Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Extraction of Flavonoid Glycosides from *Ginkgo biloba* Leaves and Their Adsorption Separations Using Hydrophobic and Anion-Exchange Membranes

Fu-Chieh Yu[a]; Shih-Ming Lai[b]; Shing-Yi Suen[a]
[a] Department of Chemical Engineering, National Chung Hsing University, Taichung, Taiwan [b] Department of Chemical Engineering, National Yunlin University of Science and Technology, Touliu, Yunlin, Taiwan

Online publication date: 27 February 2003

To cite this Article Yu, Fu-Chieh , Lai, Shih-Ming and Suen, Shing-Yi(2003) 'Extraction of Flavonoid Glycosides from *Ginkgo biloba* Leaves and Their Adsorption Separations Using Hydrophobic and Anion-Exchange Membranes', Separation Science and Technology, 38: 5, 1033 — 1050
To link to this Article: DOI: 10.1081/SS-120018122
URL: http://dx.doi.org/10.1081/SS-120018122

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

SEPARATION SCIENCE AND TECHNOLOGY
Vol. 38, No. 5, pp. 1033–1050, 2003

Extraction of Flavonoid Glycosides from *Ginkgo biloba* Leaves and Their Adsorption Separations Using Hydrophobic and Anion-Exchange Membranes

Fu-Chieh Yu,[1] Shih-Ming Lai,[2] and Shing-Yi Suen[1,*]

[1]Department of Chemical Engineering, National Chung Hsing University, Taichung, Taiwan
[2]Department of Chemical Engineering, National Yunlin University of Science and Technology, Touliu, Yunlin, Taiwan

ABSTRACT

In this work, the extraction of flavonoid glycosides from *Ginkgo biloba* leaves and their adsorption separation performance using C18 hydrophobic and strong anion-exchange membranes were investigated. First, the preparation of crude *Ginkgo biloba* L. extracts was carried out using 70% ethanol, and the effects of different extraction conditions were evaluated. The results show that the extraction temperature (50–70°C) and extraction time (1–5 h) did not significantly influence the performance, but a lower solvent amount (50 g per 10 g dry leaves) resulted in a better extraction. Before the adsorption separations,

*Correspondence: Shing-Yi Suen, Department of Chemical Engineering, National Chung Hsing University, Taichung 402, Taiwan; Fax: +886-4-22854734; E-mail: sysuen@nchu.edu.tw.

DOI: 10.1081/SS-120018122 0149-6395 (Print); 1520-5754 (Online)

the extracts were dissolved in 30% ethanol to remove the undesirable biflavones and then used as feed solutions. Eighty percent ethanol was used as the eluent in the elution stage. Comparing the performances for different processes, higher yield for total flavonoid glycoside amount (46–60% for hydrophobic membranes and 39–53% for anion-exchange membranes) was accomplished in the batch process. On the other hand, a higher flavonoid glycoside content at the pinnacle fraction of elution peak (10 wt% for hydrophobic membranes and 7–13 wt% for anion-exchange membranes) and a shorter process period were achieved in the flow process.

Key Words: Flavonoid glycosides; *Ginkgo biloba* leaves; Extraction; Hydrophobic membranes; Anion-exchange membranes; Adsorption.

INTRODUCTION

Extracts from *Ginkgo biloba* leaves have been utilized in the treatment of cerebrovascular and peripheral circulatory insufficiency for many years.[1–9] The most important active ingredients of *Ginkgo biloba* L. extracts are flavonoid glycosides and terpene lactones (including ginkgolides and bilobalide).[2,4,5,7,9,10] Flavonoid glycosides are believed to have many helpful biological activities such as dilating coronary vessels, improving peripheral and brain blood circulation, and preventing intravascular thrombogenesis.[9] On the other hand, terpene lactones are potent antagonists against platelet-activating factors.[3,9,11] In addition to these two substances, the other constituents in *Ginkgo biloba* L. extracts may be undesirable, e.g., biflavones, or even toxic, e.g., ginkgolic acids that cause contact dermatitis and allergy.[7,9]

Ginkgo biloba L. extracts are usually standardized in terms of flavonoid glycoside (24 wt%) and terpene lactone (6 wt%) contents.[4,8] To achieve these standards, better extraction methods (e.g., supercritical fluid extraction) or enrichment of flavonoid glycosides and terpene lactones from the crude extracts (e.g., liquid–liquid extraction, precipitation by alkalinization or adding ammonium solution, and adsorption separation) is required.[1,3,8,9,12] A few limitations and problems for these methods, however, have been observed and discussed in the literature.[3,6,8,12] For example, the applicability of supercritical fluid extraction method to extract the intermediate polar flavonoid glycosides is doubtful. For liquid–liquid extraction methods, large quantities of variable organic solvents are consumed, phase separation is required, and a trace of undesirable solvents may be retained in the final

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

products. In the precipitation method by means of alkalinization using metal hydroxides, the remaining metals would cause troubles. In comparison to the previously discussed methods, adsorption separation methods may be a better way to increase the active ingredient contents because the problems in the adsorption separation process, such as gradient elution and longer separation period, could be resolved by selecting appropriate adsorbents and designing better operation conditions. The adsorbents adopted or mentioned in the literature[8,9] include polyamide, synthetic MA (methacrylate)–DVB (divinylbenzene) copolymer beads, and macroporous resins such as Duolite S-761 and Diaion HP-20 with either polar or hydrophobic adsorption characteristics.

In this study, adsorptive membranes are applied to the adsorption separation process for *Ginkgo biloba* L. extracts with the consideration of their low mass-transfer limitations for a more efficient process.[13–15] The adsorptive membranes adopted include commercial hydrophobic and anion-exchange membranes. This paper emphasizes the flavonoid glycoside content in the extracts, and the results for the adsorption separations of terpene lactones using adsorptive membranes will be presented in the near future. Prior to the adsorption process, the crude *Ginkgo biloba* L. extracts were prepared using ethanol aqueous solution. Different extraction conditions were attempted and their effects were investigated. For batch and flow adsorption processes, the performance was evaluated in terms of the yield and the concentration ratio for the flavonoid glycoside content in the concentrated extract.

EXPERIMENTAL

Materials

Flavonoid aglycone standards were purchased from commercial sources: quercetin dihydrate from ACROS (Geel, Belgium), isorhamnetin (90% purity) from Extrasynthese (France), and kaempferol (90% purity) from Sigma (St. Louis, MO, USA). Commercial *Ginkgo biloba* L. extract (Cerenin® Drops) was obtained from Dr. Willmar Schwabe (Karlsruhe, Germany). Hydrochloric acid (HCl), methanol, and tetra-hydrofuran (THF) were bought from TEDIA (Fairfield, OH, USA). Ethanol was from SCI (Kyungki-do, Japan) and ortho-phosphoric acid (H_3PO_4, 85% purity) was from Riedel-de haen (Seelze, Germany). Dry green *Ginkgo biloba* leaves were from mainland China.

Hydrophobic membranes employed in this study were C18 discs (ENVI-18 DSK) from Supelco (Bellefonte, PA, USA), with a diameter of 47 mm and a thickness of 0.6 mm. The materials of these membranes are glass fibers

embedded with surface-modified silica and are usually applied for solid-phase extraction. The anion-exchange membranes adopted were strong anion-exchange discs (SB6407) from Pall (Ann Arbor, MI, USA), with a diameter of 47 mm and a thickness of 0.152 mm. The SB6407 membranes are made from polyethersulfone and have an average pore size of 0.45 μm. Their ion-exchange capacity is 2.3 μeq/cm^2, reported by the manufacturer.

Extraction of Flavonoid Glycosides from *Ginkgo biloba* Leaves

Prior to use, dry *Ginkgo biloba* leaves were further dried in an oven at 60°C for 5 h and then pulverized. Ten grams of dried and pulverized *Ginkgo biloba* leaves were dissolved in certain amount of 70% ethanol. The mixture was shaken at 150 rpm under a constant temperature for a specific time. The extract was then filtrated through a 0.2-μm nylon membrane (Lida Manufacturing Corp., Kenosha, WI, USA) and the insoluble material was collected. The filtrate (Extract A) was concentrated using a rotary evaporator and the concentrated extract was weighed. The concentrate was finally dissolved in 100 ml methanol as crude extract solution (Extract B). In addition, these same procedures under identical conditions were repeated for the extraction of the collected insoluble material.

The effects of different extraction conditions were investigated as follows. First, the extraction procedures were carried out at 50°C. Other extraction factors, such as solvent amount and extraction time, were varied in each extraction. The resulted extract solutions (Extract B) were hydrolyzed and then analyzed by HPLC, following the procedures described in the next section (quantitative analysis). After the optimal situations for 70% ethanol amount and extraction time were decided, the extraction temperature was varied to investigate its effect.

Quantitative Analysis of Flavonoid Glycosides

The total amount of flavonoid glycosides is not easy to estimate due to their great variety and the difficulty to get the pure standards. Fortunately, by hydrolysis, even the great variety of flavonoid glycosides can be reduced to three major aglycones: isorhamnetin, kaempferol, and quercetin.[2,16] According to the analysis conducted by Hasler et al.,[2] the aglycone content could be correlated to the flavonoid glycoside content. In this study, the hydrolysis of flavonoid glycosides was carried out using 24.7% (v/v) HCl. The volume ratio of *Ginkgo biloba* L. extract to 24.7% HCl was 3:1.

The hydrolysis was conducted at 70°C for 1 h. It may be worthy noting that a higher temperature (80°C) and a higher HCl ratio (the volume ratio of *Ginkgo biloba* L. extract solution to HCl = 2:1) have been tested to confirm the completion of hydrolysis. The result for either 80°C or higher HCl ratio was almost identical to that for 70°C and ratio 3:1. However, HCl evaporation was fast at 80°C or at the higher HCl ratio and caused a strong odor, which should be avoided.

The flavonoid aglycone standards (quercetin, isorhamnetin, and kaempferol) and the hydrolyzed extract solution were analyzed by HPLC under 370 nm. The HPLC system consisted of a pump (Series II, Lab Alliance, Lemont, PA, USA) and a UV–Vis detector (V^4, ISCO, Lincoln, NE, USA). The column was Hypersil, HS C18 5μ, 250 × 4.6 mm (ThermoQuest Hypersil Division, Runcorn, UK), and the mobile phase was methanol:THF:0.5% H_3PO_4 = 27.5:27.5:45. The flow rate was 1 ml/min and the sample amount for each injection was 20 μl. When each aglycone content in the chromatogram of the hydrolyzed extract solution was determined using the aglycone calibration curves, the total flavonoid glycoside content was evaluated using the formula: $\sum$(amount of each aglycone) × 2.51 = total amount of flavonoid glycosides.[2]

Adsorption Separations of Flavonoid Glycosides from Crude *Ginkgo biloba* L. Extracts

Batch Adsorption Experiments

The feed solution was prepared as follows. First, 20 g dry leaves were extracted under the optimal extraction conditions. The same extraction and filtration procedures as in the section of preparation of crude *Ginkgo biloba* L. extracts were adopted. Next, the filtrate (Extract A) was concentrated by a rotary evaporator. The concentrated extract was then dissolved in 100 ml of 30% ethanol. The resulted solution was centrifuged at 12,000 rpm for 15 min and then filtrated using disposable syringe filter (PVDF, 0.2 μm, Pall). The filtrate (Extract C) was used as the feed solution.

Prior to use, ENVI-18 DSK membranes were washed with 30% ethanol, whereas SB6407 membranes were sequentially rinsed by 10% NaOH, deionized water, and 30% ethanol. After the liquid on the membrane surfaces was removed, four (for ENVI-18 DSK) or eight (for SB6407) pieces of adsorptive membranes were incubated with 10 ml feed solution (Extract C) at room temperature for 2 h. After adsorption, an elution using 10 ml of 80% ethanol was conducted for another 2 h. The total flavonoid glycoside contents in the solutions (feed, after adsorption, elution) were analyzed by HPLC

following the procedures described in the section of quantitative analysis. Lastly, all the solutions were concentrated using a rotary evaporator and the concentrates were weighed.

Flow Adsorption Experiments

The equipment for flow adsorption experiments included a peristaltic pump (AC-2120 PERISTA® BIO-MINIPUMP, ATTO, Tokyo, Japan), a 47-mm membrane disc holder (made of polypropylene), and a fraction collector (Retriever® 500, ISCO). The feed solution for flow adsorption experiments was prepared similar to the batch adsorption experiments. However, re-extraction for the insoluble leaf material was conducted (using 200 g of 70% ethanol at 50°C for 3 h). The extract from the second extraction was filtrated and the filtrate was concentrated. The concentrate was then dissolved in 20 ml of 30% ethanol. Both the extract solutions in 30% ethanol from the first and second extractions were mixed together, centrifuged at 12,000 rpm for 15 min, and then filtrated using disposable syringe filter. The final filtrate (Extract D) was the feed solution for the flow adsorption experiments. In this case, the total flavonoid glycoside content in the feed solution was increased.

Four (for ENVI-18 DSK) or eight (for SB6407) pieces of membranes were placed in the polypropylene membrane disc holder (custom-made). An O-ring was used to prevent fluid from lateral leaking and the holder was tightly attached. The flow process was conducted at 1 ml/min at room temperature. For ENVI-18 DSK membranes, the holder was first equilibrated with 30% ethanol. For SB6407 membranes, the holder was sequentially rinsed by 10% NaOH, deionized water, and 30% ethanol. The solutions loaded were 20 ml feed solution (adsorption step), 10 ml deionized water (washing step), and 40 ml of 80% ethanol (elution step), in sequence. The effluent fractions were collected, and the flavonoid glycoside content in each fraction was analyzed following the procedures described in the section of quantitative analysis. Finally, each effluent fraction was concentrated and each concentrate was weighed. The experiment for each kind of membrane was repeated twice.

RESULTS AND DISCUSSION

Effects of Different Extraction Conditions

The crude *Ginkgo biloba* L. extracts were prepared using 70% ethanol in this study. Ethanol was chosen because of its nontoxicity for dietary purpose,

and a high-percentage ethanol solution was employed for the expectation of better extraction performance. The results for extraction at 50°C under different conditions are displayed in Table 1. With the increasing solvent amount, the yield of crude extract (weight of concentrated extract/weight of dry leaves used) increased for the first extraction cases, but not for the second extraction cases (re-extractions of insoluble materials). The weights of extracted flavonoid glycosides were close in most cases for the first extraction, whereas the weight of flavonoid glycosides from the re-extraction decreased with the increasing solvent amount. Although the influence of solvent amount was different for the first and second extraction cases, the decreasing flavonoid glycoside content in the concentrated extract was observed in both cases with the increasing solvent amount. In addition, the effect of extraction time on both the yield of crude extract and the flavonoid glycoside content was insignificant for all the cases.

Table 1. Results for extraction of flavonoid glycosides from 10 g of *Ginkgo biloba* leaves using 70% ethanol at 50°C under different conditions.

Extraction time (h)	Solvent amount (g)	Extraction	Weight of concentrated extract (g)	Yield of crude extract (%)	Weight of flavonoid glycosides (mg)	Wt% of flavonoid glycosides in concentrated extract
1	50	1st	1.18	11.8	62.90	5.33
		2nd	0.48	4.8	11.59	2.41
	100	1st	1.55	15.5	59.51	3.84
		2nd	0.44	4.4	7.63	1.73
	200	1st	1.98	19.8	60.94	3.08
		2nd	0.44	4.4	5.56	1.26
3	50	1st	1.02	10.2	44.52	4.36
		2nd	0.62	6.2	14.03	2.26
	100	1st	1.46	14.6	78.55	5.38
		2nd	0.46	4.6	8.31	1.81
	200	1st	1.78	17.8	64.97	3.65
		2nd	0.55	5.5	6.83	1.24
5	50	1st	1.03	10.3	52.60	5.11
		2nd	0.52	5.2	12.54	2.41
	100	1st	1.65	16.5	61.26	3.71
		2nd	0.48	4.8	9.12	1.90
	200	1st	1.79	17.9	60.03	3.35
		2nd	0.36	3.6	5.86	1.63

Before the investigation of temperature effect, the optimal conditions for other extraction-influencing factors should be chosen based on the results in Table 1. According to the analysis in the previous paragraph, the optimal solvent amount should be 50 g per 10 g dry leaves. However, to prevent the possible deposition of powdered leaves on the bottom of the container during the extraction process, a larger solvent amount, 100 g per 10 g dry leaves, was adopted for the subsequent experiments. On the other hand, 3 h (the middle value used in this work) was selected as the suitable extraction time since the effect of extraction time was not important. Based on these conditions, the results for different temperatures are presented in Table 2. Comparing those results, the temperature effect was also insignificant. To save energy, a lower temperature, 50°C, was chosen as the optimal extraction temperature to prepare the *Ginkgo biloba* L. extracts.

It should be noted that the same extraction conditions may not lead to identical results in each batch. For example, the result shown in Table 1 under the conditions of 50°C, 3 h, 100 g solvent, and first extraction has a flavonoid glycoside content of 5.67 wt%, whereas the result in Table 2 shows a lower content at 4.42 wt%. The difference is about 20% of the better content, which may be attributed to many factors such as nonuniform flavonoid glycoside contents distributed in different leaves, or probable loss at each stage (extraction, filtration, concentration, or hydrolysis) during the whole process. Summing up the results in Tables 1 and 2, the total flavonoid glycoside content in the concentrated extract (for the first extractions) ranged from 3.2 to 5.7 wt%. These results are close to the values reported in the literature.[9]

Batch Adsorption Performance

For batch adsorptions, 30% ethanol was used as the solvent for feed solutions, instead of 70% ethanol [the extract chromatogram is similar to

Table 2. Results for extraction of flavonoid glycosides from 10 g of *Ginkgo biloba* leaves using 100 g of 70% ethanol at different temperatures for 3 h.

Extraction temperature (°C)	Weight of concentrated extract (g)	Yield for crude extract (%)	Weight of flavonoid glycosides (mg)	Wt% of flavonoid glycosides in concentrated extract
50	1.46	14.6	64.52	4.42
60	1.59	15.9	57.50	3.62
70	1.51	15.1	63.63	4.21

Fig. 1(a)]. This change is based on the fact that biflavones were not dissolved in 30% ethanol, as shown in Fig. 1(b). In general, biflavones are undesirable substances in the commercial products, as presented in the chromatogram of Cerenin® Drops [see Fig. 1(c)]. Moreover, the removal of biflavones may avoid the possible competitive adsorption of biflavones onto the adsorptive membranes. On the other hand, picking 30% ethanol as the solvent for adsorption could also make the use of a high-percentage ethanol solution as eluent accessible. In this work, 80% ethanol was adopted as the eluent.

Flavonoid glycosides are a kind of phenolic derivative.[8] The cyclo- or benzene structures in their molecular configuration are hydrophobic, while the OH residuals in the phenolic groups possibly form anions. Therefore, both hydrophobic and anion-exchange membranes were employed as the adsorbents to separate flavonoid glycosides. In this study, C18 hydrophobic (ENVI-18 DSK) and strong anion-exchange (SB6407) membranes were adopted. The batch process of adsorption and elution was repeated for each kind of membrane, and the results are presented in Table 3. It may be worthy to mention that C8 hydrophobic and weak anion-exchange membranes were also tried, but their batch adsorption results (data not shown) were either worse than or close to those presented in this work.

During the batch experiments, certain white substances from ENVI-18 DSK membranes were released to the solution. Recalling that the ENVI-18 DSK membrane materials are glass fibers embedded with surface-modified silica, the cause for the white substance discharge should be attributed to the membrane material problem. Those released substances were filtrated and discarded before the subsequent analyses.

In Table 3, the flavonoid glycoside content for the feed solution (1.3 or 1.6 wt% in the concentrated extract) was lower than those obtained in the section about crude extract preparation, which is attributed to the use of 30% ethanol as solvent. In Table 3, the sum of yields for concentrated extracts after adsorption and after elution was 86 and 96% for the two trials of ENVI-18 DSK membranes, and 78% for SB6407 membranes. The loss in the concentrated extract amount was higher for SB6407 membranes, which could be explained from the observations on the membrane color change. During the experiments, the color for both kinds of membranes turned brownish yellow after adsorption. After elution, the color returned to white for ENVI-18 DSK membranes, while the brownish yellow color on the SB6407 membranes could not be completely removed. This difference indicates that some substances were strongly adsorbed on the strong-basic anion-exchange membranes and could not be completely eluted by 80% ethanol.

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

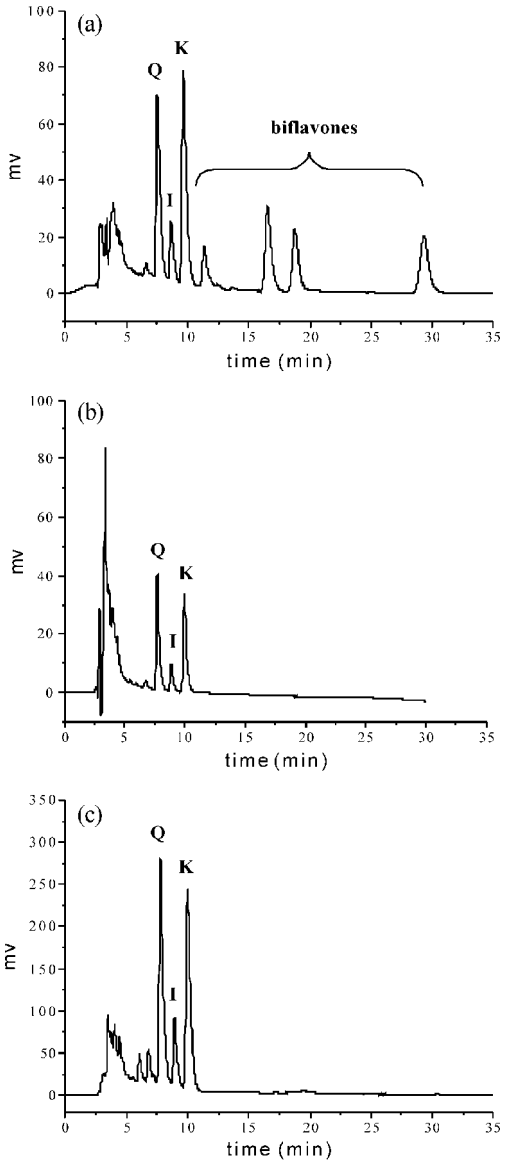

Figure 1. HPLC analysis results. (**Q**: Quercetin, **I**: isorhamnetin, **K**: kaempferol): (a) Extract B (extracted under the optimal conditions), dissolved in methanol. (b) Extract C (extracted under the optimal conditions), dissolved in 30% ethanol. (c) Cerenin® Drops, dissolved in methanol (dilution ratio = 1:3).

Table 3. Batch adsorption and elution results using different adsorptive membranes.

Trial	Condition	Weight of concentrated extract (mg)	Yield of concentrated extract (%)	Weight of flavonoid glycosides (mg)	Yield of flavonoid glycosides (%)	Wt% of flavonoid glycosides in concentrated extract	Concentration ratio
1	Feed solution	320.3		5.14		1.60	
	After adsorption with 4 pieces of ENVI-18 DSK membranes for 2 h	240.4	75.1	4.35	84.6	1.81	
	After elution using 80% ethanol for 2 h	66.0	20.6	2.37	46.1	3.59	2.2
	After adsorption with 8 pieces of SB6407 membranes for 2 h	204.7	63.9	3.29	64.0	1.61	
	After elution using 80% ethanol for 2 h	42.7	13.3	2.00	38.9	4.67	2.9
2	Feed solution	234.3		3.07		1.31	
	After adsorption with 4 pieces of ENVI-18 DSK membranes for 2 h	157.5	67.2	2.68	87.3	1.70	
	After elution using 80% ethanol for 2 h	44.4	18.5	1.85	60.3	4.16	3.2
	After adsorption with 8 pieces of SB6407 membranes for 2 h	152.3	65.0	2.28	74.3	1.50	
	After elution using 80% ethanol for 2 h	29.9	12.8	1.64	53.4	5.47	4.2

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

In Table 3, the yield of total flavonoid glycoside amount was 46–60% for hydrophobic membranes and 39–53% for anion-exchange membranes. However, the sum of yields for flavonoid glycosides after adsorption and after elution was greater than 100% for most cases, especially for ENVI-18 DSK membranes. There are several possibilities to cause these errors, such as nonuniform flavonoid glycoside contents distributed in the feed solutions for doing only quantitative analysis and for doing the adsorption separations, or probable greater loss of flavonoid glycosides in the feed solution during the hydrolysis and quantitative procedures.

The results in Table 3 show that the flavonoid glycoside content after elution was raised to 2–3 times for ENVI-18 DSK membranes, and to 3–4 times for SB6407 membranes. The concentration performance for SB6407 membranes is better than ENVI-18 DSK membranes. It is worth noting that, in both trials, the eluted flavonoid glycoside amount was close for both kinds of membranes, but the concentrated extract amount was quite different. Remembering that some extract substances adsorbed on the SB6407 membranes could not be completely eluted, it can be concluded that a larger proportion of these retained substances was not flavonoid glycosides (possibly chlorophyll and lutein). This contributed to a higher flavonoid glycoside content in the concentrated extract collected from the elution stage for SB6407 membranes.

In this study, four pieces of ENVI-18 DSK membranes were used and eight pieces were adopted in the case of SB6407 membranes. However, considering that the ENVI-18 DSK membrane was almost fourfold thicker than the SB6407 membrane, the employed volume (4.16 cm^3) for ENVI-18 DSK membranes was double of that (2.11 cm^3) for SB6407 membranes. Consequently, SB6407 anion-exchange membranes exhibit a better adsorption separation performance in terms of the concentration ratio for the flavonoid glycosides in the batch process.

Flow Adsorption Performance

The flow adsorption experiments were conducted at 1 ml/min. The feed solution was 20 ml of *Ginkgo biloba* L. extract in 30% ethanol with a flavonoid glycoside content of 2.35 wt% (407.5 mg concentrate, 9.6 mg flavonoid glycosides, $c_0 = 0.48$ mg/ml) or 4.55 wt% (643 mg concentrate, 29.2 mg flavonoid glycosides, $c_0 = 1.46$ mg/ml). The washing solution was deionized water and the eluent was 80% ethanol. The results for hydrophobic and anion-exchange membranes are illustrated in Figs. 2 and 3, respectively. The results from two different trials are close for ENVI-18 DSK membranes, so that

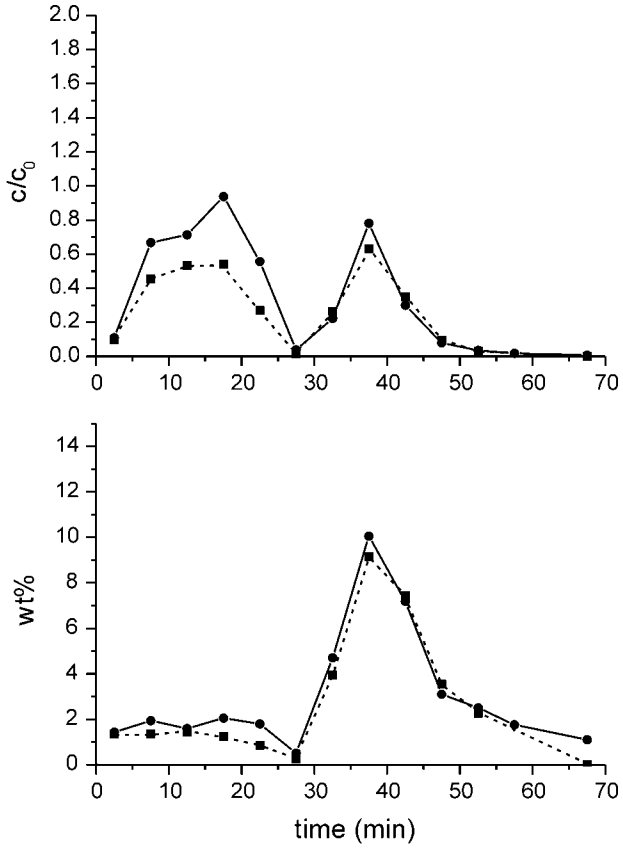

Figure 2. Flow adsorption results using ENVI-18 DSK membranes at 1 ml/min. Loading (Sample D, dissolved in 30% ethanol): 0–20 min; washing (deionized water): 20–30 min; elution (80% ethanol): 30–70 min. First trial: –●– (feed with 2.35 wt% flavonoid glycosides, $c_0 = 0.48\,mg/ml$); second trial: – –■– – (feed with 2.35 wt% flavonoid glycosides, $c_0 = 0.48\,mg/ml$). wt%: flavonoid glycoside weight/extract concentrate weight.

the flow adsorption results are considered reproducible. During the flow experiments, the release of white substances from ENVI-18 DSK membranes was not observed. In addition, the phenomenon of membrane color change was similar to that in the batch process: The ENVI-18 DSK membrane color returned to white after elution, while the SB6407 membrane color remained brownish yellow.

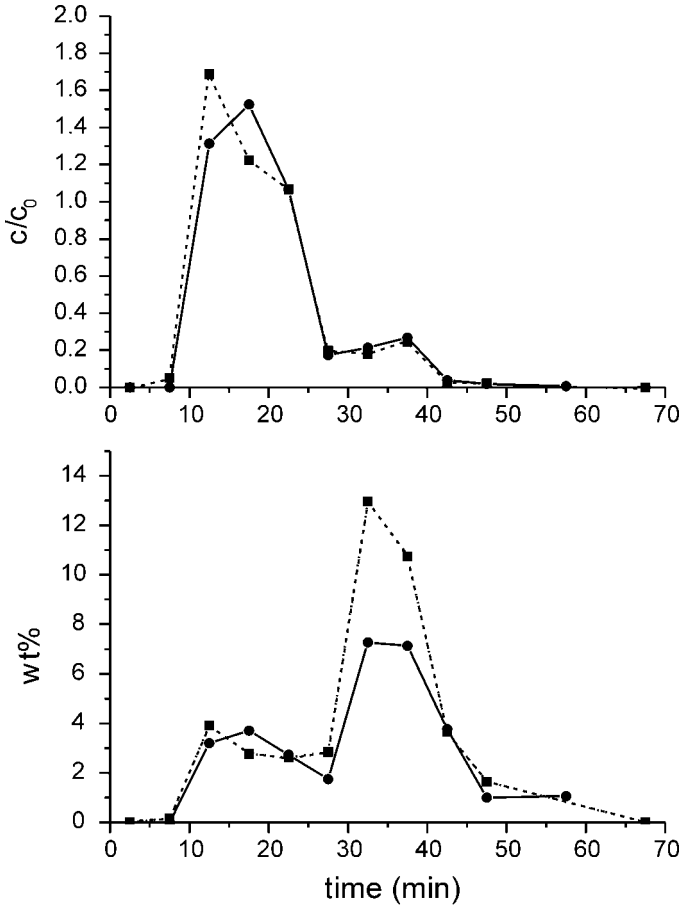

Figure 3. Flow adsorption results using SB6407 membranes at 1 ml/min. Loading (Sample D, dissolved in 30% ethanol): 0–20 min; washing (deionized water): 20–30 min; elution (80% ethanol): 30–70 min. First trial: –●– (feed with 2.35 wt% flavonoid glycosides, $c_0 = 0.48$ mg/ml); second trial: – –■– – (feed with 4.55 wt% flavonoid glycosides, $c_0 = 1.46$ mg/ml). wt%: flavonoid glycoside weight/extract concentrate weight.

In the loading stage (0–20 min), the breakthrough point was near the beginning for ENVI-18 DSK membranes and after that, the concentration rose to a value below the feed one for both trials. In the elution stage (30–70 min), the elution peak concentration for ENVI-18 DSK membranes was about

$0.7 \sim 0.8c_0$. The adsorption/elution performance for SB6407 membranes was different. The breakthrough occurred around 8 min and then the concentration was raised far above the feed concentration. Moreover, the elution peak concentrations for SB6407 membranes were very low.

According to the above analyses on breakthrough curve performance for both kinds of membranes, all the flavonoid glycoside concentrations at elution peaks were smaller than their feed concentrations. It may be concluded that the flow process using adsorptive membranes was not effective. However, it should be noted that the flavonoid glycoside content (flavonoid glycoside weight/the whole extract concentrate weight) in the product, not its concentration in solution, is more important in practical application. Therefore, the flow adsorption results were also compared by examining the flavonoid glycoside content in the concentrated extract. In the elution stage, the pinnacle flavonoid glycoside content could be raised to about 10 wt% for ENVI-18 DSK membranes (a feed with a flavonoid glycoside content of 2.35 wt%), while the corresponding content for SB6407 membranes was around 7.3 wt% for the first trial (a feed with a flavonoid glycoside content of 2.35 wt%) and 13 wt% for the second trial (a feed with a flavonoid glycoside content of 4.55 wt%). The concentration ratio for this effluent fraction (at elution peak pinnacle) could reach 4.3 for ENVI-18 DSK membranes, but was about threefold for SB6407 membranes (the ratio was close for both trials). The elution performance presented by flavonoid glycoside content was much better than that by concentration. It could be attributed to a smaller portion being adsorbed and eluted for other constituents in the extract, compared to that for flavonoid glycosides. The resulted extract concentrate weight was reduced more than the flavonoid glycoside weight. Consequently, the flavonoid glycoside content (wt%) was increased and became larger than the feed one.

Considering the whole elution process, the sum of concentrates for all the effluent fractions collected weighed around 56.5 mg for ENVI-18 DSK membranes and 27.5 mg for SB6407 membranes (the amount was almost identical in both trials). The total flavonoid glycoside amount for all the effluent fractions collected at elution was around 3.5 mg for ENVI-18 DSK membranes and 1.2 mg (for the first trial) and 1.8 mg (for the second trial) in the case of SB6407 membranes. Accordingly, the total flavonoid glycoside content for the whole elution stage was 6.2 wt% for ENVI-18 DSK membranes and 4.4 wt% (for the first trial) and 6.5 wt% (for the second trial) for SB6407 membranes. The whole concentration ratio was 2.6 for hydrophobic membranes and only 1.9 (for the first trial) and 1.4 (for the second trial) for SB6407 membranes. Furthermore, based on the flavonoid glycoside amount for the 20-ml feed solution (9.6 mg or 29.2 mg), the yield for

flavonoid glycosides was about 36.5% for ENVI-18 DSK membranes and 12.5% (for the first trial) and 6.2% (for the second trial) for SB6407 membranes. In the case of SB6407 membranes, a higher flavonoid glycoside content at feed resulted in a higher content at elution (for either pinnacle or overall value) but a lower yield and a lower concentration ratio. In comparison with both kinds of membranes at flow adsorption process, ENVI-18 DSK membranes show a better performance on flavonoid glycoside yield and concentration ratio, which is partly different from the batch performance.

CONCLUSIONS

Summing up the results obtained in this study, a better method for preparing the crude *Ginkgo biloba* L. extracts is suggested as follows. First, a high-percentage ethanol (e.g., 70%) should be employed, and the optimal extraction conditions with 50 g (or 100 g) solvent per 10 g dry leaves at 50°C for 3 h are suggested. After filtrating the raw extract, the insoluble materials should be re-extracted, and the solvent amount used in this extraction should be minimized (e.g., 50 g or less solvent per 10 g original dry leaves). After filtrating the extract from the second extraction, the filtrates from both extractions are collected together and then concentrated. Next, a low-percentage ethanol (e.g., 30%) should be adopted to dissolve the concentrated extract. Lastly, the extract solution is filtrated to remove the undesirable biflavones. The final extract filtrate is believed to contain a flavonoid glycoside content of about 2.5 wt%. To further improve the extraction process, better mixing design and reflux operation may be necessary.

In this study, the possibility to increase the flavonoid glycoside content by the adsorption separation methods using the hydrophobic or anion-exchange membranes was verified. A high-percentage ethanol (e.g., 80%) should be used as the eluent. The performance for the whole elution stage in the flow adsorption process may be slightly worse than the batch process, but a higher flavonoid glycoside content (about 10 wt%) could be achieved in the pinnacle fraction of elution peak. The flow process may be preferred because of its advantages in a shorter process time and in preventing the possible release of membrane materials. In addition, it is observed that C18 hydrophobic membranes (larger membrane volume) and strong anion-exchange membranes (smaller membrane volume) have close adsorption performance. The yield of concentrated extract, however, was lower for strong anion-exchange membranes due to the difficulty in removing certain strongly adsorbed constituents, and it is accordingly not possible for the anion-exchange membranes to regenerate and reuse. For further improvement of

the adsorption performance, several suggestions include using even larger membrane volume (stacking even more membranes in the holder), mixing hydrophobic and anion-exchange membranes in a stack, trying different membrane configurations or modules (e.g., hollow fibers), etc.; and a more extensive study is needed.

ACKNOWLEDGMENTS

The authors are grateful for financial support provided by the National Science Council of the Republic of China (NSC 89-2815-C-005-071R-E) and Taiwan Sugar Co. (NSC 89-TSC-7-224-001).

REFERENCES

1. Ayroles, G.; Rossard, R.-M.; Cadiou, M. Method for Obtaining an Extract or *Ginkgo biloba* Leaves. US Patent 4,981,688, Jan 1, 1991.
2. Hasler, A.; Sticher, O.; Meier, B. Identification and determination of the flavonoids from *Ginkgo biloba* by high-performance liquid chromatography. J. Chromatogr. **1992**, *605*, 41–48.
3. Hwang, D.-Y.; Lin, S.-J. Improved extraction method, chromatographic separation and analyses of ginkgolides from leaves of *Ginkgo biloba* L. Formos. Sci. **1995**, *48*, 106–111.
4. Li, C.L.; Wong, Y.Y. The bioavailability of ginkgolides in *Ginkgo biloba* extracts. Planta Med. **1997**, *63*, 563–565.
5. Chen, P.; Su, X.-L.; Nie, L.-H.; Yao, S.-Z.; Zeng, J.-G. Analysis of ginkgolides and bilobalides in *Ginkgo biloba* L. extract for its production process control by high-performance liquid chromatography. J. Chromatogr. Sci. **1998**, *36*, 197–200.
6. Lang, Q.; Wai, C.M. An extraction method for determination of ginkgolides and bilobalide in Ginkgo leaf extracts. Anal. Chem. **1999**, *71*, 2929–2933.
7. Ndjoko, K.; Wolfender, J.-L.; Hostettmann, K. Determination of trace amounts of ginkgolic acids in *Ginkgo biloba* L. leaf extracts and phytopharmaceuticals by liquid chromatography–electrospray mass spectrometry. J. Chromatogr. B **2000**, *744*, 249–255.
8. Xu, M.-C.; Shi, Z.-Q.; Shi, R.; Liu, J.-X.; Lu, Y.; He, B. Synthesis of the adsorbent based on macroporous copolymer MA–DVB beads and its application in purification for the extracts from *Ginkgo biloba* leaves. React. Funct. Polym. **2000**, *43*, 297–304.

9. Zhang, D.C.; Yu, Z.; Cooper, R.; Chang, M. Methods of Preparation of Bioginkgo. US Patent 6,174,531 B1, Jan 16, 2001.
10. Pietta, P.; Mauri, P.; Rava, A. Reversed-phase high-performance liquid chromatographic method for the analysis of biflavones in *Ginkgo biloba* L. Extracts. J. Chromatogr. **1988**, *437*, 453–456.
11. van Beek, T.A.; Lelyveld, G.P. Preparative isolation and separation procedure for ginkgolides A, B, C, and J and bilobalide. J. Nat. Prod. **1997**, *60*, 735–738.
12. Zhang, C.; Hu, X.; Yue, H. Progress of extraction and analytical methods of flavones in *Ginkgo biloba* leaves. Chem. Ind. Eng. Prog. **1999**, *18* (6), 27–29.
13. Brandt, S.; Goffe, R.A.; Kessler, S.B.; O'Connor, J.L.; Zale, S.E. Membrane-based affinity technology for commercial scale purifications. Biotechnology **1988**, *6*, 779–782.
14. Roper, D.K.; Lightfoot, E.N. Separation of biomolecules using adsorptive membranes. J. Chromatogr. A **1995**, *702*, 3–26.
15. Charcosset, C. Purification of proteins by membrane chromatography. J. Chem. Technol. Biotechnol. **1998**, *71*, 95–110.
16. Hasler, A.; Sticher, O.; Meier, B. High-performance liquid chromatographic determination of five widespread flavonoid aglycones. J. Chromatogr. **1990**, *508*, 236–240.

Received May 2002
Revised August 2002