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On-line cation-exchange preconcentration and capillary electrophoresis coupled by tee joint interface

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Abstract

An on-line preconcentration method based on ion exchange solid phase extraction was developed for the determination of cationic analytes in capillary electrophoresis (CE). The preconcentration–separation system consisted of a preconcentration capillary bonded with carboxyl cation-exchange stationary phase, a separation capillary for zone electrophoresis and a tee joint interface of the capillaries. Two capillaries were connected closely inside a 0.3 mm i.d. polytetrafluoroethylene tube with a side opening and fixed together by the interface. The preparations of the preconcentration capillaries and interface were described in detail in this paper. The on-line preconcentration and separation procedure of the analysis system included washing and conditioning the capillaries, loading analytes, filling with buffer solution, eluting analytes and separating by capillary zone electrophoresis (CZE). Several analysis parameters, including sample loading flow rate and time, eluting solution and volume, inner diameter and length of preconcentration capillary etc., were investigated. The proposed method enhanced the detection sensitivity of CE–UV about 5000 times for propranolol and metoprolol compared with normally electrokinetic injection. The detection limits of propranolol and metoprolol were 0.02 and 0.1 μg/L with the proposed method respectively, whereas those were 0.1 and 0.5 mg/L with conventional electrokinetic injection. The experiment results demonstrate that the proposed technique can increase the preconcentration factor evidently.

Keywords: Capillary electrophoresis; Cation-exchange; Preconcentration

1. Introduction

Capillary electrophoresis (CE) has become an effective separation tool and has been used in different area of chemistry, biology, medicine and pharmaceutics etc. However, one of the drawbacks of CE–ultraviolet detection (UV) is its poor detection sensitivity due to its extremely short light path and very small sampling volume. Two convenient approaches have been employed to improve its detection sensitivity. One is to extend its optical path, such as bubble cell or Z-cell [1], and the other is on-column preconcentration. On-column preconcentration of large sampling volume is an effective way to improve the detection sensitivity of CE–UV.

Various preconcentration methods have been reported. Field-amplified sample injection [2,3] and isotachophoresis [4] are commonly adopted for on-column preconcentration. Xiong et al. [8] developed a base-stacking preconcentration method for DNA fragments by injecting sample and alkaline plugs electrokinetically. A reaction between fast migrating OH^- and tris (hydroxymethyl) aminomethane cation ($Tris^+$) of buffer solution created a weak base zone of low conductivity, in which analyte stacking occurred. Similarly, Lunte and coworkers [9–11] presented an acid-stacking preconcentration method. Filling a separation capillary with weak-acid buffer solution, an acid zone was introduced following a sample one electrokinetically. Fast migrating H^+ from the acid zone reacted with the buffer anions and produced a low-conductivity region, in which sample ions were concentrated. Dynamic pH junction, an analogous method, was proposed for selective analyte stacking by Chen et al. [5–7].

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The migration velocity of analyte ions was greatly regulated by the pH values on both sides of pH junction and the complexation between analyte and buffer ions (such as borate). Therefore, the selective stacking can be achieved. Recently, Cao et al. [12,13] proposed a "transient moving chemical reaction boundary" method. Zwitterionic analytes were prepared in a weak-base solution and a running buffer solution was acidic. When the analytes migrated from the basic sample zone into the acidic buffer region, their charge polarities were changed and the analytes could be stacked at the moving boundary, of which the analyte migration directions were reversed on each side.

In order to improve CE–UV sensitivity further, some preconcentration techniques by combining different enrichment methods have been proposed. Britz-Mckibbin et al. [14] proposed a dynamic pH junction—sweeping method for flavin derivative preconcentration. The preconcentration factors of analytes were influenced by the pH value of buffer solution and the concentrations of borate and sodium dodecylsulfate (SDS). The detection sensitivity was improved more than 1200-fold compared with conventional electrokinetic injection. In addition, several methods combining field amplified sample injections with sweeping were developed and afforded million-fold improvement of detection sensitivity in micelle electrokinetic capillary chromatography (MECC) [15–17].

Another efficient approach is the preconcentration of solid phase extraction (SPE). Kalberg and coworkers [18] presented an on-line anion-exchange preconcentration of flow injection analysis (FIA), which was connected to CE via a specially designed interface. But only a small part of anions concentrated was introduced into separation capillary. Novič and Guček [19] improved ion-exchange preconcentration with a suppressor column, which eliminated the influence of sample and eluent matrices. However, the preconcentration factors of both the methods above mentioned were less than 10. Bonneil and Waldron [20] developed an on-line SPE method for discontinuous preconcentration in CE. The concentrator was a short piece of polyethylene tube containing 225 nl (1.5 mm in length) C_{18} stationary phase and was connected with two capillaries at both the tube ends. Up to 500-fold preconcentration factor was achieved for peptide by the method. Petersson et al. [21] developed a SPE-CE method. The extractor consisted of a short 200 μm i.d. capillary packed with C18 alkyl-diol silica. The capillary introduced glass fiber to retain the sorbent and was connected to a 50 μm i.d separation capillary. After sample loading and eluting, the analytes were separated by capillary zone electrophoresis (CZE). The proposed method enhanced the detection sensitivity by a factor of 7000. Breadmore et al. [22,23] developed an on-capillary SPE method for the preconcentration of inorganic anions, in which a single capillary possessed a preconcentration and a separation section. An eluotropic gradient based on a transient isotachophoretic boundary was adopted. The method with 10 min sample loading achieved 100-fold improvement in detection sensitivity compared with conventionally hydrodynamic injection.

In this paper, an on-line ion-exchange preconcentration method is presented for improving the detection sensitivity of CE–UV. The preconcentration and separation system consisted of an open preconcentration capillary bonded with cation-exchange stationary phase and a separation capillary untreated. Two capillaries were connected closely through a 0.3 mm i.d. polytetrafluoroethylene (PTFE) tube with a side opening, and fixed by a PTFE tee joint connecting to a PTFE valve. After washing two capillaries, conditioning the capillaries, loading analytes and filling with buffer solution, the analytes were eluted from the preconcentration capillary by 2 mol/L NH_4Cl solution and separated in the separation capillary by CZE. With the proposed method, two model cations, propranolol and metoprolol, were preconcentrated and separated, and their detection sensitivities were improved 5000-fold compared with normally electrokinetic injection.

2. Experiment

2.1. Apparatus

A 1229-HPCE Analyzer (New Tech. Appl. Institute, Beijing, China) detected at 214 nm and a N-2000 double-channel chromatography processor (Intel. Inform. Engineering Institute, Zhejiang University, Zhejiang, China) were employed throughout the work. A preconcentration capillary (100 μm i.d., 12 cm in length) and a separation capillary (50 μm i.d., total length 48 cm and effective length 33 cm) were purchased from Yongnian Chromatogr. Components Ltd. (Hebei, China). A column chamber of FULI 9790 gas chromatography (GC, Wenling Anal. Instrument Ltd., Zhejiang, China) was adopted to prepare the preconcentration capillaries for temperature control and nitrogen drying. 0.3 and 0.5 mm i.d. PTFE tubes were purchased from HI-TECH Corp. (Dandong, Liaoning, China). A three-way solenoid valve (161T031) was purchased from NResearch Inc. (Caldwell, NJ, USA). A two-way PTFE valve and a PTFE tee joint were homemade.

2.2. Chemicals

Propranolol hydrochloride and metoprolol tartrate were used as two model cations in this work and purchased from Wujin Pharmacy Factory (Jiangsu, China) and ASTRA (Wuxi, Jiangsu, China), respectively. They are β-adrenergic blockers for cardiopathy. The pK_bs of propranolol and metoprolol are 4.5 [24] and 4.3 [25], their molecular weights are 259 and 267 and their molar extinction coefficients measured in the buffer solution of CE are 28.0×10^3 L/mol cm and 5.2×10^3 L/mol cm, respectively. Other reagents were of analytical grade and all aqueous solutions were prepared with deionized water (Hefei Kesheng Co., Anhui, China). γ-(Trimethoxysilyl) propyl methacrylate and 1,1-diphenyl-

2-picrylhydrazyl (DPPH) were purchased from Alfa (Ward Hill, MA, USA). 2-Acrylamido glycolic acid monohydrate was from ACROS (Geel, Belgium). Acetone, methanol, methylene chloride, dimethylformamide (DMF), sodium acetate (NaAc), acetic acid glacial (HAc), sodium hydroxide (NaOH), hydrochloric acid (HCl) and ammonium chloride (NH_4Cl) were from Chem. Reagent Co. (Shanghai, China).

Propranolol and metoprolol stock solutions were prepared by dissolving the pharmaceuticals in deionized water, filtrating with 0.22 μm cellulose acetate membrane and diluting to 0.800 and 2.000 g/L, respectively. Two stock solutions were kept in brown flasks and in a refrigerator. Their analytical solutions were obtained by diluting the stock solutions to proper concentrations with deionized water. NaAc buffer solution was prepared by adjusting the acidity of 100 mmol/L NaAc solution to pH 4.0 with concentrated acetic acid. All water solutions were filtered with 0.22 μm filter membrane before use.

2.3. Preparation of preconcentration capillary

A weak cation-exchanger with glycolic acid (pK_a 3.83) was chosen to concentrate the cationic analytes in this work. In the preconcentration capillary, the cation-exchange stationary phase provided by 2-acrylamido glycolic acid was covalently bonded to the inner surface of the capillary through siloxane (Si–O–Si–C–) linkage. The preparation procedure of the stationary phase in the capillary was primarily based on the method described by Horváth and coworkers [26,27]. One hundred centimeter fused silica capillaries of 75, 100, 150 and 250 μm i.d. were washed by deionized water and 1.0 mol/L NaOH. Then the mixed solution of organosilane reagent (γ-(trimethoxysilyl) propyl methacrylate) and free radical reagent (1,1-diphenyl-2-picrylhydrazyl), and the solution of ion-exchange reagent (2-acrylamido glycolic acid) were introduced into the capillaries, respectively. The capillaries for silanization were treated at 120 °C for 6 h and the cation-exchange stationary phase was bonded by controlling the temperature at 70 °C for 6 h in a GC column chamber. After each bonding step, the capillaries purged and were dried by nitrogen. In order to ensure the preconcentration reproducibility, 100 cm capillaries of different inner diameter were bonded with the stationary phase one time, and were cut to different length in accordance with the experimental requirements. The working life of preconcentration capillaries can be more than 300 runs without deterioration.

2.4. Interface of preconcentration and separation capillaries

The schematic diagram of the tee joint interface of preconcentration and separation capillaries is shown in Fig. 1.

In the figure, the outer diameters of both the preconcentration and separation capillaries were about 0.37 mm and the inner diameter of the PTFE tube was 0.30 mm. The PTFE sheath tube was exsected a little exterior at its middle point

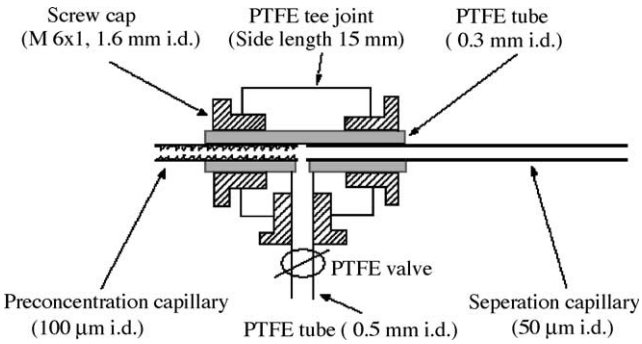

Fig. 1. Schematic diagram of tee joint interface.

(not pierced), and then was pierced with a needle. Two capillary ends were burnished with a P4000 sand paper and inserted into the opposite ends of the PTFE tube softened by an electric hair drier. The joint position of two capillaries was carefully adjusted under a scale microscope (JS27, Jiangnan Optics & Electronics Ltd., Nanjing, China), and the gap of two capillaries just located at the opening of the PTFE tube. The distance between two capillaries was 40–60 μm measured by the microscope. After cooled to room temperature, the PTFE tube fixed two capillaries tightly because its inner diameter was less than the outer diameter of two capillaries. So two capillaries were combined together by the PTFE tube. The assembled tube was inserted into a PTFE tee joint with 1.6 mm i.d. channel and immobilized by two screw caps. A two-way PTFE valve was connected to the side channel of the tee joint with a 0.5 mm i.d. PTFE tube. The valve was used to expel waste during sample loading.

Since the preconcentration and separation capillaries are detached, the preconcentration capillaries with different inner diameter can be selected according to the preconcentration factors. By using the tee joint and valve, a large volume of sample solution can be introduced and concentrated. In addition, the preconcentration and separation conditions can be optimized independently.

For the operation safety, the tee joint interface and two-way valve were made of PTFE material and installed inside the CE instrument, and the waste outflow tube was placed in a homemade insulating PTFE container during high voltage operation.

2.5. Preconcentration and separation procedure

A typical preconcentration and separation procedure is illustrated with Fig. 2.

The connected preconcentration and separation capillaries should be washed once daily. The washing steps are illustrated with Fig. 2a. The other ends of the preconcentration and separation capillary were connected to a flow system with two linkers (L, assembled by a female pipe adapter and a tube end fitting) and sealed by silicone. The flow system consisted of a peristaltic pump and a three-way solenoid valve. Firstly, three-way valve (V_1) switched to position 2

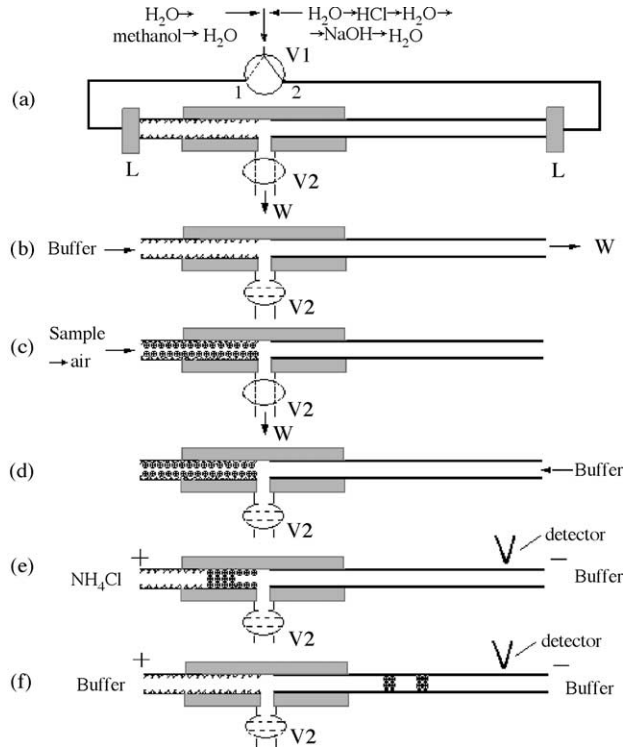

Fig. 2. Schematic diagrams of preconcentration and separation procedure. Arrows indicate flow direction. V, valve; L, linker; W, waste.

and V_2 opened, the separation capillary was washed in the order of H_2O (2 min), 1.0 mol/L HCl (5 min), H_2O (2 min), 1.0 mol/L NaOH (15 min), H_2O (2 min). The preconcentration capillary cannot be washed by 1.0 mol/L HCl or NaOH, so a three-way valve was adopted. Then V_1 turned to position 1, the preconcentration capillary was treated as follows: H_2O (5 min), methanol (10 min), H_2O (5 min). All the wastes were expelled through V_2. The second step was to fill the capillaries with buffer solution for 10 min by pressure (3.0 kPa) after disconnecting the linkers, closing V_2 and moving a buffer vial to the loading position of the preconcentration capillary inlet, as shown in Fig. 2b. Thirdly, by opening V_2 and moving a sample vial to the loading position, the sample solution was introduced into the preconcentration capillary with a flow rate of 0.03 mL/min (measured by liquid interface migration with a 0.2 mL serological pipette connected to V_2) for 10 min by pressure, the analyte ions were adsorbed by the ion-exchange stationary phase on the inner capillary wall, and the waste of sample matrix was removed through V_2, as shown in Fig. 2c. After loading sample solution and moving an empty vial to the loading position, the sample solution remaining in the preconcentration capillary was expelled with gas purging by pressure. The fourth step was to backfill the preconcentration capillary with buffer solution again. Closing V_2 and replacing an expelled buffer vial with an unpolluted buffer one at the end of the separation capillary, the separation and preconcentration capillaries were filled with buffer solution from the separation capillary, as shown in Fig. 2d. The fifth step was analyte elution. Replacing the loading position with an eluent vial and turning on the electrophoretic power supply for 10 s at 10 kV, the eluent plug was introduced into the preconcentration capillary by electroosmotic flow and the analyte ions was desorbed from the stationary phase, as shown in Fig. 2e. At last, the ends of preconcentration and separation capillary were replaced by the buffer and expelled buffer vial, respectively, and CZE separation was carried out at 18 kV, as shown in Fig. 2f.

Between two analytical operations, the capillaries were conditioned with the buffer solution; meanwhile, the ion-exchange stationary phase on the inner wall of preconcentration capillary could be regenerated. According to the experiment results, a satisfactory reproducibility can be obtained when the conditioning time of buffer solution was longer than 3 min. In order to ensure the precision, 4 min was selected as the conditioning time throughout the work. So the next analysis cycle repeated from the second step to the sixth step, viz., from Fig. 2b–f.

3. Results and discussion

3.1. Sample loading

3.1.1. Back-filling preconcentration capillary

For the elimination of air and the prevention of the analyte loss from the preconcentration capillary, a back-filling step was designed with the buffer solution filled from the separation capillary, as shown in Fig. 2d. In this step, a small amount of buffer solution can flow out from the inlet of the preconcentration capillary and may result in analyte loss. After the analyte loss investigation, it was found that the analyte loss in one drop of outflow from the preconcentration capillary inlet could be negligible. In addition, any front edge deterioration of the analyte peaks was not observed in the electropherograms, which might result from the analyte elution by the buffer solution during the back-filling step. It implies that the analytes cannot be eluted by the buffer solution because of the preconcentration capillary conditioned by the NaAc–HAc buffer solution.

In order to eliminate air in preconcentration capillary completely, the optimal back-filling times of different length and inner diameter preconcentration capillaries were measured with an electric current method. For example, the electrophoretic current could not be measured stably when the filling time was shorter than 2 s at 3.0 kPa for a 12 cm, 100 μm i.d. preconcentration capillary, because of residual air in the capillary. However, when the time was longer than 2 s, a stable current could be observed. So a back-filling time of 2 s was chosen for the preconcentration capillary in the fourth step.

3.1.2. Sample loading parameters

In the selection of sample loading conditions, the breakthrough time (viz. breakthrough volume) of preconcentration capillaries should be measured. When an analyte solution

with a fixed concentration is introduced directly into a detector, the observed absorbance corresponds to 100% signal value. However, when the analyte solution passes through an extraction column, the absorbance can be zero at the beginning of the introduction for all the analytes are retained in the column. Eventually, the column will be saturated by the analytes and then be broken through. When the absorbance reaches 10% signal value, the consumed volume of the analyte solution is defined as breakthrough volume, which multiplied by analyte concentration represents the capacity of the extraction column [28]. However, it is difficult to measure the breakthrough analytes volume directly in a short and open capillary connected to a longer separation one. So the breakthrough volume was measured indirectly, viz., those obtained from the analytes retained in the preconcentration capillaries rather than the analyte breakthrough. For a 12 cm, 100 μm i.d. preconcentration capillary, the breakthrough time was 16 min with a mixture solution of 84 μg/L propranolol and 210 μg/L metoprolol, and a flow rate of 0.03 mL/min. By increasing the loading time longer than 16 min, the peak areas of propranolol and metoprolol eluted were not enhanced because of the analyte loss after the column breakthrough. Here, the breakthrough time of 16 min corresponded to a breakthrough volume of 0.48 mL and breakthrough capacity of 0.53 nmol analytes, respectively. By reducing the loading flow rate to 0.02 and 0.01 mL/min with the same solution and capillary, the breakthrough time was 23 and 42 min, which corresponded to the breakthrough volume of 0.46 and 0.42 mL, respectively. Although the breakthrough volume of the preconcentration capillary decreased with the reduced flow rate of sample loading, the peak areas of the eluted analytes were almost the same. Compared the breakthrough volume at 0.01 mL/min with that at 0.03 mL/min, the analyte loss of the latter was about 12%. It implies that sample loading with lower flow rate can lead to more analytes adsorbed from a unit volume of sample solution in the preconcentration capillary because of mass transfer effect. However, when the flow rate is higher than 0.05 mL/min, the flow system cannot endure its pressure. So 16 min, 0.48 mL and 0.53 nmol can be considered as the breakthrough time, volume and capacity of the 12 cm, 100 μm i.d. preconcentration capillary, respectively. By considering preconcentration efficiency and analytical velocity, 0.03 mL/min was used as the flow rate of sample loading.

In the investigation of sample loading time, the loading flow rate was 0.03 mL/min and the mixed solution was the same one mentioned above. Normally, a high absorption response can be observed with a long loading time. In fact, the sample loading time is only limited by the ion-exchange capacity of the preconcentration capillary. With the loading time longer than breakthrough time, the capillary will be saturated by excessive analytes. Fig. 3 shows the variation of peak height and separation efficiency with different sample loading time. In the figure, the linear ranges of peak height could be observed up to the loading time of 12 min, but the separation efficiency had decreased obviously when the loading time was longer than 10 min. Furthermore, if the loading time were longer than 12 min, two analyte peaks could not be separated completely. It manifests that the sample loading time can influence not only analyte adsorption, but also their elution. It may result from the axial dispersion in the preconcentration capillary, thus broadening the eluted zones, as shown in Fig. 3.

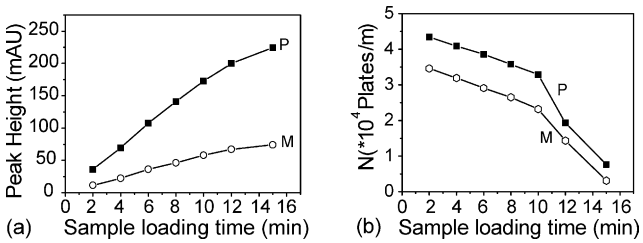

Fig. 3. Effect of sample loading time on peak height (a) and separation efficiency (b). P, propranolol; M, metoprolol. The running buffer solution is pH 4.0, 100 mmol/L NaAc–HAc and the sample solution contains 84 μg/L propranolol and 210 μg/L metoprolol. A 12 cm, 100 μm i.d. preconcentration capillary is employed and its inner wall is bonded with carboxyl cation-exchanger. The total and effective length of 50 μm i.d. separation capillary is 48 and 33 cm, respectively. The separation voltage is 18 kV and the detection wavelength is 214 nm. The sample loading flow rate is 0.03 mL/min and the elution is carried out with 2.0 mol/L NH_4Cl injected at 10 kV for 10 s.

The effect of sample loading time on the analyte peak height and separation efficiency of 6–14 cm length and 75–250 μm i.d. preconcentration capillaries was investigated. And the breakthrough time of the capillaries (except for larger than 150 μm i.d.) was in the range of 8–18 min. It was found that the separation efficiency deteriorated when the capillary inner diameter was larger than 150 μm. So the sample loading time of 10 min was allowable according to the results of peak height and separation efficiency of 12 cm, 100 μm i.d. preconcentration capillary, and breakthrough times of different dimension preconcentration capillaries.

3.2. On-line preconcentration

With 12 cm preconcentration capillary, 10 min sample loading time and 0.03 mL/min loading flow rate, the effect of preconcentration capillaries with different inner diameter was investigated on analyte enrichment. Fig. 4 displays the electropherograms with different inner diameter capillaries. It was found that the preconcentration capillary of large inner diameter could lead to the increase of ionic exchange capacity and enhancement of preconcentration factor. According to the results, the peak height with 100 μm i.d. preconcentration capillary was larger than that with 75 μm i.d capillary. However, by increasing inner diameter to 150 and 250 μm, the separation efficiency and resolution of propranolol and metoprolol decreased obviously. It may result from the dispersion of eluting volume in large inner diameter capillaries, and then lead to the axial dispersion of separation zone further. Therefore, 100 μm i.d. preconcentration capillary was proved to be favorable and was adopted in this work.

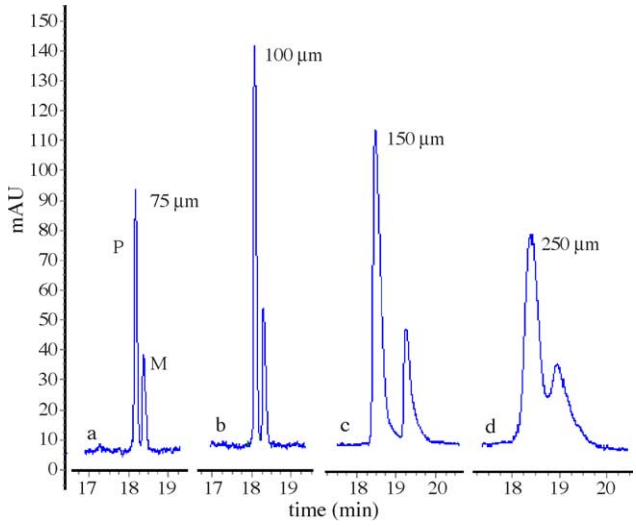

Fig. 4. Electropherograms of preconcentration capillary with different inner diameter. a, b, c, and d represent the capillary inner diameter of 75, 100, 150 and 250 μm in turn. Propranolol (P) is 64 μg/L and metoprolol (M) is 160 μg/L. The length of preconcentration capillaries is 12 cm. Sample loading time is 10 min with a flow rate of 0.03 mL/min. Other conditions are the same as in Fig. 3.

The influence of 100 μm i.d. preconcentration capillary length from 6 to 14 cm on the peak height, separation efficiency and retention time of propranolol and metoprolol was tested with the mixed solution containing 84 μg/L propranolol and 210 μg/L metoprolol. Other conditions were the same as those mentioned above. When the length of preconcentration capillary increased to 12 cm, the breakthrough capacity of preconcentration capillaries also increased and, as a result, the peak heights enhanced because of more analyte cations adsorbed and eluted. However, by increasing the preconcentration capillary length from 12 to 14 cm, a similar peak height was observed. It indicates that the ion-exchange capacity of preconcentration capillaries less than 12 cm has been saturated with the sample loading time of 10 min, so the peak height can be enhanced by increasing the capillary length from 6 to 10 cm. Nevertheless, the peak height cannot enhance obviously with the same loading time when the length of preconcentration capillary is longer than 12 cm. In addition, by increasing the sample loading time, the separation resolution decreases obviously. For instance, when the sample loading time was 12 min, the baseline separation could not be achieved between propranolol and metoprolol with 12 or 14 cm preconcentration capillaries. Meanwhile, the retention time was also delayed from 11.5 to 19.8 min for propranolol with the preconcentration capillary length from 6 to 14 cm under 18 kV. Therefore, 12 cm length was chosen for the preconcentration capillary.

3.3. Analyte elution

An efficient elution can be carried out with a small eluent volume to avoid zone broadening and to obtain a high enrichment factor; in addition, it can regenerate the ion-exchange stationary phase conveniently after each operation. Na^+, NH_4^+ and H^+ are often used as eluting ions for cation-exchange. However, the covalent siloxane bond of the stationary phase may be damaged by strong acidity of eluting solutions. In this paper, 0.5–2.5 mol/L NaCl and NH_4Cl were investigated as the eluent for the analytes. Fig. 5 shows the effect of NH_4Cl (a) and NaCl (b) concentration on the peak height and separation efficiency. It can be found that the NH_4Cl eluent is better than NaCl one for propranolol and metoprolol elution. When NH_4Cl concentration increased to 2.0 mol/L, the peak height reached maximal. And then kept almost the same level by increasing the concentration further. However, the variation of separation efficiency for the analytes was not observed obviously by changing the eluent concentration from 0.5 to 2.0 mol/L.

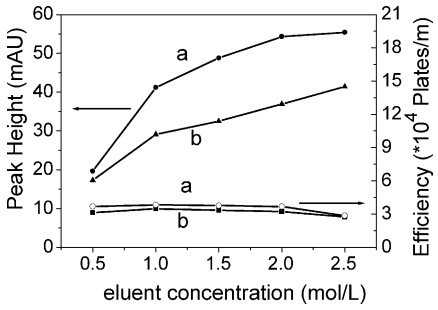

Fig. 5. Effect of NH_4Cl (a) and NaCl (b) concentration on the peak height and separation efficiency of propranolol. Propranolol concentration is 32 μg/L. Sample loading time is 10 min with a flow rate of 0.03 mL/min and eluting time is 10 s at 10 kV. Other conditions are the same as in Fig. 3.

Since the inner wall of preconcentration capillary was bonded with cation-exchange group of glycolic acid, electroosmotic flow (EOF) generated by preconcentration capillary was smaller than that in untreated capillary, so the EOF of whole system was mainly generated by separation capillary. The eluent volume can be decided by the product of eluting time and voltage. For a 12 cm, 100 μm i.d. preconcentration capillary and an eluting voltage 10 kV fixed, by increasing the eluting time to 10 s, the enhancement of the analyte peak height was observed. However, if the time was longer than 10 s, the peak height increased inconspicuously, moreover, the separation efficiency decreased rapidly for excessive zone broadening. The former may result from the incomplete elution and the latter manifests the broadening of eluting zone. So 2.0 mol/L NH_4Cl was chosen as the eluting solution and the eluting time was 10 s at 10 kV in this work.

3.4. CZE separation

Buffer system, including its pH and concentration, can influence CZE separation significantly. According to our previous work [29], pH 4.0 NaAc–HAc buffer solution was also used in this work. The effects of the buffer concentration on peak height and separation efficiency are illustrated with Fig. 6. By increasing the buffer concentration, the peak height

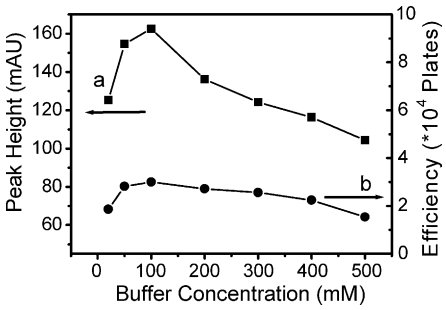

Fig. 6. Effect of buffer concentration on peak height (a) and separation efficiency (b) of propranolol. The concentration of propranolol is 84 μg/L. A 12 cm, 100 μm i.d. preconcentration capillary is adopted. Other conditions are the same as in Fig. 5.

of propranolol enhanced firstly and then reduced. From the experimental data in the figure, 100 mmol/L NaAc was a suitable concentration for the analyte separation. Meanwhile, the separation efficiency increased with the increase of NaAc concentration up to 100 mmol/L. In addition, the curves of peak height and separation efficiency became declivous when the buffer concentration increased further. It may be the effect of Joule heating produced by large current of high buffer concentration. Therefore, 100 mmol/L NaAc was chosen as the buffer solution in this work.

3.5. Analytical characteristics

According to the separation voltage experiments, 15 kV was used for a CZE separation without preconcentration, and 18 kV was adopted for the proposed preconcentration method. It can be found that their electric field strengths are similar and about 300 V/cm. With the selected conditions discussed above, two electropherograms of propranolol and metoprolol are illustrated by comparing the proposed method with normally electrokinetic injection, as shown in Fig. 7. The limit of detection (LOD, 3σ of continuous 11 noise peaks of baseline near the analyte peaks) was 0.1 and 0.5 mg/L for propranolol and metoprolol, respectively, by normally electrokinetic injection at 6 kV for 8 s and with a sample solution containing 32 mg/L propranolol and 80 mg/L metoprolol (Fig. 7b). However, the LODs of 0.02 and 0.1 μg/L were obtained for propranolol and metoprolol, respectively, by the preconcentration injection (Fig. 7a), in which a sample solution of 84 μg/L propranolol and 210 μg/L metoprolol was introduced into a 12 cm, 100 μm i.d. preconcentration capillary with a flow rate of 0.03 mL/min for 10 min. Based on the similar separation efficiencies, the preconcentration factors were calculated and the conditions of sample and eluent injections were selected, viz., the electrokinetic injection at 6 kV for 8 s without preconcentration and the eluent introduction at 10 kV for 10 s with the preconcentration. It indicates a 5000-fold improvement in the detection sensitivity for both of propranolol and metoprolol can be obtained under the similar separation efficiencies, which were 2.8×10^4/m and 2.2×10^4/m for propranolol and metoprolol with the proposed preconcentration, and 3.1×10^4/m and 2.3×10^4/m for the analytes with the normally electrokinetic injection, respectively. The analytical reproducibility of propranolol and metoprolol was 3.7 and 3.1% ($n = 5$), respectively.

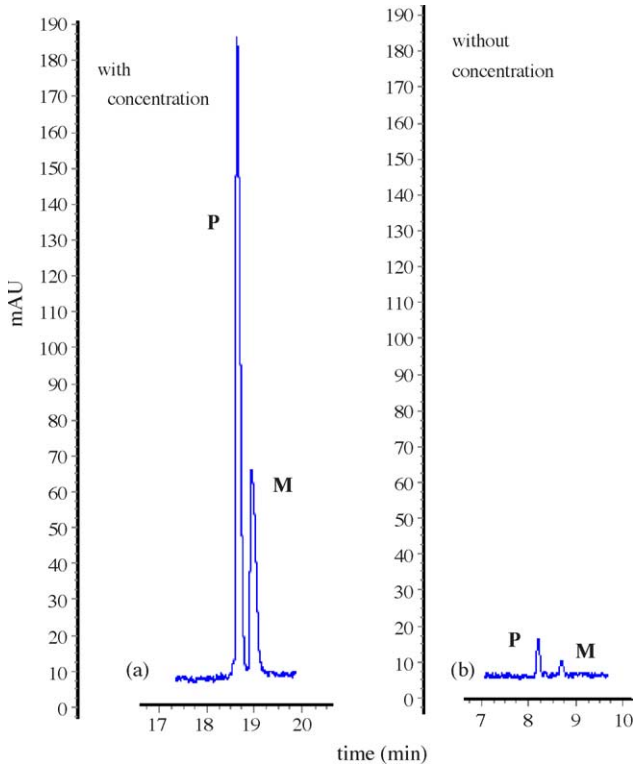

Fig. 7. Electropherograms of propranolol (P) and metoprolol (M) with (a) and without (b) on-line preconcentration. Conditions: (a) The preconcentration capillary is 12 cm in length and 100 μm i.d.; total and effective length of 50 μm i.d. separation capillary is 48 and 33 cm, respectively; the sample solution contains 84 μg/L propranolol and 210 μg/L metoprolol; the sampling flow rate and loading time is 0.03 mL/min and 10 min, respectively; the elution solution is 2.0 mol/L NH_4Cl injected at 10 kV for 10 s; the running buffer solution is pH 4.0, 100 mmol/L NaAc–HAc; the separation voltage is 18 kV; and UV detection wavelength is 214 nm. (b) The sample solution contains 32 mg/L propranolol and 80 mg/L metoprolol, respectively; the electrokinetic injection is carried out at 6 kV for 8 s; separation voltage is 15 kV; The separation capillary, running buffer solution and detection wavelength are the same as in (a).

4. Conclusions

The on-line cation-exchange preconcentration method with a tee joint interface for CZE separation has been developed in this paper. Since the preconcentration and separation capillary are connected by the interface, large sample volume can be loaded in the preconcentration capillary and sample waste after preconcentration can be expelled through the interface, different dimensions of preconcentration capillaries can be selected to achieve a satisfied preconcentration, and the treatment of two capillaries can be independent and convenient. Meanwhile, the sampling volume is limited by

the capacity of preconcentration capillary and can reach a milliliter level larger than a mono-capillary method. A 5000 times of preconcentration factor can be obtained for both the model cations of propranolol and metoprolol. The methodology can improve the detection sensitivity of CE–UV to less than μg/L, thus it can be employed as a trace analysis technique for CE. By bonding preconcentration capillaries with different solid phase and using different eluting conditions, the proposed interface can be adopted for the enrichment of anions and neutral compounds for CE. This on-line preconcentration method can be improved and performed automatically by using a high-insulation solenoid valve (V_2).

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References

[1] M. Albin, P.D. Grossman, S.E. Moring, Anal. Chem. 65 (1993) 489A.
[2] L.Y. Zhu, H.K. Lee, Anal. Chem. 73 (2001) 3065.
[3] S. Locke, D. Figeys, Anal. Chem. 72 (2000) 2684.
[4] S.J. Chen, M.L. Lee, Anal. Chem. 72 (2000) 816.
[5] P. Britiz-McKibbin, M.G. Bebault, D.D.Y. Chen, Anal. Chem. 72 (2000) 1729.
[6] P. Britiz-McKibbin, D.D.Y. Chen, Anal. Chem. 72 (2000) 1242.
[7] P. Britiz-McKibbin, A.R. Kranack, A. Paprika, D.D.Y. Chen, Analyst 123 (1998) 1461.
[8] Y. Xiong, S.R. Park, H. Swerdlow, Anal. Chem. 70 (1998) 3605.
[9] S. Park, C.E. Lunte, J. Microcolumn Sep. 10 (1998) 511.
[10] D.M. Osbourn, D.J. Weiss, C.E. Lunte, Electrophoresis 21 (2000) 2768.
[11] D.J. Weiss, K. Saunders, C.E. Lunte, Electrophoresis 22 (2001) 59.
[12] C.X. Cao, Y.Z. He, M. Li, Y.L.T. Qian, L. Yang, Q.S. Qu, S.L. Zhou, W.K. Chen, J. Chromatogr. A 952 (2002) 39.
[13] C.X. Cao, Y.Z. He, M. Li, Y.L.T. Qian, M.F. Gao, L.H. Ge, S.L. Zhou, L. Yang, Q.S. Qu, Anal. Chem. 74 (2002) 4167.
[14] P. Britiz-McKibbin, K. Otsuka, S. Terabe, Anal. Chem. 74 (2002) 3736.
[15] J.P. Quirino, S. Terabe, Anal. Chem. 72 (2000) 1023.
[16] L.Y. Zhu, C.H. Tu, H.K. Lee, Anal. Chem. 73 (2001) 5655.
[17] L.Y. Zhu, C.H. Tu, H.K. Lee, Anal. Chem. 74 (2002) 5820.
[18] L. Arce, P. Kuban, A. Ríos, M. Valcárcel, B. Karlberg, Anal. Chim. Acta 390 (1999) 39.
[19] M. Novič, M. Guček, J. Chromatogr. A 868 (2000) 135.
[20] E. Bonneil, K.C. Waldron, J. Chromatogr. B 736 (1999) 273.
[21] M. Petersson, K.G. Wahlund, S. Nilsson, J. Chromatogr. A 841 (1999) 249.
[22] M.C. Breadmore, A.S. Palmer, M. Curran, Anal. Chem. 74 (2002) 2112.
[23] M.C. Breadmore, M. Macka, N. Avdalovic, P.R. Haddad, Anal. Chem. 73 (2001) 820.
[24] http://www.windrug.com/pic/30/11/75/064.htm.
[25] http://www.drugs.com/MMX/Propranolol_Hydrochloride.html.
[26] X. Huang, C. Horváth, J. Chromatogr. A 788 (1997) 155.
[27] J. Zhang, S.H. Zhang, C. Horváth, J. Chromatogr. A 953 (2002) 239.
[28] J.A. Bert Ooms, G.J. Mark Van Gils, A.R. Duinkerken, O. Halmingh, Am. Lab. 9 (2000) 52.
[29] M. Li, Y.Z. He, W.E. Gan, Chin. J. Chromatogr. 19 (2) (2001) 176.