Journal of Chromatography A, 1445 (2016) 158–165

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

CEval: All-in-one software for data processing and statistical evaluations in affinity capillary electrophoresis☆

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ARTICLE INFO

Article history:
Received 13 February 2016
Received in revised form 31 March 2016
Accepted 1 April 2016
Available online 2 April 2016

Keywords:
Affinity capillary electrophoresis
Complexation
Cyclodextrin
Electrophoretic mobility
Software
Statistical evaluation

ABSTRACT

We introduce CEval software (downloadable for free at echmet.natur.cuni.cz) that was developed for quicker and easier electrophoregram evaluation and further data processing in (affinity) capillary electrophoresis. This software allows for automatic peak detection and evaluation of common peak parameters, such as its migration time, area, width *etc.* Additionally, the software includes a nonlinear regression engine that performs peak fitting with the Haarhoff-van der Linde (HVL) function, including automated initial guess of the HVL function parameters. HVL is a fundamental peak-shape function in electrophoresis, based on which the correct effective mobility of the analyte represented by the peak is evaluated. Effective mobilities of an analyte at various concentrations of a selector can be further stored and plotted in an affinity CE mode. Consequently, the mobility of the free analyte, μ_A, mobility of the analyte-selector complex, μ_{AS}, and the apparent complexation constant, K', are first guessed automatically from the linearized data plots and subsequently estimated by the means of nonlinear regression. An option that allows two complexation dependencies to be fitted at once is especially convenient for enantioseparations. Statistical processing of these data is also included, which allowed us to: i) express the 95% confidence intervals for the μ_A, μ_{AS} and K' least-squares estimates, ii) do hypothesis testing on the estimated parameters for the first time.

We demonstrate the benefits of the CEval software by inspecting complexation of tryptophan methyl ester with two cyclodextrins, neutral heptakis(2,6-di-*O*-methyl)-β-CD and charged heptakis(6-*O*-sulfo)-β-CD.

1. Introduction

Common software for data acquisition and evaluation in capillary (zone) electrophoresis (CE) is designed for analytical and quality control applications. Peak identification, integration and calibration; calculation of separation characteristics such as peak resolution and asymmetry; or the HPLC-equivalent measures such as the number of theoretical plates are commonly available. The next step may be to gain separation parameters necessary for modelling and thus understanding of the separation processes in CE and the subsequent optimization of the separation. Alternatively, gaining the physical-chemical data from the measured electrophoregram may even be the prime interest of the user. Both situations are typical for affinity capillary electrophoresis (ACE) [1–3], which however has its additional particularities concerning data evaluation in CE. This method extends applicability of the traditional CE to complex mixtures of structurally related analytes, often enantiomers, including neutral compounds. The separation mechanism in ACE is based not only on the difference in the electrophoretic mobilities of the analytes of their own but also on their complexation with the so-called selector. Complexation constants and electrophoretic mobilities of the analyte-selector complexes are often determined. These parameters provide insight into the separation mechanism [4] and serve for analytical method optimisations [5], as well as input parameters for computer simulations [6,7]. It should be noted that in the case of ACE the obtained parameters may only be conditional (upon pH, background electrolyte composition, ionic strength) or may not even represent any actual physical-chemical constants [8]. Nevertheless, such parameters can still be useful for analytical method optimisations [9].

This paper introduces CEval, an all-in-one open-source software solution for data evaluation in CE and ACE. When developing this software, the prime intention was to help the analysts with

☆ Selected paper from the 22nd International Symposium on Electro- and Liquid Phase-Separation Techniques (ITP2015) and the 8th Nordic Separation Science (NoSSS) Symposium, 30 August-3 September 2015, Helsinki, Finland.

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http://dx.doi.org/10.1016/j.chroma.2016.04.004
0021-9673/

evaluating (possibly conditional) complexation constants and moblities of complexes from the ACE electropherogram under the 1:1 complexation stoichiometry. Such evaluations are often encountered in the related literature but remain a tedious and time-consuming process. Additionally, reading the electromigration time from the apex of the peak, linearization of the data, ignoring viscosity effects and making conclusions without proper statistical testing are all faults that still prevail in the literature and are addressed by the CEval software. The entire evaluation procedure consists of several steps, each of which had to be done in a separate piece of software previously. These steps are:

- Assessment of the peak shape, reading the proper migration time from this peak shape, and calculating the effective electrophoretic mobility of an analyte.
- Plotting the effective mobilities as a function of the selector concentration and performing nonlinear regression on these data.
- Statistical evaluation of the results.

Each of these steps has its own specifics, which–for the sake of consistency and better readability–we are not addressing in this introduction but will discuss along with the results.

Secondly, we utilize this software for statistical evaluation of the equilibria parameters determined in ACE experiments for the first time.

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical-grade purity. Lithium hydroxide monohydrate, L-tryptophan methyl ester hydrochloride (L-Trp-Me), D-tryptophan methyl ester hydrochloride (D-Trp-Me), heptakis(6-O-sulfo)-β-cyclodextrin heptasodium salt (S-β-CD) and dimethylsulfoxide (DMSO) were purchased from Sigma-Aldrich (Prague, Czech Republic). Heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CD) was purchased from Cyclolab (Budapest, Hungary). Acetic acid was produced by Lachema (Brno, Czech Republic). NaOH solution used for rinsing the capillary was purchased from Agilent Technologies (Waldbronn, Germany). Water used for preparation of all solutions was deionized by Rowapur and Ultrapursystem (Watrex, Prague, Czech Republic). The IUPAC buffers, pH 4.005 and 7.000 (Radiometer, Copenhagen, Denmark), were used for calibration of the pH meter.

2.2. Instrumentation

All CE experiments were performed using an Agilent 7100 capillary electrophoresis system operated by ChemStation software (Agilent Technologies, Waldbronn, Germany). The instrument was equipped with a built-in photometric diode array detector (DAD). Fused silica capillary (50 μm I.D., 375 μm O.D.) was provided by Polymicro Technologies (Phoenix, AZ, USA). The total length of the capillary and distance from inlet to DAD was 50.2 cm and 41.7 cm, respectively. The pH meter PHM 240 pH/ION Meter (Radiometer, Copenhagen, Denmark) was employed to measure pH.

2.3. Experimental conditions and procedures

All experiments were measured in buffer composed of lithium hydroxide and acetic acid (LiOH/Ac buffer). Buffer containing 240 mM acetic acid and 120 mM lithium hydroxide (experimental pH = 4.72, ionic strength (IS) = 120 mM) was employed for all measurements with neutral DM-β-CD. Concentration of DM-β-CD in BGE was varied from 0 mM to 30 mM. Stock solution of 30 mM DM-β-CD was prepared by dissolving appropriate amount of the selector in the stock buffer. The background electrolytes (BGE) containing lower concentrations of the selectors were prepared by diluting the 30 mM solution with the LiOH/Ac buffer.

Increasing IS of BGEs caused by presence of 0 mM to 15 mM charged S-β-CD was compensated by appropriate reduction of LiOH/Ac buffer concentration. Buffers for the individual S-β-CD concentration levels were designed by means of PeakMaster software [10]. Composition of individual buffers, as well as their IS and buffer capacity is shown in Table S1 of the Supplementary material. The buffers were prepared by dilution of original 120 mM/240 mM LiOH/Ac buffer. Appropriate amount of S-β-CD were dissolved in particular buffers to obtain BGE with 0 mM to 15 mM of S-β-CD and constant IS = 120 mM.

1 mg/mL stock solutions of individual enantiomers of tryptophan methyl ester hydrochloride (L- and D-Trp-Me) were prepared by dissolving appropriate amounts of the analyte in the 120 mM/240 mM LiOH/Ac buffer. The samples were prepared by mixing 5 μL of the stock solution of the enantiomer (1 mg/mL in the stock buffer), 5 μL of 20 % aqueous solution of DMSO (EOF marker) and 100 μL of the running buffer. All the solutions were filtered using syringe filters, pore size 0.45 μm (Sigma-Aldrich, Prague, Czech Republic).

The signal was collected at 217 nm. The capillary was thermostated at 25 °C. New capillary was flushed with deionized water for 5 min, then with 0.1 M NaOH for 15 min and again with water for 10 min. Prior to each run, the capillary was flushed for 3 min with the relevant BGE. The samples were injected hydrodynamically at 25 × 5 mbar s. A voltage of only 15 kV (anode on the injection side) was applied to keep the electric current always below 50 μA, to avoid the effects of excessive Joule heating. Due to the slow velocity of EOF, additional pressure of 30 mbar was applied during electrophoretic measurements. Each experiment was repeated four times.

The data were evaluated i) by the common procedure, using the mathematical software Origin8.1 (OriginLab Corporation, Northampton, USA), the statistical package R [11], and Microsoft Office Excel 2010 (Microsoft) and ii) by CEval, the new software presented in this paper.

3. Results and discussion

The collected data were evaluated both by the common procedure and by the CEval software.

The common procedure consists of:

i. Export of the data from the ChemStation software to a text file.
ii. Loading of the text file in the Origin 8.1 software (OriginLab Corporation, Northampton, USA).
iii. Defining of the peak and performing a nonlinear regression on it in order to assess its proper shape and migration time.
iv. Transferring of the migration times of the analytes and EOF into a Microsoft Excel spreadsheet and calculating effective mobilities of the peaks.
v. Loading of the effective mobilities back in the Origin 8.1 and plotting them against concentrations of a selector.
vi. Performing a nonlinear regression on these data in order to observe mobility of the complex and complexation constants.

If needed the latter nonlinear regression (steps v–vi) were performed by the means of R statistical package [11] rather than the Origin software.

All steps mentioned above are performed by the CEval software, which additionally estimates the regression parameters for both