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Pressurized capillary electrochromatographic analysis of water-soluble vitamins by combining with on-line concentration technique

Li Jia*, Yaling Liu, Yanyan Du, Da Xing

MOE Key Laboratory of Laser Life Science & Institute of Laser Life Science, South China Normal University, Guangzhou 510631, China

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Abstract

A pressurized capillary electrochromatography (pCEC) system was developed for the separation of water-soluble vitamins, in which UV absorbance was used as the detection method and a monolithic silica-ODS column as the separation column. The parameters (type and content of organic solvent in the mobile phase, type and concentration of electrolyte, pH of the electrolyte buffer, applied voltage and flow rate) affecting the separation resolution were evaluated. The combination of two on-line concentration techniques, namely, solvent gradient zone sharpening effect and field-enhanced sample stacking, was utilized to improve detection sensitivity, which proved to be beneficial to enhance the detection sensitivity by enabling the injection of large volumes of samples. Coupling electrokinetic injection with the on-line concentration techniques was much more beneficial for the concentration of positively charged vitamins. Comparing with the conventional injection mode, the enhancement in the detection sensitivities of water-soluble vitamins using the on-line concentration technique is in the range of 3 to 35-fold. The developed pCEC method was applied to evaluate water-soluble vitamins in corns.

Keywords: Pressurized capillary electrochromatography; On-line concentration; Water-soluble vitamins

1. Introduction

As it is well known, vitamins are a broad group of organic compounds that are minor, but essential constituents of food required for the normal growth, self-maintenance and functioning of human and animal bodies. They can be categorized into two groups: water-soluble and fat-soluble vitamins. So far, several techniques for the determination of some water-soluble vitamins have been developed, including high-performance liquid chromatography (HPLC) [1–5] and capillary electrophoresis (CE) [6–8]. However, it is difficult to separate most water-soluble vitamins in a single run using these techniques. To the best of our knowledge, capillary electrochromatographic (CEC) separation of water-soluble vitamins has not been investigated.

CEC is a hybrid separation technique that combines capillary liquid chromatography (cLC) with CE, in which, the separation mechanism is based on both chromatographic partition and electrophoresis [9–14]. Owing to the contribution of the dual mechanisms, CEC has the advantages of high efficiency, high selectivity, fast speed, low sample and solvent consumptions, and so on. However, when CEC was performed using a packed column on a commercially available CE system without pressure, there often arose some problems associated with column drying-out and bubble formation. In order to solve these problems, a pressurized CEC (pCEC) system is developed, in which, pressure is applied to the capillary column to suppress bubble formation and protect the column from drying-out [15–18]. In addition to this solution, commercial CE instruments are also available in which pressure is applied on both inlet and outlet vial, thus preventing bubble formation [19]. In a pCEC system, a mobile phase is driven by both a pressurized flow and an electroosmotic flow (EOF), resulting in the fast speed analysis. And sample can be introduced into the column quantitatively through a rotary-type injector.

In CEC, bubbles are formed at the frit adjacent to the detection window and occur as a result of the change in EOF as the mobile phase passes from the packed bed into the unpacked region of the capillary column through this frit since the frit often serves as nucleation site for gas bubble formation [19]. Hence, the use of a fritless column in CEC is one of the methods to prevent bubble formation. A monolithic column, as a fritless column, is a good choice in CEC. Moreover, monolithic columns

* Corresponding author. Tel.: +86 20 85211543; fax: +86 20 85216052.
E-mail address: jiali@scnu.edu.cn (L. Jia).

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can overcome some inherent limitations possessed by conventional commercially packed columns, such as low permeability [20–26]. Therefore, the evolution of monolithic columns will facilitate the faster development of CEC technique.

CEC, as one of micro-separation techniques, also suffers from low detection sensitivity due to a minute sample volume and limited optical path length for on-capillary UV photometric detection. In order to enhance the detection sensitivity in CEC with octadecylsilica (ODS) packed columns and enable the technique to analyze dilute samples, several on-line sample concentration strategies, including solvent gradient elution, preparation of sample in a non-eluting solvent, and injection of a water plug after sample injection, have been reported [18,27–32]. Taylor and Teale first reported the use of solvent gradient elution for the concentration of drug mixtures [18]. Hilhorst et al. reported the use of a non-eluting matrix and solvent gradient elution for the concentration of structurally related steroids and achieved seven to nine times increase in sensitivity [27]. Stead et al. achieved up to 17-fold increase in detection sensitivity of a mixture of steroids using a non-eluting sample matrix [28]. Zhang et al. combined solvent gradient elution and field-enhanced sample stacking as on-line concentration technique to improve the detection sensitivity of benzoin and mephenytoin by two orders of magnitude and that of positively charged analyte (propatenene) by four orders of magnitude [29]. Yang and Rassi reported on-line concentration of pesticides based on the introduction of a relatively large plug of a dilute sample preceded by the introduction of a short plug of pure water [30]. The combination of solvent gradient elution and a non-eluting matrix was also used by Tegeler and Rassi to preconcentrate a mixture of carbamate insecticides [31,32]. Quirino et al. reported the use of photopolymerized sol–gel (PSG) as a monolithic stationary phase in CEC for the concentration of a variety of neutral and charged analytes with 20–100-fold increase in the detection sensitivity based on the high mass-transfer rates possible in the porous structure [33]. Quirino et al. achieved a further increase in the detection sensitivity by combining solvent gradient elution and sample stacking using PSG monolithic column [34]. Liu et al. coupled field-enhanced sample injection to CEC as well as cLC to improve the detection sensitivity by one order of magnitude [35].

The aim of the report was to study on-line concentration methods in pCEC and investigate the applicability of pCEC technique in the analysis of water-soluble vitamins. In the report, a monolithic silica-ODS column was used as the separation column and three on-line concentration techniques were studied to enhance the detection sensitivity. The applicability of the method was demonstrated by assessment of water-soluble vitamins content from corns.

2. Experimental

2.1. Apparatus and procedure

All pCEC experiments were performed on a TriSep-2100 pCEC system (Unimicro Technologies, Pleasanton, CA, USA), which comprised of a solvent gradient delivery module, a high voltage power supply (−30 kV and +30 kV), a variable wavelength UV/Vis detector, a micro-fluid manipulation module with a six-port injection valve and a data acquisition module. Two mobile phases were driven by two micro-volume high-pressure pumps, respectively, then merged in a mixer and entered into a six-port injection valve. Samples were injected into the injection valve and introduced in the sample loop, and then carried to a four-port split valve by the mobile phase flow. After splitting in the four-port valve, the flow entered into a capillary column under a constant flow rate controlled by a constant flow rate ratio regulator. A negative voltage was applied to the outlet of the column and the inlet of the column was grounded, which was connected to the split valve.

A monolithic silica-ODS column (250 mm × 0.2 mm I.D.) was obtained from GL Sciences (Tokyo, Japan). Detection wavelength was set at 210 nm.

2.2. Chemicals

Thiamine hydrochloride (VB1), riboflavin (VB2), nicotinic acid (NA), nicotinamide (VB3), calcium pantothenate (CP), pyridoxal hydrochloride (VB6-H), pyridoxine hydrochloride (VB6-OH), pyridoxamine dihydrochloride (VB6-NH2), folic acid (FA), D-biotin (VH), cyanocobalamin (VB12), and ascorbic acid (VC) were purchased from Chem Service (West Chester, PA, USA). Stock solutions of each vitamin were prepared as 2 mg/mL in deionized water and stored at 4 °C prior to use. Methanol was of HPLC grade and purchased from SK Chemicals (Ulsan, South Korea). Water used for mobile phase and sample preparations was obtained from an Elga water purification system (ELGA, London, UK).

2.3. Vitamins extraction

The water-soluble vitamins extraction procedure was similar to the method described previously with some modification [36,37]. Briefly, 4 mL of cold methanol (−20 °C) was added to the tube containing the corn sample (120 mg) and incubated at −20 °C for 30 min. Then, the solution was centrifuged at 4 °C and 5000 rpm for 25 min (Sigma 2–16 K centrifuge, Osterode am Harz, Germany). The supernatants were withdrawn and centrifugally filtered through a Millipore 5 kDa-cutoff filter at 4 °C to remove proteins and other debris. The filtrate was evaporated using a micro-centrifugal vacuum concentrator Christ RVC 2–18 (Osterode am Harz, Germany) at 30 °C. Prior to analysis, the dried sample was reconstituted in 1 mL of deionized water.

3. Results and discussion

3.1. Optimization of pCEC conditions

In pCEC using a monolithic silica-ODS column, the separation of neutral analytes is based on the partitioning of analytes between the mobile phase and stationary phase, while the separation of charged analytes is based on both partitioning and electrophoretic mobilities of analytes. The mobile phase is driven by both EOF introduced by an applied electrical field and

high-pressure from pumps. Therefore, in order to obtain better separation results, the type and concentration of organic modifier, the type, concentration and pH of buffer, applied voltage and flow rate were investigated and optimized in the following section.

In the optimization experiments, the volume of sample loop was 1 μL. The injection volume after splitting was 82.5 nL. The flow rate of mobile phase in the column after splitting was measured to be 1.65 μL/min using thiourea as a standard when total flow rate was 20 μL/min. Sample injection was carried out by applying a negative voltage same as the separation voltage to the outlet of the column before loading sample. The mixture of 12 vitamins in water was used as a test sample, where the concentration of each vitamin was 20 μg/mL.

3.1.1. Effects of type and concentration of organic modifier

The type and concentration of organic modifier in the mobile phase play key roles in the separation in pCEC. At first, two most commonly used organic modifiers (including methanol and acetonitrile) were investigated. The experimental results showed that the separation effect was better when methanol was used as the organic modifier. Next, the concentration of methanol in the range of 20 to 40% (v/v) was optimized while keeping phosphate at 4 mM (pH 4.0). When the concentration of methanol was 26% (v/v), 12 vitamins obtained the best separation. Hence, 26% (v/v) methanol in the mobile phase was used for further experiments.

3.1.2. Effects of type, concentration and pH of buffer

At first, two types of buffer solution (acetate and phosphate) at pH 4.0 were tested to investigate the influence of buffer type on the separation effect. The experimental results showed that the separation was fairly better and the baseline was stable when the phosphate buffer was utilized.

Next, the concentration of phosphate in the range of 2 to 8 mM was optimized. From the experimental results, we can find that a higher concentration of phosphate buffer tend to yield a higher current, and this can give rise to Joule heating, followed by a decrease in the signal to noise ratio as a result of an increase in the noise, resulting from a poor baseline, as discussed by Knox and Grant [11,12]. Based on the experimental results, 4 mM phosphate solution was chosen as the buffer in the mobile phase for the following experiments.

Then, the effect of pH of buffer in the range of 2.0 to 4.5 was discussed. In pCEC using a monolithic silica-ODS column, the EOF is very dependent on pH of buffer since the variety of pH determines the number of dissociated residual silanol groups on the surface of the capillary wall and the skeleton of the monolithic column. Thiourea was used as a marker to measure the EOF velocity. Our experimental results showed that the EOF velocity was from 0.008 to 0.024 mm/s in the pH range of 2.0 to 4.5. The linear velocity of the mobile phase in pCEC was 0.9 mm/s. Hence, the main contribution to the driving force of the mobile phase in pCEC is from pump under our experimental conditions.

The pH of buffer has dramatic effect on the separation resolution of 12 water-soluble vitamins as all the vitamins contain acidic and basic groups. The pH of buffer affected the dissociation of acidic and basic groups, resulting in affecting the hydrophobicity and electrophoretic mobility of the vitamins containing these groups. In the pH range of 2.0 to 4.5, the effect of the pH of buffer on the separation resolution of water-soluble vitamins was investigated. The experimental results showed that the 12 vitamins obtained best separation at pH 2.5. Under the condition, VC eluted together with the solvent peak. Comparing the result in pCEC with that in cLC, the detection window in pCEC was widened since the hydrophilic vitamins having positive charges eluted earlier than neutral compounds not retaining on the column owing to the effect of their electrophoretic mobilities. Due to the dual separation mechanisms including HPLC and CE, 12 vitamins were separated better using pCEC than cLC.

3.1.3. Relationship of applied voltage and flow rate

At first, the effect of electric field strength in the range of 4 to 14 kV on the separation was studied by varying applied voltages at a certain flow rate. The change of applied voltage had dramatic effect on the resolution of hydrophilic vitamins through affecting their electrophoretic velocities. With the increase in the applied voltage, the resolution of VB6-OH and VB6-H, VB3 and NA became better, while the resolution of VC and VB6-OH became worse.

Next, the effect of flow rate in the range of 10 to 20 μL/min on the separation was investigated. Due to the dual separation mechanisms in pCEC, the applied voltage and the flow rate combined to influence the separation. The experimental results showed that at a lower flow rate, the optimum separation effect was obtained at a smaller applied voltage. With the same ratio of applied voltage to flow rate (12/20, 9/15, 6/10) (kV/(μL/min)), the vitamins obtained better separation at corresponding flow rate. While at corresponding optimum applied voltage, the vitamins were separated better at a lower flow rate. Based on the discussion, we can suggest that the ratio of applied voltage to flow rate obtaining optimum separation effect at a certain flow rate can guide the selection of the optimum applied voltage at other flow rate. Considering the speed of analysis, the flow rate (20 μL/min) and the applied voltage (12 kV) were selected for studying on-line sample concentration.

3.2. On-line sample concentration

3.2.1. Effect of sample matrix

The effect of sample matrix was first discussed while keeping the sample loop 1 μL. Three types of sample matrix (mobile phase, 4 mM phosphate solution and water) were investigated. When the sample matrix was mobile phase, the half peak width of analytes was largest since under the condition there was not solvent gradient zone sharpening effect and field-enhanced sample stacking, as shown in Fig. 1A. When the sample matrix was water, the half peak width of analytes was smallest due to the existence of the two zone sharpening effects, resulting in the increase in the peak height of analytes (see Fig. 1C). Hydrophilic charged vitamins are concentrated by field-enhanced sample stacking, more hydrophobic neutral vitamins are concentrated by solvent gradient zone sharpening, and more hydrophobic

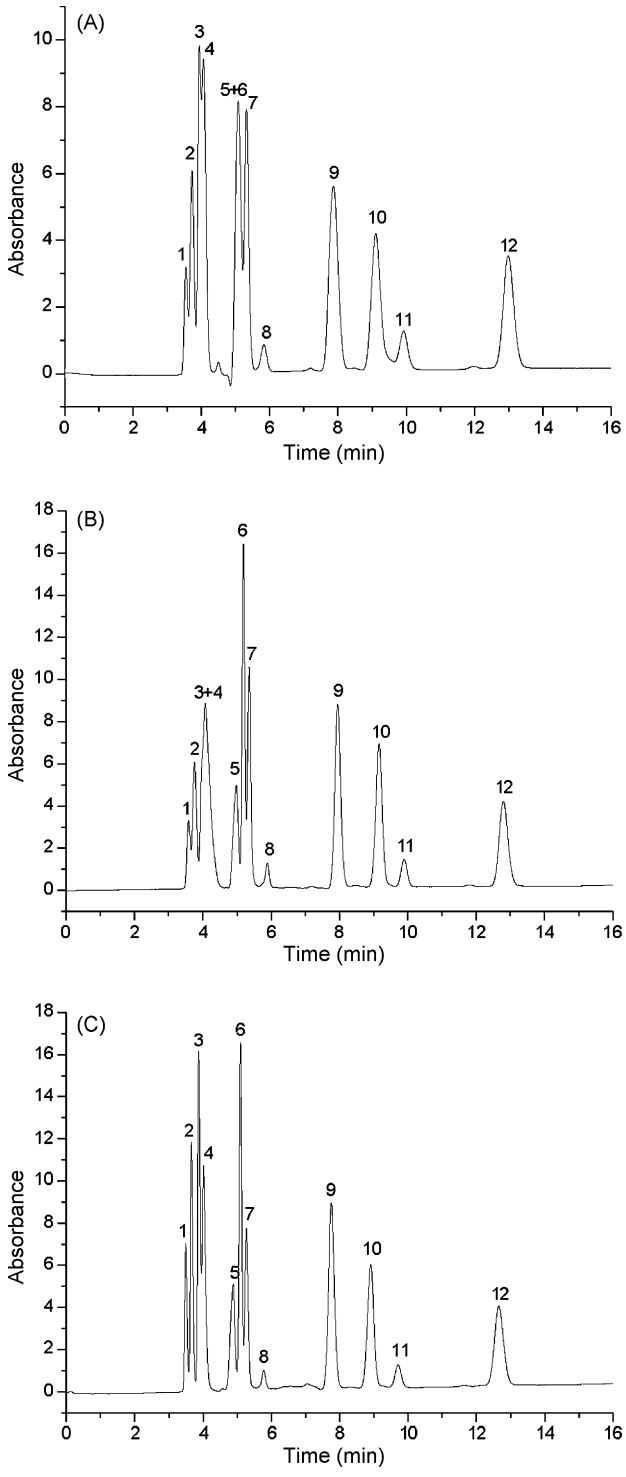

Fig. 1. Effect of sample matrix. (A) Mobile phase; (B) 4 mM phosphate solution (pH 2.5); (C) water. Analytes: 1, VB1; 2, VB6-NH2; 3, VB6-OH; 4, VB6-H; 5, VC; 6, VB3; 7, NA; 8, CP; 9, VB12; 10, FA; 11, VH; 12, VB2. Isocratic elution was performed with 4 mM phosphate (pH 2.5) in methanol–water (26/74, v/v) as the mobile phase at a flow rate of 20 μL/min. Detection wavelength was set at 210 nm. Sample loop was 1 μL. Injection was carried out by applying a negative voltage (12 kV) same as the separation voltage to the outlet of the column before loading sample.

charged vitamins in the same sample mixture are concentrated by the two zone sharpening effects. In order to observe the separation results with solvent gradient zone sharpening effect and without field-enhanced sample stacking, 4 mM phosphate solution was used as the sample matrix. Fig. 1B shows that when the sample matrix was 4 mM phosphate solution, the hydrophobic vitamins (VB3, NA, CP, VB12, FA, VH and VB2) obtained similar separation result to that using water as sample matrix owing to the existence of solvent gradient zone sharpening effect. The four hydrophilic vitamins eluting earlier than solvent obtained poor separation as field-enhanced sample stacking did not exist. The above experimental results suggested that using water as the sample matrix can benefit the simultaneous on-line concentration of hydrophobic and charged vitamins due to the existence of the two zone sharpening effects. Hence, water was used as the sample matrix for the following experiments.

3.2.2. Effect of injection mode

The effects of two types of injection mode on the separation and on-line concentration of vitamins were investigated, which were pressure injection combined with electrokinetic injection (pressure-electrokinetic injection) and pressure injection, respectively. The pressure-electrokinetic injection mode was carried out by applying a negative voltage to the outlet of the column before loading sample. The pressure injection mode was performed by applying a negative voltage to the outlet of the column after sample passing through the four-pore split. With the pressure injection mode, for different volumes of sample loop, the time applying a negative voltage after loading sample was different, which was calculated in terms of the volume of sample loop and the total flow rate of mobile phase. At a total flow rate of 20 μL/min, when the volume of sample loop was 1, 5, 10, 50 and 100 μL, respectively, applying a negative voltage was performed 20, 20, 35, 155 and 505 s after loading sample in our experiments, respectively.

Using the pressure-electrokinetic injection mode, since the sample matrix was water and the pH of the phosphate buffer in the mobile phase was 2.5, EOF can be negligible, indicating that the sample was introduced into the column mainly by the pressure and the electrophoretic velocities of vitamins, resulting in the difference in the injection quantity of cations, negative ions and neutral compounds. And there existed field-enhanced sample stacking injection using the pressure-electrokinetic injection mode. Comparing with the pressure injection mode, the peak areas and heights for VB12 and VB2 using the pressure-electrokinetic injection mode were similar, larger for VB1, VB6-H, VB6-OH, VB6-NH2, and VB3, while less for NA, CP, FA and VH. For the hydrophilic vitamins eluting earlier than solvent (VB1, VB6-NH2, VB6-OH and VB6-H) at the injection volume 82.5 nL (sample loop 1 μL), the increases in the peak area and height of VB1 were highest, which were 2.8 and 2.5-fold, respectively, comparing with the pressure injection mode.

3.2.3. Effect of sample injection volume

The detection sensitivity problem in pCEC arises from the short optical path length for on-column detection and low

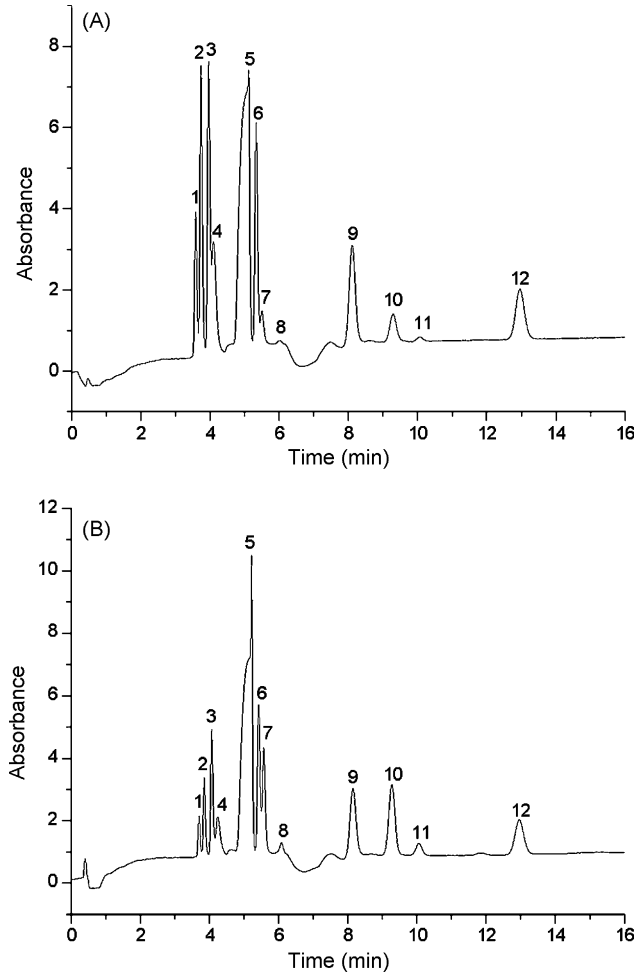

Fig. 2. Chromatograms of the vitamins mixture at 0.413 μL of injection volume with the two injection modes. (A) Pressure-electrokinetic injection; (B) pressure injection. Analyte peak numbering is the same as in Fig. 1C. Concentration of each vitamin is 2 μg/mL. Other experimental conditions are the same as in Fig. 1C.

sample injection volume. In our experiments, with UV absorbance as a detector, the strategy for enhancing the detection sensitivity is to perform on-line concentration by increasing the sample injection volume. It should be noted that larger injection volumes would deteriorate separation efficiency of pCEC. In order not to compromise the separation efficiency of pCEC, two types of on-line concentration techniques, namely solvent gradient zone sharpening effect and field-enhanced sample stacking, were utilized based on the above studies. The effects of different injection volumes on the separation efficiency and on-line concentration of the vitamins using the two injection modes were carried out. Fig. 2 shows the chromatograms at 0.413 μL of injection volume with the two injection modes. For VB12 and VB2, when the injection volume was 8.25 μL (sample loop 100 μL) using the pressure-electrokinetic injection mode, the half widths of the peaks were slightly larger than those with the injection volume 82.5 nL (sample loop 1 μL) and gained up to 35 (VB12) and 35-fold (VB2) sensitivity enhancement in terms of peak height. For FA and VH achieving better separation with the injection volume 4.125 μL (sample loop 50 μL) at the pressure injection, the sensitivity enhancement factors (SEFs) in terms of peak height were 22 and 17-fold, respectively. For NA and CP achieving better separation with the injection volume 0.413 μL (sample loop 5 μL) at the pressure injection, the SEFs of them were all 4-fold. Comparing with the injection volume 82.5 nL at the pressure injection, VB1, VB6-H, VB6-OH, VB6-NH2, and VB3 with the injection volume 0.413 μL at the pressure-electrokinetic injection mode achieved up to 10, 9, 6, 3, and 3-fold sensitivity enhancement, respectively.

The experimental results indicated that the combination of solvent gradient zone sharpening effect and sample stacking was useful for simultaneously enhancing the detection sensitivity of hydrophobic and charged compounds in a single run. Coupling electrokinetic injection with the two on-line concentration techniques was much more beneficial for the concentration of positively charged compounds. The larger the injection volume was, the more was the effect of the electrokinetic injection on the injection quantity of cationic and negative vitamins. For VB1, the increase in the peak height was 2.5 and 2.8-fold, respectively, at 82.5 nL and 0.413 μL of injection volume with the pressure-electrokinetic injection relative to the pressure injection. While for FA, the decrease in the peak height was 1.6 and 3.3-fold, respectively, at 82.5 nL and 0.413 μL of injection volume with the pressure-electrokinetic injection relative to the pressure injection.

3.3. Validation

The injection volume 0.413 μL (sample loop 5 μL) was used for the investigation of the quantification of the developed method. A series of vitamins with the concentration ranging from 1 to 40 μg/mL was used to determine the calibration parameters for the 11 vitamins in pCEC. The sample with the concentration of each vitamin at 2 μg/mL was used for the determination of the reproducibility. The limits of detection (LOD) for the vitamins were calculated at signal-to-noise ratio equal to 3 (S/N = 3). Table 1 shows the reproducibility data obtained for the retention time and peak area, the calibration function (peak area versus concentration in μg/mL), linearity of the calibration function, and sensitivity (LOD) for the determination of the vitamins with the two injection modes.

3.4. Analysis of corn samples

The developed pCEC method was applied to evaluate the contents of water-soluble vitamins in a corn sample. In the sample analysis experiments, the pressure injection mode was utilized. The water-soluble vitamins in the sample were identified by two methods: (1) comparing their retention times with those of standards and (2) spiking the standards to the sample. Fig. 3 shows the chromatogram of the sample. VB6-OH, NA, VB12, FA and VB2 were detected in the corn sample. Due to the insufficient resolution of NA peak from matrix interferences in the corn sample, NA was not quantified. The contents of VB6-OH, VB12, FA and VB2 in the corn sample

Table 1
Reproducibility, linearity and sensitivity for the vitamins using the two injection modes (the volume of sample loop was 5 μL)

Injection mode	Analyte	Calibration line		RSD ($n = 6$) (%) (each vitamin 2 μg/mL)		LOD (S/N = 3)
		$y = ax + b$	r^2	Retention time	Peak area	μg/mL
Pressure injection combining with electrokinetic injection	VB1	$y = 6.7x + 0.2$	0.9997	2.0	4.5	0.04
	VB6-NH2	$y = 12.2x + 0.4$	0.9980	0.8	4.8	0.02
	VB6-OH	$y = 16.1x + 0.1$	0.9928	1.3	2.7	0.02
	VB6-H	$y = 11.2x + 0.1$	0.9980	1.2	2.6	0.03
	VB3	$y = 16.2x + 0.5$	0.9978	1.2	2.5	0.02
	NA	$y = 10.1x + 0.4$	0.9950	0.5	7.8	0.2
	CP	$y = 0.6x + 0.1$	0.9995	1.1	7.1	1.4
	VB12	$y = 17.9x + 0.01$	0.9999	1.5	6.6	0.06
	FA	$y = 9.6x + 0.2$	0.9996	2.2	4.0	0.2
	VH	$y = 2.6x + 0.05$	0.9982	1.1	5.4	0.7
	VB2	$y = 14.3x + 0.04$	0.9987	0.7	2.0	0.1
Pressure injection	VB1	$y = 3.6x + 0.1$	0.9997	1.1	2.3	0.1
	VB6-NH2	$y = 7.1x + 0.3$	0.9997	1.2	1.8	0.05
	VB6-OH	$y = 12.0x + 0.4$	0.9989	1.4	1.0	0.03
	VB6-H	$y = 8.7x + 0.05$	0.9991	1.2	5.2	0.06
	VB3	$y = 15.9x + 0.3$	0.9998	0.9	1.1	0.03
	NA	$y = 12.7x + 0.02$	0.9995	0.8	2.5	0.04
	CP	$y = 1.5x + 0.1$	0.9985	0.8	2.5	0.6
	VB12	$y = 17.9x + 0.3$	0.9981	1.1	2.1	0.05
	FA	$y = 20.3x + 0.1$	0.9995	3.0	2.7	0.06
	VH	$y = 3.0x + 0.1$	0.9981	0.5	2.3	0.3
	VB2	$y = 12.6x + 0.03$	0.9999	1.8	1.0	0.1

y: Peak area; x: analyte concentration (μg/mL); r: correlation coefficient.

were found to be 3.08 ± 0.18, 12.75 ± 0.63, 9.83 ± 0.29, and 2.67 ± 0.17 μg/g, respectively, by triplicate analysis of the corn sample. The recovery experiments were carried out based on a certain concentration of each vitamin added to the sample. The recoveries for the four vitamins were determined to be 92% (VB6-OH), 92% (VB12), 96% (FA) and 90% (VB2) by triplicate analysis, with the RSD lower than 5.2%. The new strategies for analysis of more water-soluble vitamins in corns will be explored.

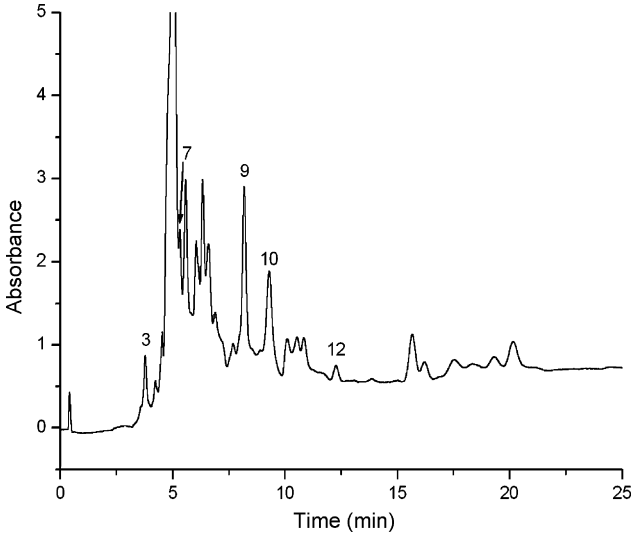

Fig. 3. Chromatogram of the corn sample analysis. Analyte peak numbering is the same as in Fig. 1C. Experimental conditions are the same as in Fig. 2B.

4. Conclusions

A pCEC method was developed for analysis of water-soluble vitamins, in which on-line concentration techniques were studied. The pCEC technique with dual separation mechanisms was beneficial for the separation of hydrophobic compounds and hydrophobic charged compounds simultaneously in a single run. The combination of solvent gradient zone sharpening effect and sample stacking was useful for simultaneously enhancing the detection sensitivity of hydrophobic and charged compounds in a single run. Coupling electrokinetic injection with the two on-line concentration techniques was much more beneficial for the concentration of positively charged compounds. From 3 to 35-fold sensitivity enhancement in terms of peak height for the determined vitamins was gained using the on-line concentration methods. The developed method can be applied to evaluate four water-soluble vitamins contents in corns. The new strategies for analyzing more water-soluble vitamins in corns will be explored.

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