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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-