Fingerprint Development for *Ginkgo biloba* Extracts by Pressurized Capillary Electrochromatography: Comparison of Column Types

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Abstract

A pressurized capillary electrochromatographic (pCEC) fingerprint of *Ginkgo biloba* leaf extract was developed on three different types of capillary columns. A commercial column packed with 3-µm particles and an in-house column packed with 5-µm particles were investigated for their performance. Additionally, a monolithic column was included in the fingerprint study as a potential alternative to the conventional packed columns. The effects of experimental parameters, such as the composition of the mobile phase, the concentration and pH of the buffer, and the applied voltage, were studied. Binary mobile phases consisting of acetonitrile and a 5 mM sodium dihydrogen phosphate electrolyte at pH 2.8 were used in gradient elution mode with an applied voltage of 5 kV. Under optimal gradient conditions, at least 45 peaks were observed within 60 min on the commercial packed column, whereas only about 20 peaks were separated on the methacrylate-based monolithic and the in-house packed columns. The commercial column thus clearly outperforms the two other. However, the properties of the monolithic stationary phase still might be adapted (i.e., by changing the polymerization-mixture composition, the porosity, and thus the selectivity of the phase might be changed), which could lead to an improved efficiency.

Introduction

Ginkgo biloba has been used in China as a traditional medicine for various diseases. Phytopharmaceutically, *G. biloba* has become one of the top selling products in the U.S. and Europe. It is used worldwide to treat cardiovascular and cerebrovascular disorders, asthma, and bronchitis. Its memory-enhancing effects are also widely known (1,2). The two major types of active components in *G. biloba* leaf extract are the flavone glucosides and terpene lactones. Terpene lactones, i.e., ginkgolides A, B, C, J, and bilobalide (approximately 6% in total amount) contain few chromophore groups. They poorly absorb in the UV range and are therefore difficult to determine with a UV detector (3–6). Another major type of compound in *G. biloba* extracts, the flavone glucosides (approximately 24% in total amount), is mostly derived from quercetin, kaempferol, and isorhamnetin. These flavonoids generally show good UV absorption (7). Several methods have been developed to analyze flavonoids in *G. biloba* leaf extracts. Thin-layer chromatography (TLC) (8,9), high-performance liquid chromatography (HPLC) (10–12), high-speed counter-current chromatography (HSCCC) (13), capillary electrophoresis (CE) (14,15), and HPLC–MS (16–18) are the most commonly used analytical techniques. However, no method to analyze flavonoids in *G. biloba* leaf extract is, to our knowledge, described using pressurized capillary electrochromatography (pCEC) as a separation technique.

pCEC is a hybrid microcolumn electro-separation technique that combines the high separation efficiency of CE with the high selectivity of capillary liquid chromatography (CLC). In pCEC, an electroosmotic flow (EOF) caused by the application of an electrical field over a capillary column is superimposed on a pressure-induced hydrodynamic flow. Because the flat electro-driven flow profile is distributed by the local laminar profile from a pressure-driven flow, the separation efficiency is intermediate between regular CEC and CLC. The major advantages of pCEC over regular CEC are the stability of the mobile phase flow due to bubble suppression by the application of pressure at the column inlet, the increased speed of separation, and on some instruments the possibility to apply gradient elution (19).

Capillary columns packed with spherical octadecylsilica (ODS) particles are commercially available and are most frequently used in pCEC. Because the same type of packing material as in HPLC is used, the chromatographic behavior of these columns in pCEC is in good agreement with that in HPLC (20–25). Because of its good precision, high separation efficiency, and resolution, pCEC has the potential to become a useful separation tool for complex mixtures (26–29). Also for fingerprint development, it has already proven its applicability (24,30,31).

Although the chromatographic properties of some polymers formed from the glycidyl esters of methacrylic acid were already evaluated in 1978 by Lukas et al. (32), molded monolithic columns manufactured from methacrylates were originally introduced for HPLC analysis by Svéc and Fréchet (33) in the

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early 1990s. As an alternative to the conventional packed columns, capillary monolithic columns have attracted great attention in recent years due to their simple preparation procedure and better hydrodynamic properties (34,35). Monolithic columns in CEC also solve some problems associated with packed columns, i.e., the necessity of frits to retain the stationary phase inside the capillary. These frits are often the cause of bubble formation, adsorption of the sample, or irreproducible results. These are some reasons why the monolithic stationary phase was considered as an alternative phase.

In this study, pCEC has been evaluated to establish fingerprints of *G. biloba* using conventional packed columns, both made in-house and commercial, and polymethacrylate-based monolithic columns. Monolithic columns are also potential candidates for fingerprint development because they allow the separation of complex mixtures (36–38). The flavonoids present in the extract were chosen as active compounds of focus because the terpene lactones poorly absorb in the UV range. The structures of some flavonoids are given in Figure 1.

Fingerprints of herbal extracts can be used for several purposes, such as in quality control to identify herbal samples, to classify or discriminate samples (for instance, based on their origin or species), or for multivariate calibration (for instance, to link a fingerprint to cytotoxic or antoioxidant activity) (39).

In a first instance, it was evaluated whether pCEC as separation technique allows developing suitable fingerprints of *G. biloba* on the commercial packed column. A second part of this study focuses on the development of a fingerprint on an in-house fabricated packed column and on a monolithic column. It allows for comparison of the performances of the three stationary phases and to indicate whether the commercial column is the most suitable column for the considered fingerprint analysis or that both other columns are possible alternatives.

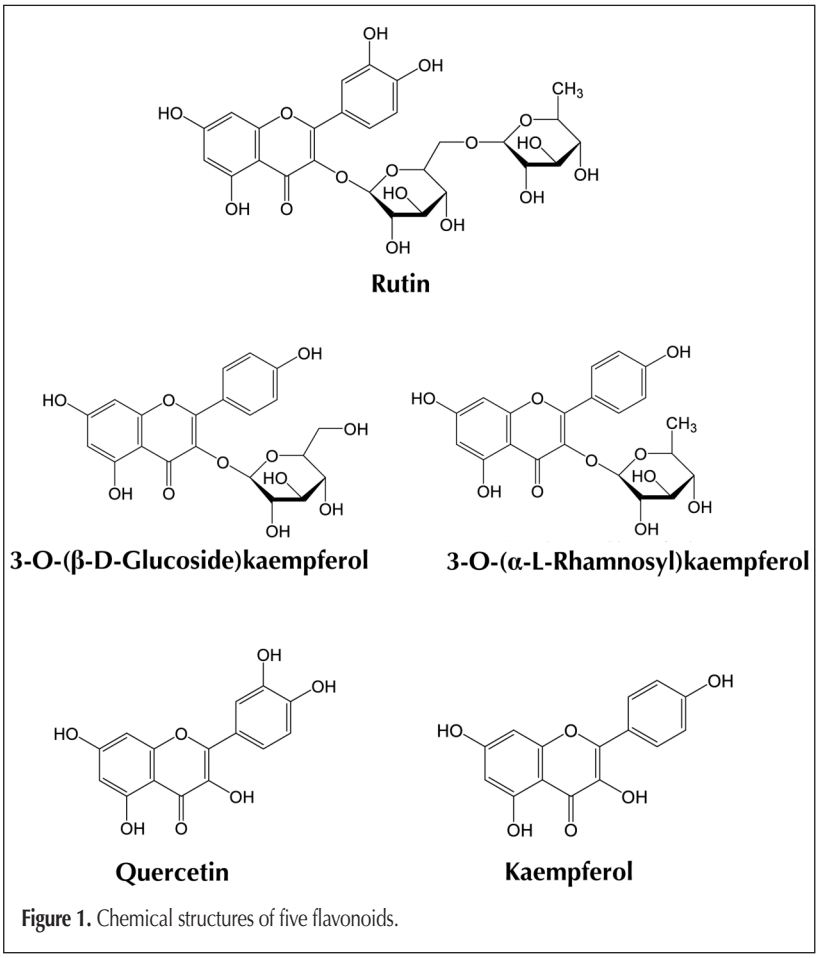

Figure 1. Chemical structures of five flavonoids.

Materials and Methods

Apparatus

pCEC was performed on a Trisep-2100 capillary electrochromatography system (Unimicro Technologies, Pleasanton, CA), composed of a binary gradient solvent delivery module, a high voltage power supply (+30 and –30 kV), a Valco six-port injection valve, and a UV–vis variable wavelength detector equipped with a cell for on-column detection and an Unimicro Trisep™ workstation 2003. A mobile phase was generated by merging two solvent flows in a mixer and then entering the Valco six-port injection valve. Samples were injected into the injection valve and introduced in a 1-µL sample loop. They then are carried to the four-port split valve by the mobile phase flow. After splitting in the four-port valve, a fraction of the flow entered the capillary column at a maximum back pressure of 74 bar.

Pressure was applied to the column inlet during the separation. The pump flow rate was set on 0.05 mL/min with the packed columns and on 0.1 mL/min with monolithic columns. However, because a constant back pressure was obtained in the experiments, the flow rate over the column is almost the same regardless of the flow rate that is set at the pumps, i.e., only the fraction going to the waste varies. Detection was performed at 360 nm. Negative voltages ranging between 2–10 kV were applied on the column outlet for the experiments on particle-based columns. Positive voltages were applied on the monolithic column outlet, also ranging between 2 and 10 kV.

Chemicals and reagents

Ginkgo biloba extract (GBE 20030106) was provided by the TSI Natural products company (Xuzhou, China). The standards, 3-O-(6''-O-(α-L-rhamnosyl)-β-D-glucosyl) quercertin (rutin), kaempferol, and quercetin were purchased from Sigma (St. Louis, MO), whereas 3-O-(β-D-glucoside)kaempferol and 3-O-(α-L-rhamnosyl) kaempferol were from Extrasynthese (Genay, France).

To prepare the mobile phases, sodium dihydrogen phosphate monohydrate (NaH_2PO_4), phosphoric acid (H_3PO_4), and formic acid (HCOOH) from Merck (Darmstadt, Germany) were used. Trifluoroacetic acid (TFA) was from Sigma Aldrich. The organic modifiers, acetonitrile (ACN) and methanol (MeOH) (both HPLC-grade), were purchased from Fisher (Leicestershire,

Loughborough, U.K.). Milli-Q water was obtained with the Milli-Q water purification system (Millipore, Molsheim, France). To synthesize monolithic columns, butyl methacrylate (BMA), ethylene dimethacrylate (EDMA), and 2-methacryloyloxyethyltrimethylammoniumchloride (META), all from Sigma Aldrich, and α,α'-azoisobutyronitrile (AIBN) from Fluka Chemie (Buchs, Switzerland) were used. The pH was measured with an Orion pH meter model 520 A (Boston, MA). Five-µm LiChrospher RP-C_{18} stationary phase particles were from Merck.

Sample and buffer solution preparation

0.49 g of dried extract was weighed into a 100.0-mL volumetric flask, and 50 mL MeOH was added. The mixture was shaken, ultrasonicated at room temperature for 30 min, diluted to volume with methanol, and homogenized. Sample solutions were filtered through a 0.2-µm filter (Macherey-Nagel, Duren, Germany) before injection.

5 mM $NaH_2PO_4.H^2O$ solutions were prepared in Milli-Q water, and phosphoric acid was used to adjust the pH to 2.8. By preparing the buffer as thus, its final concentration deviates somewhat from 5 mM.

Columns

pCEC experiments were performed with three types of columns, all having a 20-cm stationary phase section and 45-cm total length. Method development was mainly executed with the commercially available packed columns (100 µm i.d. × 375 µm o.d.) containing 3 µm octadecyl silica particles (Unimicro Technologies). The in-house packed columns (100 µm i.d. × 375 µm o.d.) contained 5 µm LiChrospher RP-C_{18} silica particles. The monolithic columns were synthesized as described further.

In-house column packing

Capillary columns were packed in-house using a slurry-based method. As stationary phase, 5 µm LiChrospher RP-18 particles were used. The stationary phase (35 mg) was suspended in 1 mL MeOH to from a slurry. Before packing, a temporary frit was created by tapping one end of the capillary into 10 µm silica gel powder and sintering it in a gas flame. The slurry was pumped through a slurry reservoir into the capillary at a pressure of 600 bar generated by an air-driven liquid pump (Haskel, Burbank, CA). A mechanical shaker was put on the slurry reservoir to prevent precipitation of the particles. After the capillary was packed for the desired length, the pressure was reduced to 400 bar, which was then kept for at least 2 h. The pressure was further released to 200 bar, and two permanent frits, 20 cm from each other, were sintered with a capillary burner (Capital HPLC, Broxburn, West Lothian, Scotland). The temporary frit was removed, and the column connected to an HPLC pump to rinse away the residual stationary phase behind the outlet frit. Finally, a detection window was burned 2–4 mm after the outlet frit through heating for 5 s with a capillary burner.

Table I. The Weight Compositions of the Monomers and Pore-Forming Solvents for the Synthesized Monolithic Columns

		Monomer			Pore-forming solvents		
	Column	BMA	EDMA	META	1,4-butanediol	1-propanol	water
Low density	L-a	11.80	7.60	0.40	19.50	55.00	5.50
	L-b	11.80	7.60	0.40	17.80	55.00	5.50
	L-c	13.60	7.60	0.40	19.50	53.20	5.50
High density	H-a	23.50	16.10	0.40	19.50	36.00	5.50
	H-b	23.50	16.10	0.40	17.80	36.70	5.50

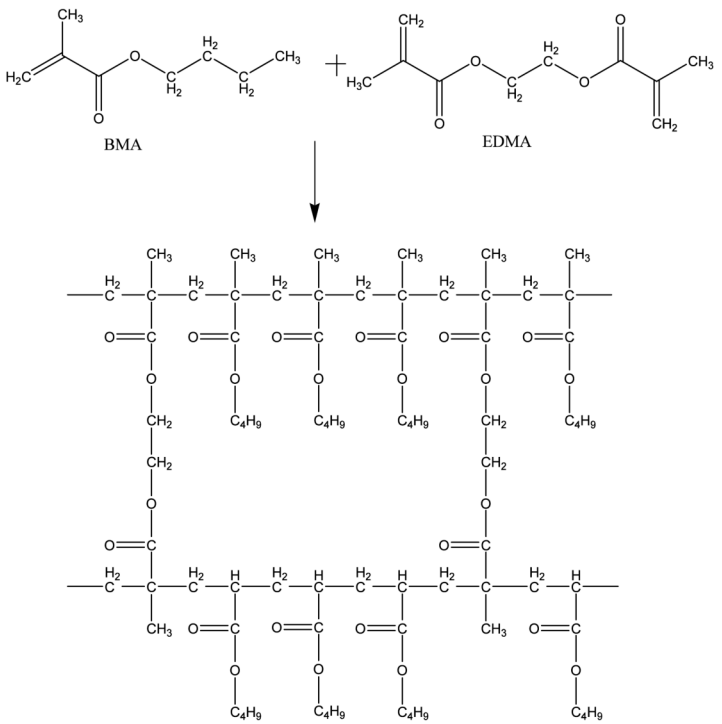

Figure 2. Copolymerization of butyl methacrylate (BMA) with ethylene dimethacrylate (EDMA).

Monolithic column synthesis

A copolymerization approach was used (Figure 2) to synthesize the monolithic columns. It concerns a thermally induced reaction in a polymerization mixture, consisting of a bulk monomer, a cross-linker, pore-forming solvents, and a charge-providing monomer. Prior to the polymerization, 3-(trimethoxysilyl)propyl methacrylate was used to modify the capillary inner-wall surface. As bulk monomer BMA was used together with EDMA as a cross-linker and a positively charged monomer (META) to generate the EOF. The pore-forming solvents were mixtures of 1,4-butanediol, 1-propanol, and water with varying ratios. As initiator, AIBN was included.

The compositions of the polymerization mixtures were according to a recipe developed by Eeltink et al. (35). Two kinds of polymeric

material were created: high-density material prepared with 40% (m/m) monomers mixture and 60% (m/m) pore-forming solvent, and low-density material with a 20:80 (m/m) ratio of monomer/pore-forming solvent. The detailed compositions of the monomer mixtures and pore-forming solvents are shown in Table I.

After mixing the components, the polymerization mixture was ultrasonicated for 10 min. The degassed mixture was introduced into a 45-cm fused-silica capillary with a syringe, and a 20 cm-long segment was filled. The ends of the capillary were plugged with a piece of rubber and placed into an oven at 70°C to polymerize for 20 h.

Results and Discussion

Method development was mainly performed on the commercial column, containing 3-µm ODS particles. After optimization of the gradient type, pH, concentration of the buffer, and applied voltage, the conditions were applied on the in-house packed column with 5-µm particles because a similar chromatographic selectivity is expected. For the monolithic stationary phases, the gradient was re-optimized.

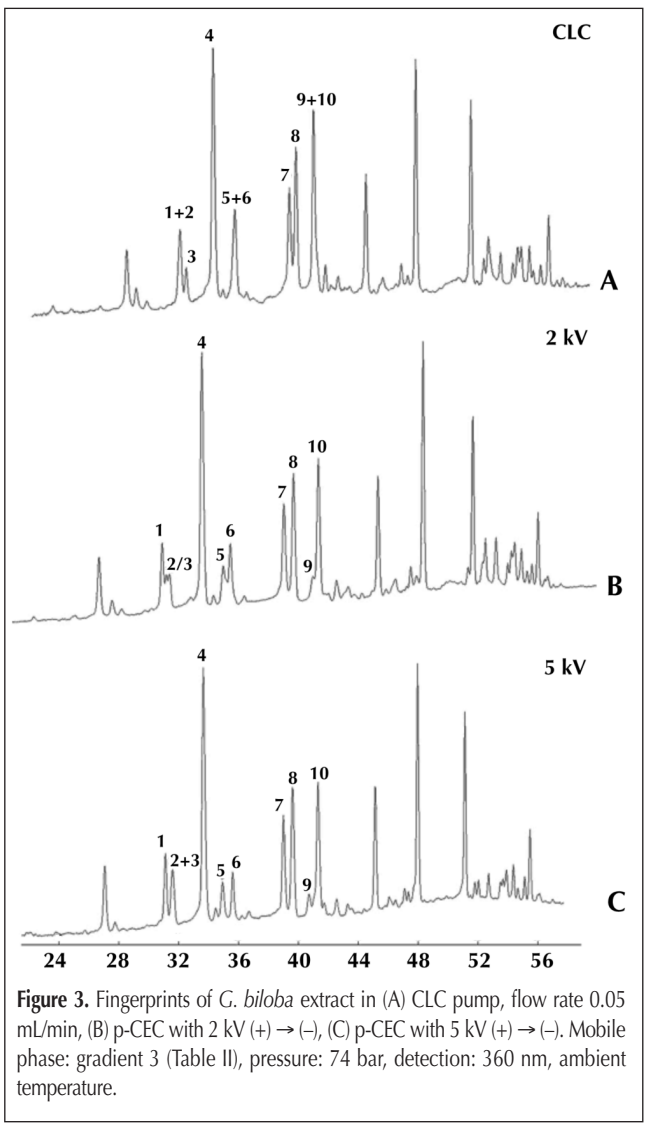

Figure 3. Fingerprints of *G. biloba* extract in (A) CLC pump, flow rate 0.05 mL/min, (B) p-CEC with 2 kV (+) → (–), (C) p-CEC with 5 kV (+) → (–). Mobile phase: gradient 3 (Table II), pressure: 74 bar, detection: 360 nm, ambient temperature.

Fingerprint development on packed columns with 3-µm particles

Optimization of the elution mode

Optimization of the gradient conditions was only performed in the CLC mode. An acetonitrile-based mobile phase is employed in most CEC applications because of the high dielectric constant and low viscosity of ACN (40,41). Preliminary studies were performed in isocratic elution mode with binary mobile phases consisting of a 2 mM phosphate solution at pH 2.8 and various volume fractions of ACN (10%, 15%, 20%, and 40%). However, because of the wide hydrophobicity of the analyzed compounds, a complete separation of all herbal compounds under isocratic conditions was not possible. Gradient elution systems were therefore developed to overcome the polarity problem. Several linear and multisegmented gradients were tested (Table II). Gradients 1, 2, 4, 5, 6, and 7 did not allow obtaining acceptable resolutions, analysis times, or stable baselines. However, significant improvements compared to isocratic elution were seen with gradients 3 and 8, and they were chosen for further study (Figure 3A–4A).

Effect of concentration and pH of the buffer

The study on the effect of buffer concentration and pH was performed with an applied voltage of 2 kV. The concentration of the electrolyte was varied from 2 to 10 mM. In each solution, the pH was adjusted to 2.8 with phosphoric acid. It was observed that with a 2 kV applied voltage, retention times decreased slightly with an increase in the phosphate concentration. However, a high ionic strength (10 mM) of the mobile phase caused an unstable baseline and was therefore not preferred. A buffer concentration of 5 mM was finally selected for further experiments.

The change of pH affects the number of dissociated residual silanol groups on the capillary wall surface and on the stationary-phase particles, as well as the surface charge density and the zeta potential. Therefore, the pH of the mobile phase is an important factor to affect EOF in pCEC. Generally, a high pH value is chosen in pCEC to maintain a high EOF (42,43). The separation

Table II. Gradients Tested

Gradient*	Time (min)	% A
1	0–40	14
	40–45	14–25
	45–100	25
2	0–35	10–18
	35–80	18–40
3	0–35	10–20
	35–60	20–36
4	0–35	15–25
	35–60	25–45
5	0–60	14–28
6	0–80	14–30
7	0–80	12–30
8	0–80	12–36

* Mobile phase: A = ACN and B = 2 mM NaH_2PO_4 (pH 2.8).

mechanism in CEC for neutral analytes is partitioned between the stationary and the mobile phase, as in conventional HPLC. However, the mechanism for charged species is more complicated because both partition and electrophoretic mobility will contribute to the separation. The flavonoids in *G. biloba* extracts are glycosides, mostly derived from three types of aglycones (kaempferol, quercetin, and isorhamnein). A relatively high selectivity is required to separate these flavonoids, which have relative similar structures. The effect of the pH was studied between 2.8 and 5 using the previously selected 5 mM NaH_2PO_4 electrolyte concentration. Resolution increased slightly when the pH decreased. This can be attributed to a decreased EOF at lower pH values. The best separations were seen at the lowest pH of 2.8. Therefore, pH values above 5 were not studied. Taking into consideration the resolution, pH 2.8 was used to further optimize the separation conditions.

Effect of the applied voltage

The effect of the applied voltage was studied using gradients 3 and 8 at 2 kV, 5 kV, and 10 kV. With gradient 3, compared to CLC, pCEC brings some improvement in resolution and analysis time. As shown in Figure 3C, resolutions of 1.5 between peaks 5–6 and 7–8 were achieved when a 5 kV voltage was applied, whereas these peak pairs were co-eluting or overlapping using CLC. A better separation of peaks 9–10 also can be observed while they co-elute in CLC. A possible explanation for the observed could be related to the properties of the applied instrument. It uses a back-pressure regulator, which fixes the maximal back pressure and thus the total flow over the column. This means that the electro-driven and pressure-driven flows are not additive in all circumstances. When the back-pressure limit is reached, the maximal flow over the columns also is. Different conditions leading to this back-pressure may result in different contributions of the pressure-driven and electro-driven fractions to the total flow (36). Thus, by changing from CLC to pCEC and by considering different voltages, the fractions of the electro-driven and pressure-driven flows over the column might change. It also means that the relative importances of the chromatographic and electrophoretic separation mechanisms of a given molecule may change, which could result in the observed differences between the CLC chromatogram and pCEC electrochromatogram.

With gradient 8, peaks 5–6, 7–8, and 9–10 were only partially separated with an applied voltage of 5 kV (Figure 4B). Therefore, this gradient was abandoned for further experiments. When 10 kV was applied, an unstable baseline was obtained in both gradients. Therefore, gradient 3 and 5 kV voltage were selected as the best conditions.

Fingerprints at finally selected conditions

A mobile phase comprising acetonitrile and 5 mM NaH_2PO_4 buffer at pH 2.8 was used in gradient elution mode (gradient 3) with an applied voltage of 5 kV, 74 bar pressure, ambient column temperature, 360 nm UV detection, and a pump flow rate of 0.05 mL/min. At these conditions, 45 peaks can easily be distinguished in less than 60 min, and baseline separation of the major *G. biloba* flavonoids was achieved (Figure 5). An electrochromatogram of a mixture of the five flavonoid standards was also shown for comparison.

Fingerprints on the in-house packed column

Using the selected optimal conditions stated earlier, a self-packed column (5 µm ODS) was tested, and the resulting electrochromatogram is shown in Figure 6. For particle-packed columns, the efficiency is generally particle size-dependent and increases as the particle size decreases. Using the in-house prepared column, a similar elution pattern as with the commercial column was observed. However, compared with the latter 3-µm particle-based column, peak shapes, resolutions, and thus efficiency were worse on the column containing 5-µm particles, which was expected. Moreover, many peaks overlapped, and they were generally broader. Therefore, this 5-µm column cannot be considered very suitable for *G. biloba* analysis. Further optimization of the analysis conditions was not considered because of the less good efficiency.

Fingerprints on the methacrylate ester-based monolithic columns

The majority of pCEC separations with monolithic columns reported to date were performed in the reversed-phase mode. Under these conditions, the hydrophobicity of the stationary phase partly determines the selectivity of the separation, and retention can be controlled by adjusting the hydrophobicities of surface and mobile phase (33). In this study, methacrylate-based monolithic columns were prepared. The polymerization reaction of BMA with EDMA is shown in Figure 2. By using BMA in the polymerization mixture, a hydrophobic butylgroup was introduced to the stationary phase, which enables a reversed-phase separation mechanism. The compositions of polymerization mixtures for columns L-a, L-b, H-a, and H-b (Table I) were selected following a recipe developed by Eeltink et al. (35), whereas that for L-c was a somewhat modified L-a composition. With simple linear and multisegmented gradients (Table II), the five types of monolithic columns were tested. Because a positively charged monomer (META) was incorporated in the mono-

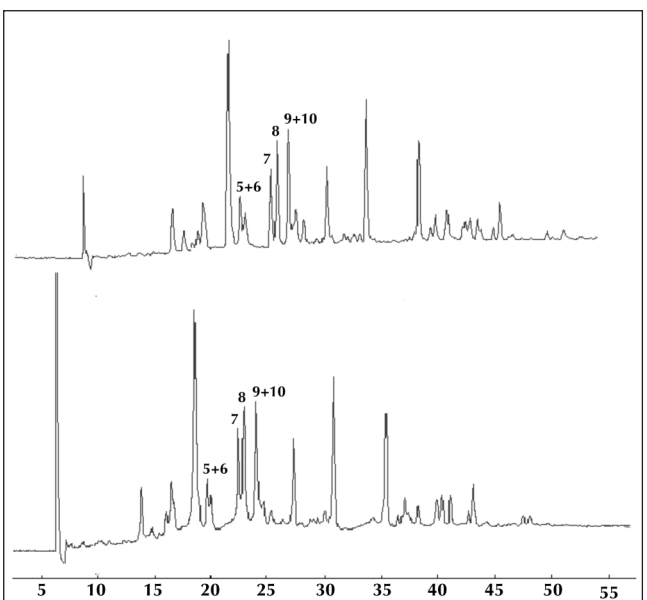

Figure 4. Electrochromatograms of *G. biloba* extract using gradient 8, (A) CLC pump, flow rate: 0.05 mL/min, (B) pCEC with applied voltage: 5kV (+) → (–), pressure: 74 bar, detection: 360 nm, ambient temperature.

lithic columns, a positive voltage ranging between 2–10 kV was applied at the column outlet in order to have an EOF directed towards the detection outlet. Thus, the EOF direction with these monoliths, i.e., from cathode to anode, is opposite to the regular EOF direction.

Effect of pore size and mononer ratio

Most authors studying monolithic columns hold the opinion that pore size plays a very important role in the retention and separation of analytes. As reported by Eeltink et al. (35), an increase in the 1,4-butanediol fraction relative to 1-propanol increases the polarity of the mixture and not only results in a strong increase in the average pore size but also in an increase of the globule dimensions and the polymeric agglomerates.

When testing the two types of low-density columns (L-a and L-b), no retention was observed, even when the weight fraction of 1,4-butanediol was reduced from 19.5 (L-a) to 17.8 % (L-b). Only one peak was observed with a retention time of approximately 5 min. Pore sizes that are too large or an insufficient amount of bulk monomer (BMA) and hence too few C_4 groups on the stationary phase may be the cause of this lack of retention.

The tested high-density columns H-a and H-b had the same 1,4-butanediol contents as L-a and L-b, respectively. However, these stationary phases displayed a bad permeability, probably due to the smaller pore sizes. They could not even be rinsed properly. A low density monolithic column (L-c), with the same 1,4-butanediol fraction as L-a but with an increased weight fraction of BMA (13.6% m/m) and a decreased 1-propanol fraction (53.2% m/m), showed a remarkable improvement in retention. Increasing slightly the weight fraction of the monomer (BMA) appeared to improve the retention and separation considerably. This might be explained by the increased number of C_4 groups introduced to the monolith. The electrochromatogram on this monolithic column L-c is shown in Figure 6.

The separation mechanism on methacrylate ester-based monolithic columns is as on traditional reversed-phase particulate packed columns. Therefore the same gradients were tested as in the study of the packed columns. Taking into account acceptable resolutions and peak shapes, the L-c column and gradient 4 were chosen as the best column and the optimal elution conditions, respectively. Although the resolution and selectivity were less ideal than with the commercial column, the distribution of peaks over the entire chromatogram is reasonable and starts earlier than on the commercial column. However, the peak broadening indicates that the polymerization-mixture composition still needs to be further optimized (i.e., efficiency should be improved). This is confirmed by the results on the other tested low- and high-density monoliths. Therefore, we believe that using a further optimized monolithic column to establish the fingerprint might be an interesting approach. In order to improve resolution and selectivity, various amounts of monomers and pore-forming solvents should be studied, for instance, in an experimental design approach (44). However, such systematic stationary-phase optimization is outside the scope of the actual paper and will be the subject of a next study.

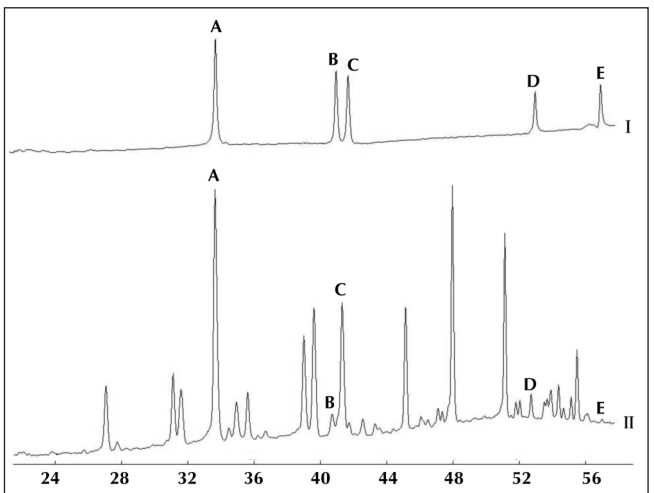

Figure 5. (I) Standard electrochromatogram of five flavonoids, (II) Fingerprint of *G. biloba* extract in p-CEC. Mobile phase: see text; gradient 3 (Table II), voltage: 5 kV(+) → (–), pressure: 74 bar, detection: 360 nm, ambient temperature. Peaks: (A) Rutin, (B) 3-O-(β-D- rhamnosyl) kaempferol, (C) 3-O-(β-D-glucoside)kaempferol, (D) quercetin, and (E) kaempferol.

Comparing the fingerprints

The best results obtained on the commercially packed, the in-house prepared, and the monolithic columns, respectively, can

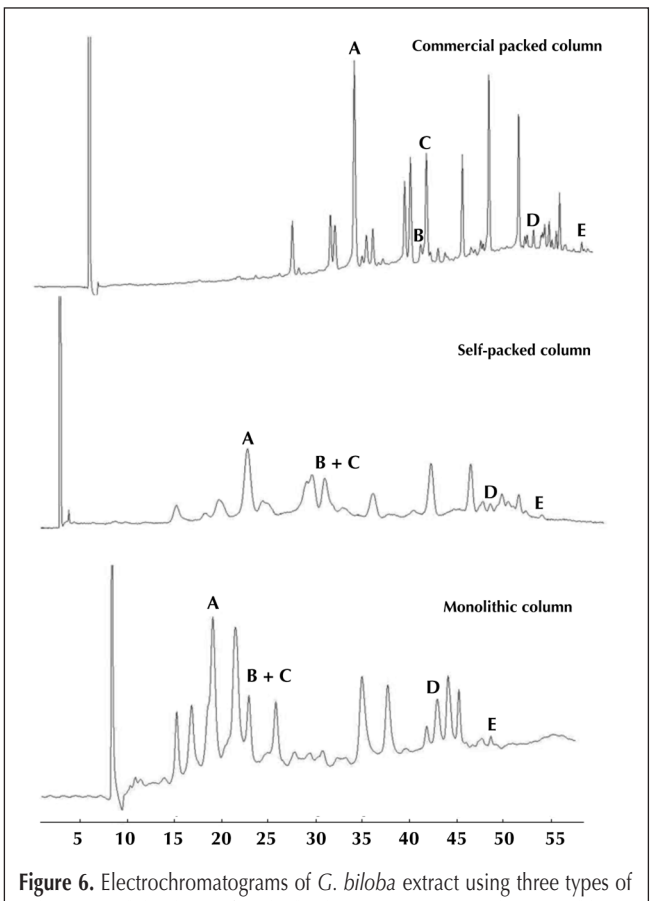

Figure 6. Electrochromatograms of *G. biloba* extract using three types of columns. Mobile phase of packed columns (see text): gradient 3 (Table II), pump flow rate: 0.05 mL/min, applied voltage: 5 kV(+) → (–), mobile phase of monolithic column (see text): gradient 4 (Table II), pump flow rate: 0.1 mL/min; applied voltage: 5 kV(–) → (+). Peaks: (A) Rutin, (B) 3-O-(β-D-rhamnosyl) kaempferol, (C) 3-O-(β-D-glucoside)kaempferol, (D) quercetin, and (E) kaempferol. Other conditions as stated in Figure 5.

be seen in Figure 6. A total of at least 45, 22, and 23 isolated peaks, respectively, were observed. Due to the larger particle size and the limitations of the packing equipment, poorer resolution and selectivity was observed for the in-house packed column. For the monolithic column, resolution and selectivity were of similar quality to the self-packed column. However, here is still possibility for improvement by optimization of the polymerization-mixture composition as discussed earlier. Such optimization might make monolithic columns an alternative for the commercial packed column in the development of fingerprints. In summary, the commercial column packed with 3-µm particles was the most powerful separation tool tested in the analysis of *G. biloba* extracts resulting in a better resolution and efficiency.

Repeatability

The commercial column was used to investigate the precision of the fingerprint measurements. The highest peak in the electrochromatogram corresponds with rutin and was used as internal standard for repeatability studies. The injection precision was determined by six replicate injections of the same sample solution. The relative standard deviations (RSD) of the relative retention times and relative peak areas of the standards were below 1.34% and 4.32%, respectively. However, the precision of the absolute retention times and peak areas was not as good (i.e., RSD below 2.21% and 7.64% were obtained for the absolute retention times and peak areas of the standards, respectively). The higher RSD of the absolute peak areas can be attributed to the instrumental set-up where the sample flow is split after injection (only a fraction goes over the column) and where the effective flow over the column is determined by a back-pressure regulator.

The inter-day precision was evaluated from measurements during six days. During this period, the sample solution was stored at room temperature. The RSD of the relative retention time and relative peak area of the standards were below 3.75% and 7.66%, respectively. Therefore, the developed pCEC fingerprint using a commercial packed-column can be considered adequate to be used in the further quality control of *G. biloba* extracts (e.g., to classify, identify, or model a given activity of these samples. Moreover, prior to such actual data treatment the fingerprints are pretreated, which involves here an alignment of the corresponding peaks in the different chromatograms.

Conclusion

The results of this study indicated that pCEC is a suitable analytical technique for the development of fingerprints and offers a high separation efficiency, resolution, and an acceptable repeatability. Compared with CLC, the introduction of an electrical field in pCEC may somewhat improve the separation of components. A comparison between two types of conventional ODS-packed columns and methacrylate-based monolithic column was made for the fingerprints of a *G. biloba* extract. Investigation of the influences of buffer composition, pH, and applied voltage in gradient elution mode allowed to derive optimal analysis conditions. The commercial particle-based column proved to be most suitable for the fingerprint development. The monolithic column might present an alternative, but further optimization of the polymerization-mixture composition is necessary to obtain columns with a better efficiency. This optimization will be the subject of a further study.

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