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Stacking and separation of fluorescent derivatives of amino acids by micellar electrokinetic chromatography in the presence of poly(ethylene oxide)

Tai-Chia Chiu [a], Huan-Tsung Chang [a,b,*]

[a] *Department of Chemistry, National Taiwan University, Taipei, Taiwan*
[b] *Department of Natural Science Education, National Taitung University, Taitung, Taiwan*

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Abstract

A new approach for the analysis of large-volume naphthalene-2,3-dicarboxaldehyde (NDA) derivatives of amino acids by micellar electrokinetic chromatography (MEKC) in conjunction with a purple light-emitting diode-induced fluorescence detection is described. In order to optimize resolution, speed, and stacking efficiency, a discontinuous condition is essential for the analysis of NDA-amino acid derivatives. The optimum conditions use 2.0 M TB (pH 10.0) buffer containing 40 mM sodium dodecyl sulfate (SDS) to fill the capillary, deionized water to dilute samples, and 200 mM TB (pH 9.0) containing 10 mM SDS to prepare 0.6% poly(ethylene oxide) (PEO). Once high voltage is applied, PEO solution enters the capillary via electroosmotic flow and SDS micelles interact and thus sweep the NDA-amino acid derivatives having smaller electrophoretic mobilities than that of SDS micelles in the sample zone. When the aggregates between SDS micelles and NDA amino acid derivatives enter PEO zone, they are stacked due to decrease in electric field and increases in viscosity. Under the optimum conditions, the concentration and separation of 0.53-μL 13 NDA-amino acid derivatives that are negatively charged has been demonstrated by using a 60-cm capillary, with the efficiencies 0.3–9.0 × 10^5 theoretical plates and the LODs at signal-to-noise ratio 3 ranging from 0.30 to 2.76 nM. When compared to standard injection (30-cm height for 10 s), the approach allows the sensitivity enhancements over the range of 50–800 folds for the derivatives. The new approach has been applied to the analysis of a red wine sample, with great linearity of fluorescent intensity against concentrations ($R^2 > 0.98$) and the RSD (three repetitive runs in one day) values of the migration times for the ten identified amino acids less than 2.8%.

Keywords: Amino acids; Light-emitting diode-induced fluorescence detection; Micellar electrokinetic chromatography; Naphthalene-2,3-dicarboxaldehyde; Polymer solutions; Sodium dodecyl sulfate; Sweeping

1. Introduction

Capillary electrophoresis (CE) is a powerful separation technique that possesses a number of advantages, including high separation efficiency, short analysis time, and small amounts of reagents and sample required [1–3]. Although CE using optical detectors has been well demonstrated for the separation of biological molecules such as proteins, nucleic acids, peptides, and amino acids [4–6], its sensitivity is inherent from the limited optical path length (e.g. 75 μm) and extremely small sample volumes (usually < 10 nL) injected to the capillary. Over the past few decades, techniques such as field amplified sample stacking, pH-mediate sample stacking, isotachophoresis, sweeping, and use of polymer solutions have been developed for improving the sensitivity [7–13]. Differences in the physicochemical properties (such as pH, ionic strength, and viscosity) of the sample matrix and the background electrolytes are essential for stacking of the analytes. Analyte molecules migrate with faster speed in the sample zone than those in the background electrolyte zone, and thus they are stacked at the boundary either front or back of the sample zone to form sharp sample zones.

Since Quirino and Terabe introduced the concept of sample sweeping almost 10 years ago, sweeping has become one of the most important techniques for improving the sensitivity of analytes, especially neutral solutes [7,14,15]. Sweeping involves picking and accumulation of analyte molecules in micellar electrokinetic chromatography (MEKC) in which micelles act as a pseudostationary phase for sweeping the analytes from a long injected sample zone into a narrow band and then for separat-

* Corresponding author: Department of Chemistry, National Taiwan University, Taipei, Taiwan. Tel.: +886 2 33661171; fax: +886 2 33661171.
E-mail address: changht@ntu.edu.tw (H.-T. Chang).

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ing the concentrated sample zones during electrophoresis [7,14]. Theoretically, neutral analytes having great partition coefficients in micelles can be effectively stacked and separated in MEKC [16,17].

In CE, laser-induced fluorescence (LIF) provides better sensitivity for amino acids than does UV–vis absorption detection. However, besides three aromatic amino acids, most amino acids are not fluorescent in nature. Thus, derivatization of amino acids with reagents to form stable and highly fluorescent derivatives is required in CE-LIF [18–24]. When using a low-cost and purple light-emitting diode (LED) as the light source of CE, naphthalene-2,3-dicarboxaldehyde (NDA) has been found to be practical for derivatization of amino acids. The maximum wavelengths of the two absorption bands of NDA-amino acid derivatives are at 420 and 440 nm and the emission wavelength is at 490 nm [25]. Owing to low partition coefficients of NDA-amino acid derivatives in sodium dodecyl sulfate (SDS) and cetyltrimmethylammonium bromide (CTAB), two most commonly used micelles in MEKC, stacking efficiencies and separation resolution in MEKC of the derivatives are not impressive [26]. In order to further improve stacking efficiency and resolution, we tested analysis of amino acids by MEKC in the presence of poly(ethylene oxide) (PEO). When using PEO, SDS is superior over CTAB because SDS micelles migrate into neutral PEO zone, which were expected to achieve great resolution and stacking efficiency based on our previous experiences [27,28].

In this study, we proposed a simple stacking and separation approach for the analysis of NDA-amino acid derivatives by CE in conjunction with LED induced fluorescence (CE-LEDIF). Several important electrophoretic parameters such as SDS concentration, PEO concentration, and injection length with respect to separation resolution and stacking efficiency of the CE-LEDIF approach were evaluated. The practicality of the proposed approach was validated by the determination of amino acids in red wine samples.

2. Experimental

2.1. Chemicals

DL-Amino acids, including alanine (Ala), asparagine (Asn), aspartic acid (Asp), leucine (Leu), cystine (Cys), glutamic acid (Glu), glutamine (Gln), glycine (Gly), histidine (His), serine (Ser), threonine (Thr), tyrosine (Tyr), valine (Val), SDS, and sodium cyanide (NaCN) were obtained from Sigma (St Louis, MO, USA). PEO (M_r 8.0×10^6 g mol^{-1}) was obtained from Aldrich (Milwaukee, WI, USA). NDA was obtained from Tokyo Chemical Industry (Tokyo, Japan), and was dissolved in analytical grade methanol that was purchased from Malinckrodt Baker (Phillipsburg, NJ, USA). Sodium tetraborate was obtained from Acros Organic (Geel, Belgium). Tris(hydroxymethyl)aminomethane (Tris) was a product from J.T. Baker (Phillipsburg, NJ, USA). Boric acid was purchased from Riedel-deHaën (Buchs, Switzerland). The stock solution of 400 mM Tris-borate (TB) was prepared by dissolving 24.23 g of Tris in 500-mL aqueous solution that was adjusted with suitable amounts of boric acid to pH 9.0. Unless otherwise noted, the molarity of Tris represents that of TB buffer. PEO (0.015–0.75 g) was gradually added to each of prepared 200 mM TB solutions (pH 9.0). During the addition of PEO, a magnetic stirring rod was used to produce a well-homogeneous suspension. After the addition was completed, the solutions were stirred for at least 12 h. Prior to use for CE separation, the solutions were degassed with a vacuum system in an ultrasonic tank for 10 min [29]. Red wine (Gracia de Chile, Las Condes, Santiago, Chile) was purchased from a local store.

2.2. Apparatus

CE-LEDIF system (CE/LIF, Model: 2100) was purchased from Pebio Scientific Company (Taipei, Taiwan). Fused-silica capillary with 75 μm I.D. and 365 μm O.D. was purchased from Polymicro Technologies (Phoenix, AZ, USA). The capillary length is either 40 cm (30 cm to detector) or 60 cm (50 cm to detector). A DV-E viscometer (Brookfield Engineering Laboratories, Middleboro, MA, USA) was employed to measure the viscosity of PEO solutions in a constant-temperature bath at 25.0 ± 0.2 °C. All measurements were performed in triplicate.

2.3. Sample handling and derivatization procedure

The derivatization procedure of amino acids with NDA in the presence of cyanide was modified from the literature [30]. The derivatization was performed in 1.5-mL centrifuge tubes. For standard NDA-amino acid derivative samples, 1.0-mL reaction mixtures (pH 9.3) containing amino acids (10 μM), NaCN (0.2 mM), NDA (0.2 mM), and sodium tetraborate (1.0 mM) were prepared. For derivatization of amino acids in red wine samples, 1.0-mL reaction mixtures (pH 9.3) containing 10-μL red wine, NaCN (0.2 mM), NDA (0.2 mM), and sodium tetraborate (1.0 mM) were prepared. The mixtures were allowed to react for 20 min at room temperature and, if necessary, were diluted to suitable concentrations with deionized water before injecting into the capillary.

2.4. Procedures

Prior to analysis, capillaries were filled with high concentration of TB (2.0 M) buffer (pH 10.0) containing SDS (0–75 mM). Hydrodynamic injection was applied at 30-cm height (the difference in the exit and entrance ends of the capillary) for 10 to 360 s. After injection, the capillaries were immersed in the PEO solutions and the separations were conducted at 15 kV. After each run, PEO in the capillaries were flushed out and the capillaries were equilibrated with 0.5 M NaOH at 1 kV for 1 s. The capillaries were then filled with TB buffers containing SDS. This treatment was quite successful to regenerate high and reproducible electroosmotic flow (EOF) (RSD < 1.5%). During separation, PEO solution that was prepared in TB buffer containing SDS was introduced to the capillary by EOF from the anodic side. The sample volume was estimated according to our previous method by using NDA-amino acid derivatives as a fluorescent marker [31]. The time of the baseline shift indicates