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Research Article

Two-dimensional strong cation-exchange liquid chromatography/reversed-phase pressurized capillary electrochromatography for separation of complex samples

A 2-D separation platform was constructed using micro strong cation-exchange liquid chromatography (μ-SCXLC) and reversed-phase pressurized capillary electrochromatography (RP-pCEC) for the analysis of complex samples. Samples were fractionated by the first-dimension μ-SCXLC with a linear solvent gradient and then injected into the second-dimension RP-pCEC for further separation. The μ-SCXLC/RP-pCEC 2-D system with three separation mechanisms, namely strong cation-exchange, reversed-phase chromatography and electrophoresis, provided high selectivity, high resolution and high peak capacity compared to one-dimensional chromatographic approaches. Separation effectiveness of this 2-D system was demonstrated by the analysis of different kinds of complex samples, such as traditional Chinese medicine *Cortex Phellodendri*, bovine serum albumin (BSA) tryptic digest and real serum tryptic digest. A theoretical peak capacity of approximately 1200 was achieved, which proves its promising potential for the separation and analysis of complex samples.

Keywords: Complex samples / pCEC / Pressurized capillary electrochromatography / Strong cation-exchange chromatography / Two-dimensional chromatography
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1 Introduction

For the purpose of analyzing complex samples and searching for traces of possible bioactive substances, powerful analytical tools with higher resolution, higher peak capacity and higher detection sensitivity are clearly required. However, it is often difficult to completely separate all components in a complex sample using one-dimensional (1-D) chromatography approach. A possible solution to this problem is the use of multidimensional (MD) system [1], which can provide higher selectivity, higher peak capacity and higher resolution. Giddings demonstrated that the total peak capacity of MD systems is the product of the peak capacities in each independent dimension and the resolution is equal to the square root of the sum of the squares of the resolution for each dimension, provided the separation mechanisms of each are orthogonal.

The development and employment of column-based MD chromatography has been increasing due to their greatly enhanced resolving power as compared with one-dimensional (1-D) approaches. Jorgenson and co-workers [2–4] have developed a number of coupled column separation schemes, based on different column sizes in the first dimension and sample loops or on-column focusing, for performing comprehensive MD separations. The column-based 2-D chromatography is becoming more and more popular for the separation of many complex samples, such as nature products [5, 6], peptide mixture of digested protein [7, 8], proteomics [9, 10]. So far, the most popular 2-D modes are 2-D HPLC [5], 2-D CE [11] and 2-D HPLC-CE [12].

Capillary electrochromatography (CEC) is a miniaturized, hybrid separation technique that combines micro-high performance liquid chromatography (μHPLC) and CE. Column efficiency in CEC is usually much higher than that in HPLC with an identical column, since electroosmotic flow (EOF) – the driving force in CEC – generates a plug-like flow profile in contrast to the parabolic flow profile in HPLC. Therefore, CEC has both high selectivity as in HPLC and high efficiency as in CE. However, the problems and

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Abbreviations: **μHPLC**, micro-high performance liquid chromatography; **μ-SCXLC**, micro strong cation-exchange liquid chromatography; **MD**, multidimensional; **pCEC**, pressurized capillary electrochromatography; **TCM**, traditional Chinese medicine

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difficulties associated with the lack of proper instrumentation and in-house column fabrication prevent CEC from becoming a mainstream separation technique. In addition, the high applied voltage across the column in ''pure'' CEC format often causes Joule heating, which leads to bubble formation that can result in dryout of the column and disruption of the current. Pressurized CEC (pCEC) with EOF combining hydraulic pressure as its driving force has gained more attention. In pCEC, the retention mechanism for neutral compounds is essentially based on chromatographic partition. However, for charged compounds, both chromatographic partition and electrophoretic mobility contribute to the separation mechanism. Therefore, high efficiency, high resolution and high peak capacity can be achieved in pCEC. If pCEC can be used in MD technology, it is obvious that the separation power of the system can be increased. In a previous study, we reported some preliminary results on the analysis of the extract of a traditional medicine by the combination of capillary LC with pCEC [13].

In this work, a new 2-D system, named as μ-SCXLC/RP-pCEC, was established for complex sample analyses. This approach combines the advantages of pCEC with the advantages of μ-SCXLC, resulting in a platform with higher sensitivity, higher resolution and higher peak capacity. The performance of this new platform was evaluated by different kinds of complex samples, such as traditional Chinese medicine (TCM) (*Cortex Phellodendri*), tryptic digest of bovine serum albumin (BSA) and real serum.

2 Materials and methods

2.1 Reagents and materials

Methanol and acetonitrile (ACN) were HPLC grade from Tedia (Fairfield, OH, USA). Water was MilliQ grade (Millipore, Bedford, MA, USA). Chemicals were analytical grade reagents from Sinopharm Chemical Reagent (Shanghai, China). All solutions were filtered through 0.22-μm filters.

The *Cortex Phellodendri* was bought from drugstore of Leiyunshang (Shanghai, China). BSA was of 99.0% purity from Shanghai Biocolor BioScience & Technolgy (Shanghai, China). Trypsin was bought from Shanghai Pufei Bio-Technology (Shanghai, China). The human sera, obtained from 20 healthy humans, were provided by Shanghai Ruijin Hospital (Shanghai, China).

2.2 Sample preparation

Extracts of *Cortex Phellodendri*: *Cortex Phellodendri* was dried and ground into powder. 0.5 g of powder was mixed with 10 mL of methanol-hydrochloric acid (99:1, v/v) for 12 h and then extracted by ultrasound for 30 min. The extract was filtered through a 0.22-μm membrane and stored at 4°C in the absence of light.

Bovine serum albumin (BSA) tryptic digest: BSA powder was dissolved in 100 mmol/L NH_4HCO_3 into 25 mg/mL and heated to boiling for several minutes. Then, 10 mg/mL trypsin was added to the BSA solution with a ratio of 45:1 BSA/trypsin. The mixture was incubated at 37°C overnight. The digest was filtered through a 0.22-μm membrane and stored at 4°C for subsequent experiments.

Human serum tryptic digest: Protein concentration of serum sample was measured by the Bradford assay using BSA as standard. The sample was diluted four times with 100 mmol/L NH_4HCO_4 into 20 mg/mL and heated to boiling for several minutes. Then, 10 mg/mL trypsin was added to the solution with a ratio of 45:1 protein:trypsin. The mixture was incubated at 37°C overnight. The digest was filtered through a 0.22-μm membrane and stored at 4°C for subsequent experiments.

2.3 μ-SCXLC/RP-pCEC 2-D system

The first dimension was micro strong cation-exchange liquid chromatography (μ-SCXLC). Experiments were carried out by using a lab-made system, which consisted of a binary solvent gradient module with a six-port injector and an on-column variable-wavelength UV/VIS detector (Unimicro Technologies, Pleasanton, CA, USA). Capillary column with dimension of 10 cm × 300 μm id packed with 2.5-μm SCX stationary phase was a gift from TOSOH Bioscience Shanghai (Shanghai, China).

The second dimension was reversed-phase pressurized capillary electrochromatography (RP-pCEC). Experiments were performed on a TriSep^{TM}-2100 pCEC system (Unimicro Technologies), which consisted of a binary solvent gradient module, an on-column variable-wavelength UV/VIS detector, a high-voltage power supply (±30 kV), a six-port injector and a chromatography workstation for data acquisition and analysis. Capillary column with dimensions of 55 cm (effective length of 30 cm) × 150 μm id was packed with 3-μm C18 stationary phase (Global Chromatography, Suzhou, China). Detection wavelength was set at 230 nm. This 2-D system was an offline system equipped with a collector after the first-dimensional column.

2.3.1 BSA and serum digests analysis

The mobile phase for the first-dimensional separation was as follows: A: H_2O–acetonitrile–TFA (95:5:0.1, v/v/v); B: 0.5 mol/L NH_4Cl–acetonitrile–TFA (95:5:0.1, v/v/v); gradient: 0–5 min, 0% B; 5–40 min, 0–50% B; 40–50 min, 50–100% B; 50–60 min, 100% B; flow rate: 3 μL/min; inject volume: 1 μL; splitting ratio: 20:1. The effluent was collected every 5 min and totally 12 (BSA digest) or 10 (serum digest) fractions were collected. Each fraction was collected three times. The mobile phase for the second-dimensional separation was as follows: A: H_2O–acetonitrile–TFA (95:5:0.1, v/v/v); B: H_2O–acetonitrile–TFA (5:95:0.1, v/v/v); gradient: 0–5 min, 0–10% B; 5–35 min, 10–50% B;

35–45 min, 50–100% B; applied voltage: 3 kV; injection volume: 15 μL without splitting; flow rate for injection: 750 nL/min; flow rate for analysis: 400 nL/min.

2.3.2 *Cortex Phellodendri* analysis

The mobile phase for the first-dimensional separation was as follows: A: 5 mmol/L PBS–acetonitrile (95:5, v/v); B: 5 mmol/L PBS + 0.5 mol/L NH_4Cl–acetonitrile (95:5, v/v); gradient: 0–5 min, 0% B; 5–40 min, 0–50% B; 40–50 min, 50–100% B; 50–55 min, 100% B; flow rate: 3 μL/min; injection volume: 1 μL; splitting ratio: 20:1. The effluent was collected every 5 min and totally 11 fractions were collected. Each fraction was collected three times. The mobile phase for the second-dimensional separation was as follows: A: 20 mmol/L NH_4Cl–acetonitrile (95:5, v/v); B: 20 mmol/L NH_4Cl–acetonitrile (5:95, v/v); gradient: 0–5 min, 0–5% B; 5–30 min, 5–20% B; 30–40 min, 20–35% B; 40–55 min, 35–50% B; 55–65 min, 50–80% B; applied voltage: 5 kV; injection volume: 15 μL without splitting; flow rate for injection: 750 nL/min; flow rate for analysis: 400 nL/min.

3 Results and discussion

3.1 Development of 2-D system

In pCEC, the retention mechanism for neutral compounds is essentially based on chromatographic partition. However, for charged compounds, both chromatographic partition and electrophoretic mobility contribute to the separation mechanism. The dual mechanism of pCEC makes it suitable for both neutral and charged compounds and dramatically enhances the separation selectivity compared to stand-alone HPLC or CE. Besides, the hydraulic pressure from the binary solvent gradient delivery pumps superimposed on EOF can increase the overall separation speed while suppressing bubble formation. SCX chromatography was chosen for the first-dimensional separation. Separation of solutes is mainly determined by the electrostatic interaction, whereas in RP-pCEC the major separation mechanism is hydrophobic interaction and electrophoretic mobility. These two dimensions are orthogonal and it is suitable for constructing a 2-D separation system.

The schematic of the μ-SCXLC/RP-pCEC 2-D system is illustrated in Fig. 1. The mobile phase in the first dimension, driven by the binary solvent delivery module, flows through the first six-port injection valve. Sample is injected into the sample loop and then carried to the first splitting tee. About 5% of the sample and mobile phase flows into the capillary SCX column. Different fractions are collected in the collector and then injected into the loading loop. Then, the mobile phase of second dimension carries the sample fraction to the second splitting cross, where the grounding electrode is connected. There is a capillary restrictor connected to every splitting cross to control the flow rate in the column and maintain a constant pressure at the inlet of the column to suppress bubble formation. During the sample injection into the second dimension, the capillary restrictor was closed off so that the sample collected from the first dimension could be totally injected without splitting. After injection, the capillary restrictor was opened again, depending on the split ratio, about 1% of the mobile phase flows into the RP capillary column. The capillary column with a 2-mm coating burned off as detection

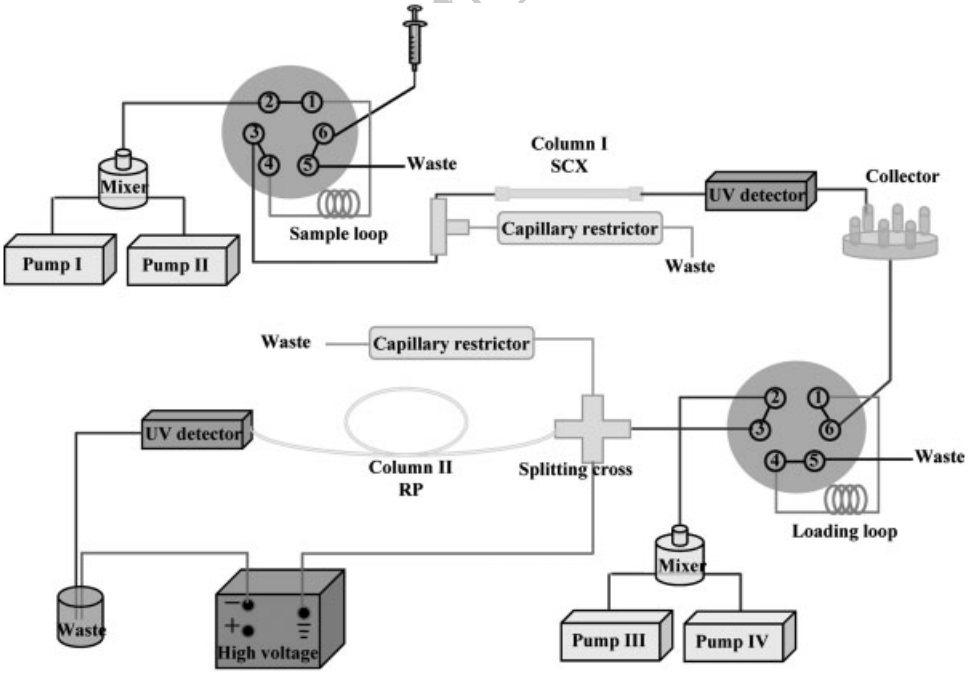

Figure 1. Schematics of 2-D μ-SCXLC/RP-pCEC.

window was installed into the UV/VIS detector. The waste vial contains both the RP capillary column outlet and the negative electrode. Thus, a high voltage can be applied to the column.

3.2 Separation of BSA and human serum digest

In the era of proteomics, both the structure and function of many proteins are identified via their peptide fragments generated by their enzymatic hydrolysis. This ''bottom-up'' or ''shotgun'' approach is one of the main directions in the proteome research. In spite of the high separation power of individual HPLC and CEC modes, for complete separation of complex peptide and protein mixtures present in body fluids, tissue extracts and enzymatic digests of large proteins, a combination of two or more complementary separation principles is necessary. Therefore, BSA and a real sample, human serum, were chosen to test this μ-SCXLC/RP-pCEC system.

Being a hybrid electrokinetic and chromatographic technique, CEC of peptides benefits from the high selectivity of numerous stationary phases developed for peptide separation by HPLC and from the low dispersion of electroosmotically driven mobile phase. pCEC is used in our system with the capacity to allow continuous gradient elution, where the mobile phase is driven by both EOF and pressurized flow, facilitating fine tuning in selectivity of neutral and charged molecules, has been shown to be more efficient for the separation of model oligopeptides compared to the isocratic CEC [14]. As shown in Fig. 2, the BSA digest and human serum digest can be separated under the gradient eluting conditions in RP-pCEC. The peak capacity was less than 100, still not enough for separation of the complex protein digest. Thus, MD peptide separations in proteomics are necessary.

While building a 2-D separation system, besides orthogonality and peak capacity, which should be considered, another key factor affecting the development of 2-D liquid chromatography is mobile phase compatibility in two dimensions. Although both anion and cation-exchange HPLC were viable options for peptide ion-exchange separations, cation-exchange HPLC was selected for the first dimension primarily on the basis of buffer pH compatibility with the subsequent reversed-phase dimension.

In order to match better for two-dimension mobile phase, 0.1% TFA solution and 5% acetonitrile was added to the mobile phase of first dimension. The elution salt for μ-SCXLC was NH_4Cl with a linear gradient. The peak capacity was about 30, which was much less than that in the second dimension.

Fractions from the first-dimension μ-SCXLC were collected every 5 min and totally 12 (BSA digest) or 10 (serum digest) fractions were collected. The 2-D chromatograms of these samples are given in Figs. 3 and 4. According to the retention mechanism of RP-pCEC (dual mechanism of reversed-phase chromatography and electrophoresis), substances in serum digest with lower hydrophobicity and lower mass-to-charge ratio eluted at the beginning of the gradient in the second-dimensional RP-pCEC.

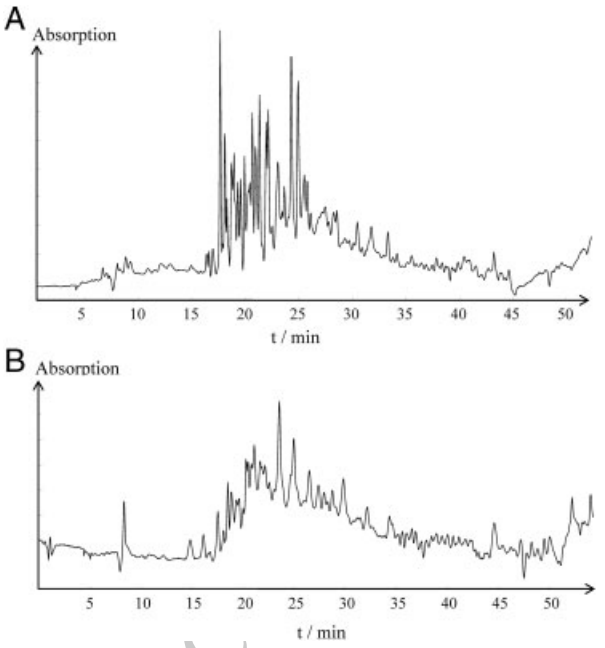

Figure 2. RP-pCEC chromatogram for digest of (A) BSA and (B) serum. Column: 55 cm (effective length of 30 cm) × 150 μm id capillary packed with 3-μm C18; mobile phase: A: H_2O–acetonitrile–TFA (95:5:0.1, v/v/v); B: H_2O–acetonitrile–TFA (5:95:0.1, v/v/v); Gradient: 0–5 min, 0–10% B; 5–35 min, 10–50% B; 35–45 min, 50–100% B; flow rate: 400 nL/min; applied voltage: 3 kV; detection: UV at 230 nm; sample: (A) BSA tryptic digests, (B) serum tryptic digests.

It is also shown that separation in the first dimension reduced the complexity of sample and the separation capability of the whole system was increased.

As in the separation of BSA digest, the peak width on the C18 column was approximately 0.5 min, estimated by the peptide insulin, representing a peak capacity of 100 in 50 min. The average peak width on the SCX column was approximately 1.6 min, representing a peak capacity of 30 in 50 min. Therefore, the peak capacity of this 2-D system could be as high as 3000 in theory according to Giddings [1]. The peak capacities in each dimension and the orthogonality both influence the practical peak capacity of 2-D system [15, 16]. Practical peak capacity (N_p) can be derived from the proposed geometric model using knowledge of the 2-D surface coverage, and is defined as $N_p = P_1 P_2 \sum bins / P_{max}$[16], where P_1 and P_2 are peak capacities of both separation dimensions, $\Sigma bins$ is the number of bins in the 2-D plot containing data points and P_{max} is the total peak capacity obtained as the sum of all bins. Thus, the practical peak capacity could be 2140. However, this practical peak capacity could be obtained only with frequent fraction collection in the first dimension (with

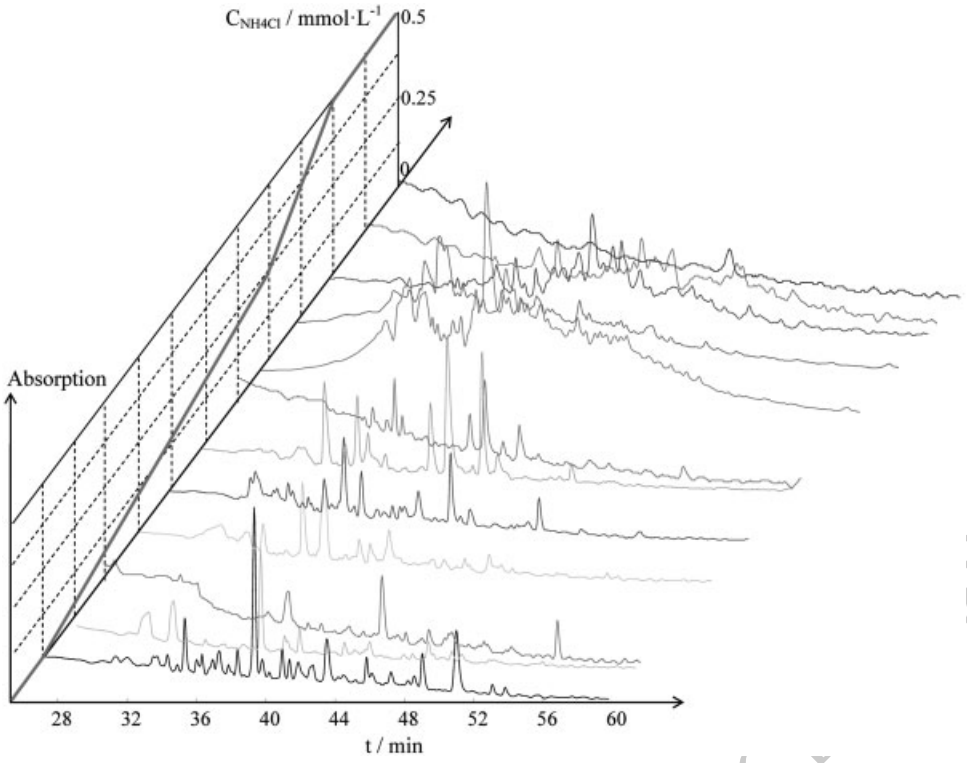

Figure 3. 2-D μ-SCX LC/RP-pCEC chromatogram for digest of BSA. I: Column: 10 cm × 300 μm id capillary packed with 2.5-μm SCX; mobile phase: A: TFA–H_2O–acetonitrile (0.1:95:5, v/v/v); B: TFA–0.5 mol/L NH_4Cl–acetonitrile (0.1:95:5, v/v/v); gradient: 0–5 min, 0% B; 5–40 min, 0–50% B; 40–50 min, 50–100% B; flow rate: 3 μL/min; sample: BSA tryptic digests. II: Column: 55 cm (effective length of 30 cm) × 150 μm id capillary packed with 3-μm C18; mobile phase: A: H_2O–acetonitrile–TFA (95:5:0.1, v/v/v); B: H_2O–acetonitrile–TFA (5:95:0.1, v/v/v); gradient: 0–5 min, 0–10% B; 5–35 min, 10–50% B; 35–45 min, 50–100% B; flow rate: 400 nL/min; applied voltage: 3 kV; detection: UV at 230 nm.

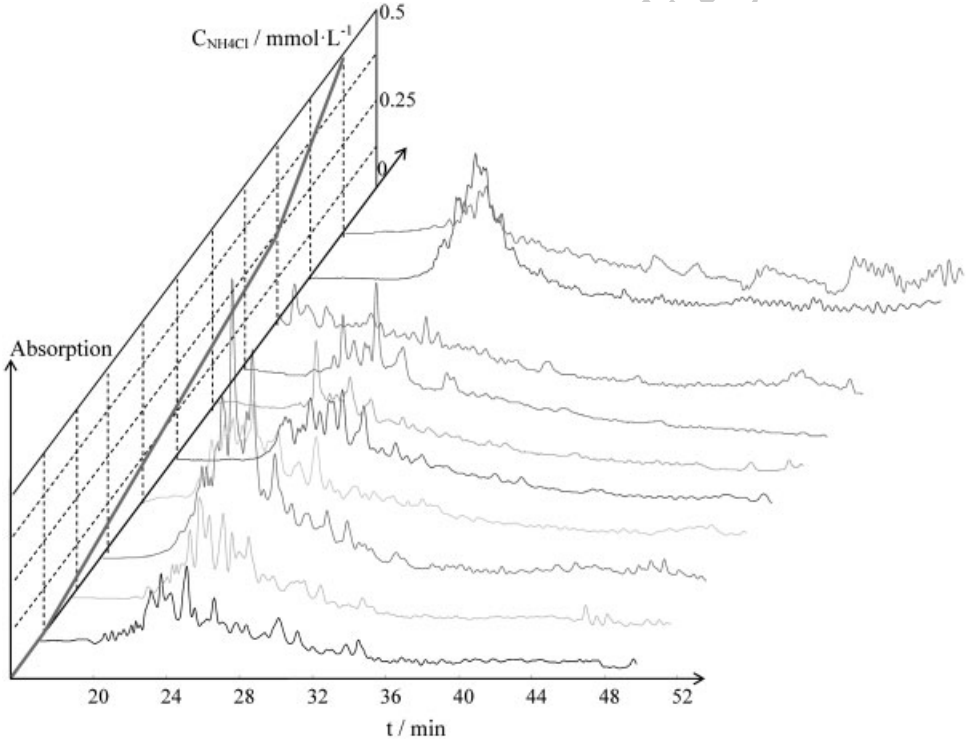

Figure 4. 2-D μ-SCX LC/RP-pCEC chromatogram for digest of serum. I: Column: 10 cm × 300 μm id capillary packed with 2.5-μm SCX; mobile phase: A: TFA–0.5 mol/L NH_4Cl–acetonitrile (0.1:95:5, v/v/v); B: TFA–H_2O–acetonitrile (0.1:5:95, v/v/v); gradient: 0–5 min, 0% B; 5–40 min, 0–50% B; 40–50 min, 50–100% B; flow rate: 3 μL/min; sample: digest of human serum. II: Column: 55 cm (effective length of 30 cm) × 150 μm id capillary packed with 3-μm C18; mobile phase: A: TFA–H_2O–acetonitrile (0.1:95:5, v/v); B: TFA–H_2O–acetonitrile (0.1:5:95, v/v); gradient: 0–5 min, 0–10% B; 5–35 min, 10–50% B; 35–45 min, 50–100% B; flow rate: 400 nL/min; applied voltage: 3 kV; detection: UV at 230 nm.

narrower fractions than the typical peak width). We calculated the practical peak capacity (N'_p) as the product of fractions in the first dimension and the peak capacity of the second dimension [17]. The peak capacity was increased from about 30 in μ-SCXLC and 100 in RP-pCEC to over 1000 in the 2-D system.

3.3 Separation of *Cortex Phellodendri*

Cortex Phellodendri (CP) is a TCM, which is derived from the dried bark of *Phellodendron chinense* Schneid (PCS) or *Phellodendron amurense* Rupr. (PAR). It is a well-known oriental folk medicine and is known as an effective anti-inflammatory, antiphlogistic and antipyretic agent in clinical usage. It contains many different components such as isoquinoline alkaloids, lactones, phenols, limonoid, triterpenoids, sterols, etc. Several analytical methods, such as TLC, capillary zone electrophoresis (CZE) and narrow-bore HPLC, etc. have been reported for studying the main components of CP [18–20]. However, because TCMs are the complex samples that contain components differing from inorganic to organic, from polar to non-polar and from small molecules to large biomolecules, those methods are still not enough for separation of components of CP.

Three extract solutions, 95% acetonitrile in water, 100% methanol and methanol–hydrochloric acid (99:1, v/v), were used to extract CP. More peaks were observed with methanol–hydrochloric acid (99:1, v/v) and, therefore, it was chosen as the final extracted solution [21].

NH_4Cl, which was a weak acid solution, was added into the pCEC mobile phase (20 mmol/L NH_4Cl–acetonitrile) for better separation of alkaloid substance. The elution salt for the first dimension was NH_4Cl, which was also used to match the second dimension. As illustrated in Fig. 5, the first fraction eluted from μ-SCXLC may contain more negative ions and neutral compounds. Many negative ions may be hydrophilic and can be eluted easily in RP-pCEC. Fractions eluted from the first dimension, when the salt concentration was relatively low, contained more compounds with less positive charge, which would be retained longer in the second dimension. And fractions eluted at the end of μ-SCXLC contained many strong cation components and compounds

Table 1. Comparison of peak capacity[a)] with different separation modes

	BSA digest	Serum digest	Extracts of CP
μ-SCXLC	30	30	20
RP-pCEC	100	100	80
2D system[b)]	1200	1000	880

a) The peak capacity was calculated by the rate of total time and average peak width.
b) The peak capacity of 2-D system was calculated as the product of fractions in the first dimension and the peak capacity of the second dimension [17].

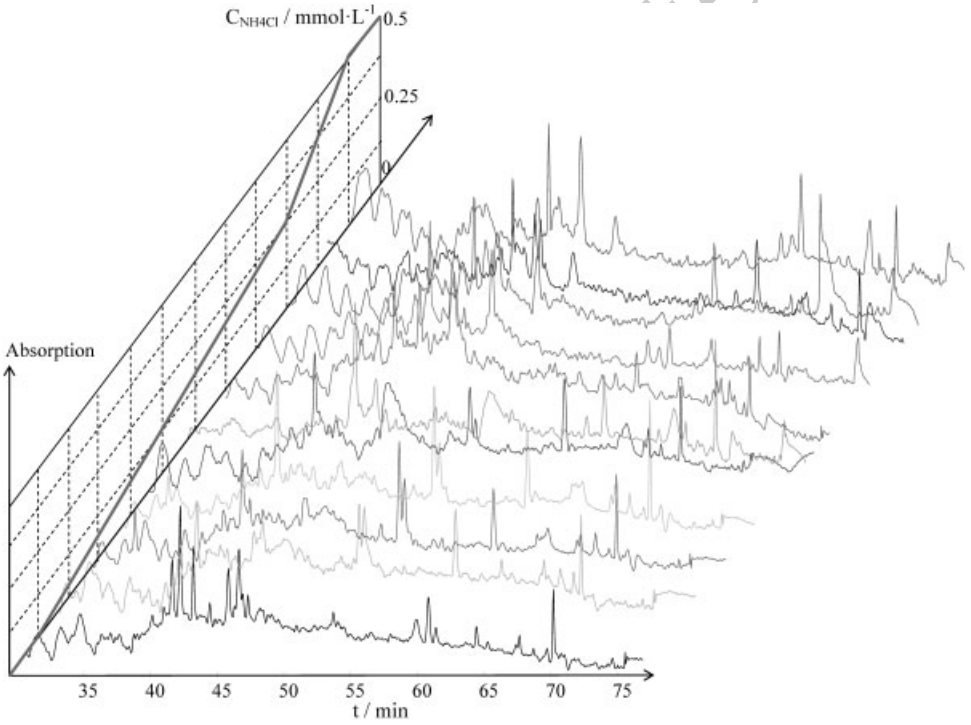

Figure 5. 2-D μ-SCX LC/RP-pCEC chromatogram for extracts of *Cortex Phellodendri*. I: Column: 10 cm × 300 μm id capillary packed with 2.5-μm SCX; mobile phase: A: 5 mmol/L PBS–acetonitrile (95:5, v/v); B: 5 mmol/L PBS + 0.5 mol/L NH_4Cl–acetonitrile (95:5, v/v); gradient: 0–5 min, 0% B; 5–40 min, 0–50% B; 40–50 min, 50–100% B; 50–55 min, 100% B, the effluent was collected every 5 min and totally 11 samples were collected; flow rate: 3 μL/min; sample: extracts of *Cortex Phellodendri*. II: Column: 55 cm (effective length of 30 cm) × 150 μm id capillary packed with 3-μm C18; mobile phase: A: 20 mmol/L NH_4Cl–acetonitrile (95:5, v/v); B: 20 mmol/L NH_4Cl–acetonitrile (5:95, v/v); gradient: 0–5 min, 0–5% B; 5–30 min, 5–20% B; 30–40 min, 20–35% B; 40–55 min, 35–50% B; 55–65 min, 50–80% B; flow rate: 400 nL/min; applied voltage: 5 kV; detection: UV at 230 nm.

with strong polarity, whose retention times were shorter also in the second-dimensional RP-pCEC.

Moreover, the sample complexity was decreased by the first-dimensional separation, and more peaks could be separated by the second-dimension. This 2-D system increased N'_p to about 900 while the peak capacity in μ-SCXLC and PR-pCEC was nearly 20 and 80 [13], respectively. From Table 1, it is clear that the 2-D system greatly enhanced the whole peak capacity compared with any 1-D method.

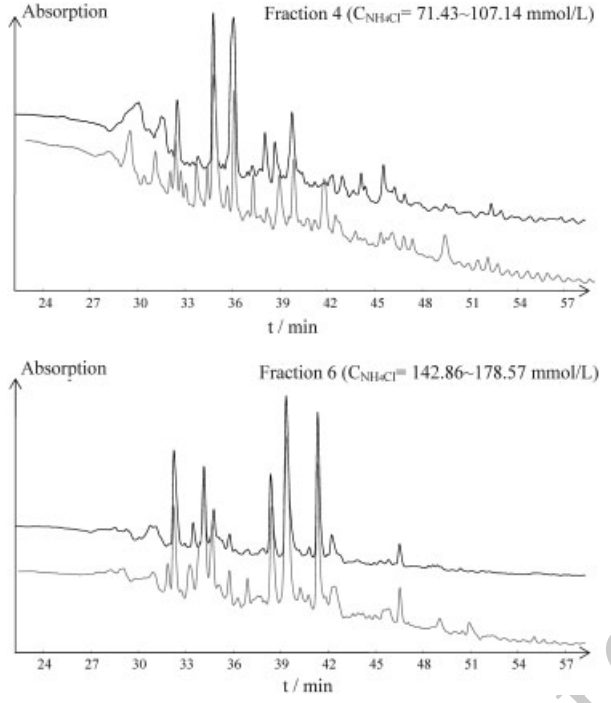

Figure 6. Reproducibility of the 4th and 6th fractions collected from the first dimension. Column: 55 cm (effective length of 30 cm) × 150 μm id capillary packed with 3-μm C18; mobile phase: A: H_2O–acetonitrile–TFA (95:5:0.1, v/v/v), B: H_2O–acetonitrile–TFA (5:95:0.1, v/v/v); gradient: 0–5 min, 0–10% B; 5–35 min, 10–50% B; 35–45 min, 50–100% B; flow rate: 400 nL/min; applied voltage: 3 kV; detection: UV at 230 nm. Sample: the 4th and 6th fractions collected from the first dimension.

3.4 Reproducibility

The reproducibility of the 2-D system was evaluated with digest of BSA. After the second separation, the chromatogram profiles of two fractions (fractions 4 and 6) were compared, as shown in Fig. 6. The observed results indicated overlap of many peaks between the two runs, indicating reasonable reproducibility of the 2-D system. On the other hand, there was still a level of variability; one of the limitations of this offline 2-D system is to precisely control the collecting fractions from one run to another. The same peak may be split into two peaks in one case and not in the other, creating a mismatch, which also existed in the reported offline 2-D LC [22].

3.5 Advantage of pCEC and μ-SCXLC/RP-pCEC 2-D system

The introduction of voltage in μHPLC could reduce the separation time and enhance peak resolution as shown in Fig. 7. In the separation of BSA digest, higher efficiency was achieved in the pCEC mode than in μHPLC (see Fig. 7A). From Fig. 7B, it can be seen that peaks that could not be separated in μHPLC were separated well when 3-kV voltage was applied. Moreover, electrophoretic mobility also contributed in pCEC.

Therefore, this new 2-D system, pCEC connected to μ-SCXLC, was established for complex samples analysis. Compared with the reported 2-D-SCX-RPLC, this approach couples the principal advantages of pCEC with the advantages of SCX. In pCEC, the retention mechanism for neutral compounds is essentially based on chromatographic partition. For charged compounds, both chromatographic

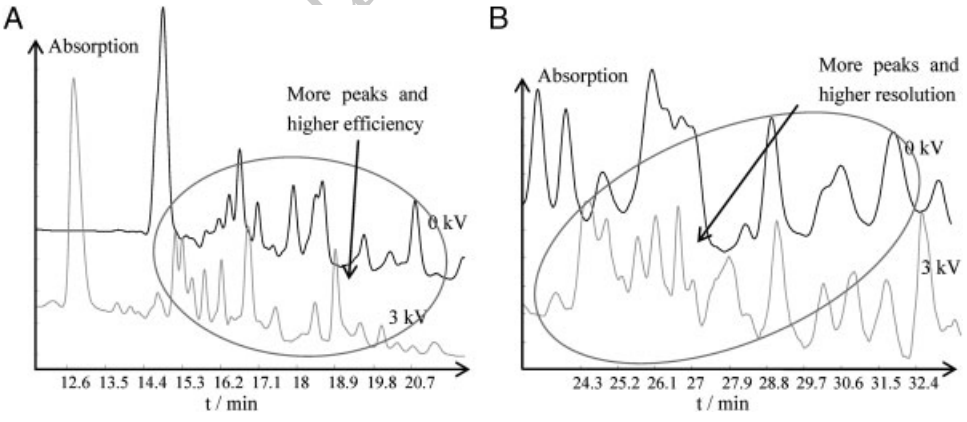

Figure 7. Comparison of pCEC and μHPLC for (A) digest of BSA and (B) digest of serum. Column: 55 cm (effective length of 30 cm) × 150 μm id capillary packed with 3-μm C18; mobile phase: A: H_2O–acetonitrile–TFA (95:5:0.1, v/v/v), B: H_2O–acetonitrile–TFA (5:95:0.1, v/v/v); Gradient: 0–5 min, 0–10% B; 5–35 min, 10–50% B; 35–45 min, 50–100% B; flow rate: 400 nL/min; detection: UV at 230 nm; Sample: (A) BSA tryptic digests, (B) serum tryptic digests.

partition and electrophoretic mobility contribute to the separation mechanism. This dual mechanism of pCEC makes it suitable for both neutral and charged compounds and dramatically enhances the separation selectivity compared to a standalone HPLC. In addition, the selectivity of the mixture in pCEC can be tuned by simply adjusting the voltages/pressure ratio, which is a unique feature of pCEC. So, our present μ-SCXLC/RP-pCEC system provides high sensitivity and high-throughput analysis platform compared to other 2-D HPLC systems.

Though the performance of the 2-D system was satisfactory in terms of peak capacity, components could not be identified. Therefore, this μ-SCXLC/RP-pCEC system combined with mass spectrometry (MS) detector would provide more molecular weight and structural information on the identification of the components. Work in these directions is in progress.

4 Concluding remarks

A new comprehensive 2-D separation technique – μ-SCXLC/RP-pCEC – was developed and evaluated for the separation of complex samples, digest of BSA, human serum and a TCM. The selectivity of the 2-D system is completely different and hence the orthogonality is ensured. This 2-D system permits three separation modes – electrophoresis, reversed-phase chromatography and strong cation exchange. Therefore, it provides higher peak capacity, selectivity and resolution compared to mono-dimensional systems.

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The authors have declared no conflict of interest.

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