract due to the growth location of *A. ebracteatus* Vahl. in brackish water. Therefore, desalting of *A. ebracteatus* Vahl. extract is required prior to the use of the aqueous extract as a herbal medicine or food supplements.

For years, membrane techniques have played an important key role in bioseparation such as purifying water, producing drugs or ultrapure chemicals and processing food. Their advantages are considered to be cost and energy efficient along with improving product quality by operating at mild conditions without phase changes. Nanofiltration (NF) membranes have remarkable features based on their separation mechanisms involving both sieving (Steric) and electrical (Donnan) effects. Transport of ions due to the flux of ions in terms of diffusion, electric field gradient, and convection. Membrane transport models describing salt rejection based on the extended-Nernst-Planck equation were well documented (5–10). Solute rejection was described as a function of volume flux and the transport parameters could be calculated from the rejection-flux curve (5, 6). The variations of rejection attributed to fouling by formation of deposits of different charges on NF membrane were reported (11, 12). Besides, solvent flux appeared to be dependent on viscosity, molecular size, and the difference in surface tension between the membrane and the solvent (13). A pore diffusion transport model incorporating surface and internal pore fouling was developed for predicting performance of NF membranes (14). Recently, an "Adsorption-Amphoteric" model was proposed for the mechanism of charge formation in NF membrane (15).

NF membranes are effective for a separation range of organic molecules (neutral or charged) and salt mixtures. In addition, NF membranes can be operated at low pressure causing the reduction in capital and operation costs and easier system maintenance (16). NF is capable of desalinization and concentrating sugars, divalent salt, proteins, particles, dyes, and other constituents that have molecular weight greater than 1000 daltons (17–20). The application of spiral-wound NF for the purification of heterocyclic drug derivatives from concentrated saline solution was reported (21).

In this present study, the separation of salts from the aqueous extract of *Acanthus ebracteatus* Vahl by NF membrane, NTR 7450 has been investigated. The objective is to reduce salt concentration in the retentate by diafiltration. Furthermore, for a better understanding of the transport phenomena across the membrane, the mathematical models for the separation are verified. Lastly, for the qualitative analysis, the cytotoxic activities against cancer cell lines of the desalted products are examined.

MATERIALS AND METHODS

Materials

The aqueous extract of *A. ebracteatus* Vahl. was prepared by The Natural Products Research Section, Research Division, National Cancer Institute, Bangkok, Thailand. The procedure for the preparation was reported elsewhere (2).

Apparatus

Figure 1 shows the diagram of the NF apparatus. The NF units were kindly provided by Prof. Shin-ichi Nakao (Department of Chemical System Engineering, the University of Tokyo). A NF membrane, NTR-7450 (Nitto Denko, Shiga, Japan) was used for the desalinization. It was a flat-sheet thin film composite membrane made of sulfonated polythersulfone with filtration area of 0.00353 m^2 and membrane pore radius of 0.70 nm (5).

Experimental Procedure

Continuous Desalinization

The experimental procedure for desalinization of aqueous extract of *A. ebracteatus* Vahl. could be divided into two parts. The first part was to determine the

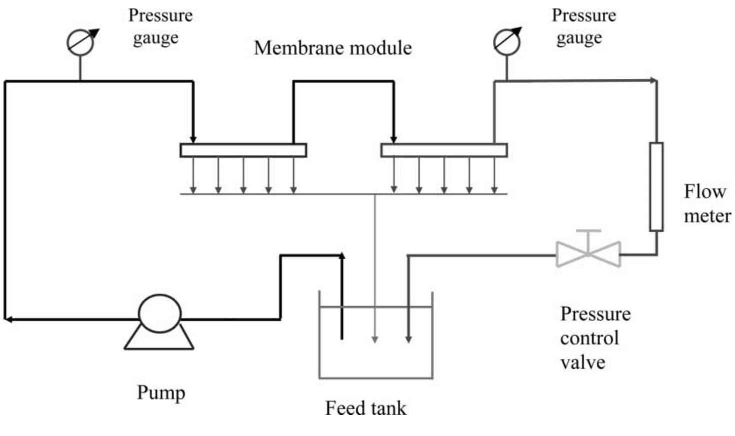

Figure 1. Schematic flow diagram of the experimental apparatus.

optimal conditions for the desalting. In this step, both the retentate and the permeate were recycled to the feed tank in order to keep the feed concentration unchanged as shown in Fig. 1. In order to investigate the effects of the operating condition, an experimental study was performed with transmembrane pressure (TMP) and feed flow velocity as key parameters where flux and salt rejection were variables. Temperature was also an important parameter. An operation at a higher temperature could reduce feed viscosity and might increase membrane pore size and solute diffusivity. Thus, the increase of temperature could improve permeate flux and/or reduce solute rejection. Although high flux was desirable, in this study an increase in temperature more than 30°C could cause a reduction in recovery and quality of the product as a result of lower rejection and degradation. Therefore, the operating temperature was controlled at 30°C.

The effects of transmembrane pressure (TMP) and feed flow velocity on the separation were determined in the range of $10\,\mathrm{kg\,cm^{-2}}$ to $30\,\mathrm{kg\,cm^{-2}}$ and 0.5 to $1\,\mathrm{m\,min^{-1}}$, respectively. The permeate flux and the rejections were measured after the flux was stable. Before and after each test, pure water permeate flux was measured to check the condition of the membrane.

Diafiltration Process

The second part was a diafiltration test. The diafiltration process comprised of two steps:concentration and diafiltration. The apparatus used for the diafitration experiments was very similar to what was shown in Fig. 1, except that in this part only the retentate was recycled to the feed tank. The permeate was collected in a volumetric cylinder and timed to estimate the flux. For the diafiltration step, two membranes connected in series were used for a higher filtration rate. In order to keep the constant volume, pure water was continuously added to the feed tank under a level control.

Analytical Method

Total salt concentrations were automatically measured using an electrical conductivity probe. The concentrations of ions such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, and Al^{3+} were determined by an atomic absorption spectrometer (AAS) with air-acetylene flame.

In order to calculate the recovery of the total product, the dry solid content was determined by measuring the weight loss after drying samples at 70°C for 48 hours in an oven. The cytotoxic activities of samples were evaluated against KB (human epidermoid carcinoma) and HeLa (human cervical carcinoma) cell lines employing the standard MTT colorimetric method.

BASIC CONCEPTS

The volume flux (J_v) and the solute flux (J_s) over the membrane surface of NF membrane can be described with Spiegler-Kedem equation (22) by the irreversible thermodynamic model in which concentration polarization close to the membranes surface is assumed to occur within a boundary film layer of thickness (Δx) (5, 6).

$$J_v = L_p(\Delta P - \sigma \cdot \Delta\pi) \tag{1}$$

$$J_s = -P' \cdot \frac{dc}{dx} + (1 - \sigma) \cdot J_v\bar{c} \tag{2}$$

For dilute concentration, by assuming that the concentration gradient through the membrane is linear, the equation (2) could be simplified as follows (23).

$$J_s = P \cdot (c_m - c_p) + (1 - \sigma) \cdot J_v\bar{c} \tag{3}$$

L_p, P', P, σ are the membrane parameters, namely pure water permeability, local solute permeability, solute permeability, and reflection coefficient respectively; where $P' = P\,\Delta\,x$. The terms of c_m and c_p refer to the concentration at membrane surface and in permeate, respectively; $\bar{c}$ is the average concentration. ΔP is the transmembrane pressure and $\Delta\pi$ is the osmotic pressure difference across the membrane. The pure water permeability (L_p) is obtained from the filtration test of pure water. The osmotic pressure difference $\Delta\pi$ can be determined by the concentration at the membrane surface (21). P and σ are dependent on the membrane structure and the electrical properties and could be obtained from the rejection-flux curve by a best-fit method (5). These models could be used for application in a continuous process in which at steady state concentration and flux are constant.

Diafiltration Model

Concentration Step

The mathematical model for diafiltration process can be described by Wang et al. (7) based on the mass balance of any component passed through the membrane.

$$\frac{d(V_f c_{f,i})}{dt} = -J_v A c_{p,i} \tag{3}$$

or

$$V_f \frac{dc_{f,i}}{dt} + c_{f,i} \frac{dV_f}{dt} = -J_v A c_{p,i} \tag{4}$$

where, A (m^2) is the total filtration area and V_f (m^3) is the volume of feed solution, whereas $c_{f,i}$ and $c_{p,i}$ are feed and permeate concentration of i, respectively. For the concentration step, the change rate of the solution volume is

$$\frac{dV_f}{dt} = -J_v A \tag{5}$$

By substitution of the equation (5) into the equation (4) gives

$$\frac{d\ln(c_{f,i})}{dt} = \left(\frac{c_{p,i}}{c_{f,i}} - 1\right)\frac{d\ln(V_f)}{dt} \tag{6}$$

The observed rejection of i is defined as:

$$R_{obs,i} = 1 - \frac{c_{p,i}}{c_{f,i}} \tag{7}$$

Therefore, the equation (6) can be rewritten as:

$$\frac{d\ln(c_{f,i})}{dt} = -R_{obs,i}\frac{d\ln(V_f)}{dt}$$

If the observed rejection can be considered as a constant, the equation (6) can be expressed in the dimensionless form

$$\frac{c_{f,i}}{c_{f,i0}} = \left(\frac{V_{f0}}{V_f}\right)^{R_{obs,i}} \tag{8}$$

Diafiltration Step

During the diafiltration step, pure water is fed into the feed tank under the level control to maintain at constant volume.

$$\frac{dV_f}{dt} = 0 \text{ and } \frac{dV_w}{dt} = J_v A \tag{9}$$

The relations of the concentration of component i ($C_{f,i}$) in the feed solution ($C_{f,io}$) and the volume of the feed solution, V_w, can be reckoned by combining the equations (4), (7), and (9),

$$\frac{c_{f,i}}{c_{f,i0}} = \exp\left[-\frac{V_w}{V_{f0}}(1 - R_{obs,i})\right] \tag{10}$$

RESULTS AND DISCUSSIONS

Effects of TMP and Flow Velocity

Figures 2 showed the dependence of the permeate flux on flow velocity and transmembrane pressure (TMP). The linear increase of the permeate

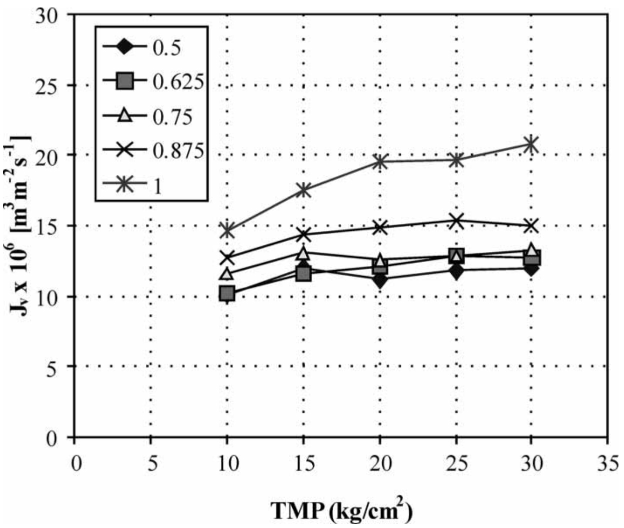

Figure 2. Permeate flux of the aqueous extract of *A. ebracteatus* Vahl. at various operating conditions. The 0.5; 0.625; 0.75; 0.875 and 1 refer to flow velocities (mmin^{-1}). The applied pressures varied from 10 to 30 kg cm^{-2}.

flux with TMP at the same flow velocity can be readily explained by Equation (1). However, at the flow velocity less than 0.875 m min^{-1}, the permeate flux only slightly increased with increasing TMP due to the fouling effect on the membrane surface. The effect of the flow velocity could be explained by shear rate effect (24). The high shear rate under the flow velocity more than 0.875 m min^{-1} led to reduction in both concentration polarization and membrane fouling by an increase of mass transfer coefficient and a decrease in concentration on the membrane surface.

The rejection of NaCl varied from 0.31 to 0.53 influenced by flow velocity and TMP (Fig. 3). The minimum of NaCl rejection could be obtained at flow velocity of 0.875 l/min and TMP of 10 kg cm^{-2}. In general, at the same flow velocity, higher TMP resulted in increasing NaCl rejection. A similar observation has been reported (7, 16 and 20).

Although the increase of both TMP and flow velocity led to the higher permeate flux, the NaCl rejection was considerably increased only with TMP but not significantly affected by flow velocity. Based on the desalting results, the optimal condition to desalinize aqueous extract of *A. ebracteatus* Vahl. was at the flow velocity of 0.875 m min^{-1} and the TMP of 10 kg cm^{-2}. Operating at flow velocity exceeding 1 m min^{-1} could reduce the fouling effect, however, foam formation occurred during the operation created difficulty to maintain the system.

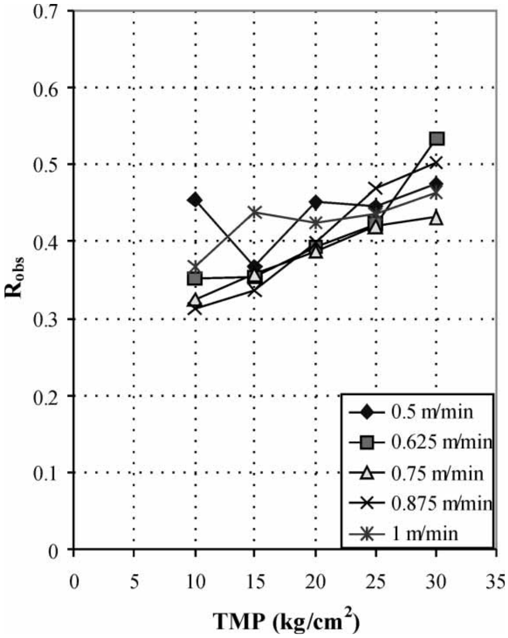

Figure 3. NaCl rejections of the aqueous extract of *A. ebracteatus* Vahl. at various operating conditions. The experiments were carried out at the TMP from 10 kg cm^{-2} to 30 kg cm^{-2} and at flow velocity ranging from 0.5 to 1 m min^{-1}.

Diafiltration Process

Concentration Step

The diafiltration process was carried out under the condition of 0.875 m min^{-1} flow velocity and TMP of 10 kg cm^{-2}. The concentration factor was at 1.5. The permeate flux against time and the NaCl concentration as a function of feed volume were demonstrated in Figs. 4 and 5. The permeate flux reduced from 9.5×10^{-6} m^3 m^{-2} s^{-1} to 6.1×10^{-6} m^3 m^{-2} s^{-1} after 14 hours of the operation due to the fouling at the membrane surface. As can be seen in Fig. 6, an excellent agreement was observed between the experimental data and the prediction using Equation (8).

Although at the operation range of this study, the salt rejection remained nearly constant at 0.31. However, it should be noted that the salt rejection could depend on operating parameters including flux and feed concentration. At low flux, contribution of diffusive transport was the most important whereas at high flux, transport by convection became more important. The permeate concentration whose mass transfer was diffusion controlled would increased with increasing feed concentration. In the previous work (25), by using TFCSR NF membrane to recovery NaCl from dye solutions, NaCl

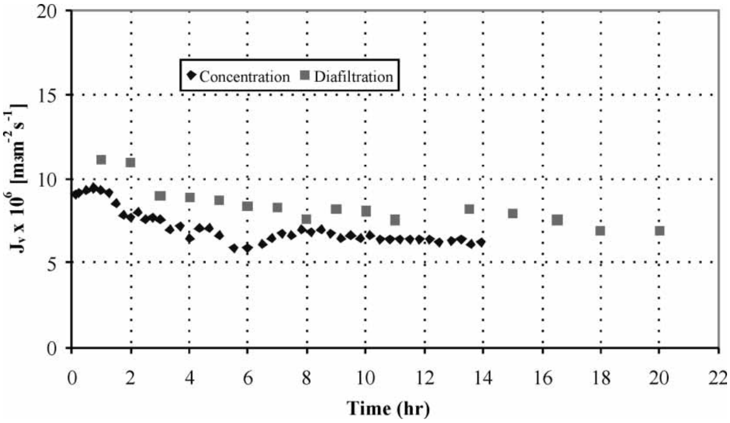

Figure 4. Permeate flux during the concentration and diafiltation steps at the TMP of 10 kg cm^{-2} and the flow velocity of 0.875 m min^{-1}.

rejection was decreased from 64.3% to 58.0% when NaCl concentration in the spent liquor was increased from 10 to 80 g/L, besides, the increase of NaCl rejection over time was also observed.

Diafiltration Step

The retentate from the concentration step was used as the initial feed for the diafiltration step. In this step, the permeate flux was slightly higher than that

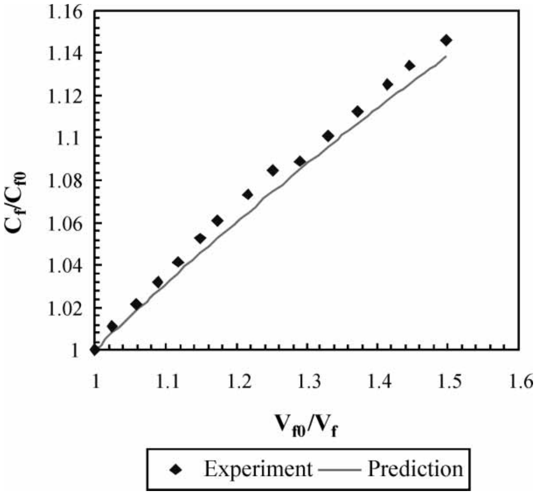

Figure 5. Ratios of NaCl concentration in feed to the initial concentration as a function of volume ratio during the concentration step. The prediction curve is plotted using Equation (8) with the constant NaCl rejection of 0.31.

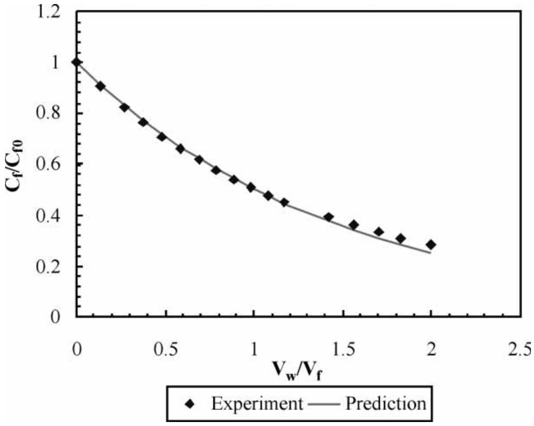

Figure 6. Ratios of NaCl concentration in feed to the initial concentration as a function of flow volume during diafiltration step. The prediction curve is plotted using Equation (10) with the constant NaCl rejection of 0.31.

of the concentration step due to the diluted concentration from the addition of pure water (Fig. 4). The concentration of NaCl as a function of feed water volume was presented in Fig. 6. The experimental data for the ratios of feed concentration to initial concentration were in good agreement with those from the calculation using Equation (10) at the constant NaCl rejection of 0.31. The total NaCl removal after the diafiltration ratio of 2 was about 80%. According to the model, the diafiltration ratio of 3 could be sufficient to remove 90% of NaCl from the aqueous extract solution. However, with the limit of our filtration area, 90% desalting could not be done in the acceptable processing time. From the dried products, the percentage of NaCl in the dry solid from the initial aqueous extract was 14.0% w/w; the amount of NaCl after the concentration and the diafiltration step were reduced to 11.5% w/w and 5.8% w/w, respectively (Table 1).

The percentages of total solid recovery after the concentration and the diafiltration step were 78.4 and 48.4, respectively (Table 1). Although the

Table 1. Recovery (%) of total solid and NaCl content in freeze dried compounds from the initial aqueous extract, the retentate from the concentration and diafiltration steps and the permeate from the concentration and diafiltration steps

Source of dried compounds	Total solid recovery (%)	NaCl (% wt)
Initial aqueous extract	100.0	14.0
Retentate from the concentration step	78.4	11.5
Retentate from the diafiltration step	48.4	5.8
Permeate at concentration step	—	30.5
Permeate at diafiltration step	—	35.3

permeate was clear and colorless, the removal of compounds other than NaCl from the concentration and the diafiltration step was detected. The high molecular weight components in the aqueous extract, for instance, polysaccharides and alkaloids could slightly, if any, pass through the membrane. Salts and very small organic compounds such as sugars and small amino acids could partially pass through the membrane due to molecular weight cut-off of NTR 7450 ranges from 600–1000 Daltons (26, 27).

Removal of Cations

In NF, combined charge and size effects were well documented (5–10). Variation of solute rejection by charged was common in the diffusion controlled process. Since the membrane NTR 7450 was a monopolar membrane of negative charge, the attractive force for counterions (cation) should occur and thus particles with larger positive charges were more likely to be less rejected than others. Tsuru et al. (27) reported that by using NTR-7450 for single-component electrolytes, the rejections of divalent counterion ($MgCl_2$, $CaCl_2$) was less than those of mono-monovalent electrolytes (NaCl, KCl) and an increase of the feed concentration resulted in a decreased rejection. A similar result was observed from the test of single-component solutions in this study (data not shown). However, with the multi-component solution, multivalent ions were found retained more than monovalent ions and the order of rejection of cations by NTR7450 membrane was as follows: $Al^{3+} > Fe^{3+}$, $Fe^{2+} > Ca^{2+} > Mg^{2+} \approx Mn^{2+} > K^{+} \approx Na^{+}$. The concentration and removal percentages of cations after the concentration and diafiltration steps were summarized as shown in Table 2.

Hereby, after the NF membrane was used, we observed an irreversible thin layer on the membrane surface. The adsorb of positive charged compounds such as amino acids or other organic acids and multivalent cations in the aqueous extract possibly would act as the upper charged layer of positive charge which caused repulsive force for cations. The similar pattern has been reported by Anne C.O. et al. (28) for the fractionation of mono and multi-valent cations from sea water separation by tubular NF membrane. The fouling effects on rejection in NF were recognized (11, 12). The rejection variation by fouling with a calcium organic deposit and a ferric hydroxide precipitate deposit was reported (12). The deposits of a highly positive charged layer on NF membrane caused the increase in cation rejection but lowered that of negatively charged organics.

Bioactivity

For qualitative analysis of the desalted product, the cytotoxic activity of freeze dried compounds from the initial aqueous extract, the concentrate and the

Table 2. Concentration and % removal of cations in the feed, retentate and permeate at the concentration and diafiltration steps

	Step								
	Concentration step				Diafiltration step				
	Concentration (ppm)				Concentration (ppm)				
Cations	Feed	Retentate	Permeate	Removal (%)	Feed	Retentate	Permeate	% Removal	Total removal (%)
Na^+	742.2	850.9	524.7	23.6	850.9	226.2	312.4	73.4	79.7
K^+	765.0	889.1	516.8	22.5	889.1	220.5	334.3	75.2	80.8
Mg^{2+}	209.5	267.3	94.0	15.0	267.3	106.0	80.6	60.3	66.3
Fe^{2+}, Fe^{3+}	124.5	186.3	1.0	0.3	186.3	123.5	31.4	33.7	33.9
Ca^{2+}	22.6	29.0	9.7	14.3	29.0	16.7	6.2	42.4	50.6
Mn^{2+}	15.4	20.0	6.2	13.5	20.0	7.7	6.1	61.3	66.5
Al^{3+}	1.7	1.9	1.1	22.7	1.9	1.9	0.0	2.3	24.5

Table 3. Cytotoxicity of freeze dried compounds from the initial aqueous extract, the desalted product[a] and the permeate from the concentration and diafiltration steps

	Cancer cell lines	
Source of compounds	KB	HeLa
Initial aqueous extract	4000	3800
Desalted product[a]	3200	3500
Permeate at Concentration step	4800	4200
Permeate at Diafiltration step	5000	4500

[a]The desalted product = the retentate from the diafiltration process.

permeate after the diafitlration process were examined against KB (human epidermoid carcinoma) and HeLa (human cervical carcinoma) cell lines employing the MTT colorimetric method (2). The results of their cytotoxicities were concluded in Table 3. The desalted product showed better cytotoxicity against both of KB and HeLa cell lines with IC_{50} values of 3200 and 3500 μg/ml, respectively. The higher cytotoxicity of the desalted extract compared to the initial extract could be a result of the higher content of bioactive compounds in the product after the desalinization. As the consequence, the permeates showed less cytotoxic activities, although high content of NaCl could also be cytoxic to the test cells.

CONCLUSIONS

The NF membrane, NTR 7450 was used for concentrating and desalting of the aqueous extract of A*canthus ebracteatus* Vahl. The permeate flux and the NaCl rejection depended on both flow velocity and transmembrane pressure (TMP). At the flow velocity less than 0.875 l/min, increasing TMP did not significantly increase the permeate flux due to the fouling effect. It was found that the optimal condition for the desalting was at flow velocity of 0.875 l/min and TMP of 10 kg/cm^2 at which the rejection of NaCl was 0.31. The application of the diafiltration process comprised 1.5 concentration followed by 2 times of diafiltration ratio allowed 80% NaCl removal. The mathematical simulation for the diafiltration process was found to be excellent to estimate the concentration profiles. The order of rejection of cations by NTR7450 membrane was as follows: $Al^{3+} > Fe^{3+}$, $Fe^{2+} > Ca^{2+} > Mg^{2+} \approx Mn^{2+} > K^+ \approx Na^+$. The aqueous extract of *A. ebracteatus* Vahl. after the diafiltration showed better cytotoxicity against both of KB and HeLa cell lines. This investigation as well highlights the significant effect of multi-components in the solution on the separation performance of nanofiltration membrane.

NOMENCLATURE

A	filtration area (m^2)
c	concentration ($mol\,m^{-3}$)
c_m	concentration at membrane surface ($mol\,m^{-3}$)
c_p	concentration in permeate ($mol\,m^{-3}$)
J_s	averaged solute flux over membrane surface ($mol\,m^{-2}\,s^{-1}$)
J_v	averaged volume flux over membrane surface ($m^3\,m^{-2}\,s^{-1}$)
L_w	pure water permeability
P	solute permeability
P'	local solute permeability
R_{obs}	observed rejection
t	time (s)
V	volume of permeate (L)
V_{fo}	initial volume (L)
V_w	volume of feed water (L)
σ	reflection coefficient
Δx	membrane thickness (m)
$\Delta\pi$	osmotic pressure across the membrane (($kg\,cm^{-2}$)
ΔP	transmembrane pressure ($kg\,cm^{-2}$)

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REFERENCES

1. Kanchanapoom, T., Kasai, R., Picheansoonthon, C., and Yamasaki, K. (2001) Megastigmane, aliphatic alcohol and benzoxazinoid glycosides from *Acanthus ebracteatus*. *Phytochemistry*, 58: 811–817.
2. Masathien, C. and Siripong, P. (1991) In vitro immunopotentiating effect(s) of *Acanthus ebracteatus* Valh. Roots on human lymphocytes. *J. Med. Technol. Assoc. Thailand*, 15: 97–103.
3. Tiwawech, D., Siripong, P., and Kupradinun, P. (1993) Inhibition of diethylnotrosamine (DNE)-included hepatic foci by pre and post treatment with *Acanthus ebracteatus* Valh. in rat. *Thai Cancer J.*, 19: 7–13.
4. Siripong, P., Kupradinun, P., Piyaviriyagul, S., et al. (2001) Chronic toxicity of *Acanthus ebracteatus* Vahl. in rats. *Bull. Dept. Med. Sci. Th.*, 43 (4) 193–204.

5. Wang, X.L., Tsuru, T., Nakao, S., and Kimura, S. (1995) Evaluation of pore structure and electrical properties of nanofiltration membranes. *J. Chem. Eng. Jap.*, 28: 186–192.
6. Wang, X.L., Tsuru, T., Nakao, S., and Kimura, S. (1997) The electrostatic and steric-hindrance model for the transport of changed solutes through nanofiltration membranes. *J. Membr. Sci.*, 135: 19–32.
7. Wang, X.L., Zhang, C., and Ouyang, P. (2002) The possibility of separating saccharides from a NaCl solution by using nanofiltration in diafiltration mode. *J. Membr. Sci.*, 204: 271–281.
8. Van der Horst, H.C., Timmer, J.M.K., Robbertsen, T., and Leenders, J. (1995) Use of nanofitration for concentration and demineralization in the dairy industry: Model for mass transport. *J. Membr. Sci.*, 104: 205–218.
9. Browen, W.R., Mohammad, A.W., and Hilal, N. (1997) Characterization of nanofiltration membranes for predictive purposes-use of salts, uncharged solutes and atomic force microscopy. *J. Membr. Sci.*, 126: 91.
10. Mohammad, A.W. and Takriff, M.S. (2003) Predicting flux and rejection of multicomponent salts mixture in nanofiltration membranes. *Desalination*, 157: 105–111.
11. Schäfer, A.I., Fane, A.G., and Waite, T.D. (1998) Nanofiltration of natural organic matter: Removal, fouling and the influence of multivalent ions. *Desalination*, 118: 109–122.
12. Schäfer, A.I., Fane, A.G., and Waite, T.D. (2000) Fouling effects on rejection in the membrane filtration of natural waters. *Desalination*, 131: 215–224.
13. Geens, J., Van der Bruggen, B., and Vandecasteele, C. (2005) Transport model for solvent permeation through nanofiltration membranes. *Sep. Purif. Technol.*, (in press).
14. Tu, S-C., Ravindran, V., and Pirbazari, M. (2005) A pore diffusion transport model for forecasting the performanance of membrane processes. *J. Membr. Sci.*, 265: 29–50.
15. Bandini, S. (2005) Modelling the mechanism of charge formation in NF membranes: Theory and application. *J. Membr. Sci.*, 264: 75–86.
16. Ratanatamskul, C. and Yamamoto, K. (1998) Low-pressure reverse osmosis as a process for treatment of anionic pollutants in water environment. *Environ. Eng. Policy*, 1: 103–107.
17. Tsuru, T., Shutou, T., Nakao, S., and Kimura, S. (1994) Peptide and amino acid separation with nanofiltration membrane. *Sep. Sci. Technol.*, 29 (80): 971–984.
18. Vandanjon, L., Jaouen, P., Rossignol, N., et al. (1999) Concentration and desalting by membrane processes of a natural pigment produced by the marine diatom *Haslea ostrearia* Simonsen. *J. Biotechnol.*, 70: 393–402.
19. Yu, S., Gao, C., Su, H., and Liu, M. (2001) Nanofiltration used for desalination and concentration in dye production. *Desalination*, 140: 97–100.
20. Koyuncu, I. and Topacik, D. (2003) Effects of operating conditions on the salt rejection of nanofiltration membranes in reactive dye/salt mixtures. *Sep. Purif. Technol.*, 33: 283–294.
21. Capelle, N., Moulin, P., Charbit, F., and Gallo, R. (2002) Purification of heterocyclic drug derivatives from concentrated saline solution by nanofiltration. *J. Membr. Sci.*, 196: 125–141.
22. Spiegler, K.S. and Kedem, O. (1966) Thermodynamic of hyperfiltration (reverse osmosis): Criteria for efficient membranes. *Desalination*, 1: 311–326.

23. Kedem, A. and Katchasky, A. (1963) Permeability of composite membranes: Part 1. Electric current, volume flow and flow of solute through membrane. *Trans. Faraday Soc.*, 59: 1918–1953.
24. Bian, R., Yamamoto, K., and Watanabe, Y. (2000) The effect of shear rate on controlling the concentration polarization and membrane fouling. *Desalination*, 131: 225–236.
25. Shu, L., Waite, T.D., Bliss, P.J., et al. (2005) Nanofiltration for the possible reuse of water and recovery of sodium chloride salt from textile effluent. *Desalination*, 173: 235–243.
26. Van der Bruggen, B., Schaep, J., Wilms, D., and Vandecasteele, C. (1999) Influence of molecular size, polarity and charge on the retention of organic molecules by nanofiltration. *J. Membr. Sci.*, 156: 29–41.
27. Tsuru, T., Urairi, M., Yabe, T., et al. (Oct 2–4, 1991) Ion separation by reverse osmosis with mono and bipolar membranes, *Proceedings of ICIE'91*, Tokyo, Japan.
28. Anne, C.O., Trebouet, D., Jaouen, P., and Quemeneur, F. (2001) Nanofiltration of seawater: fractionation of mono and multi-valent cations. *Desalination*, 140: 67–77.