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Generalized model of electromigration with 1:1 (analyte:selector) complexation stoichiometry: Part II. Application to dual systems and experimental verification

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ABSTRACT

Interactions among analyte forms that undergo simultaneous dissociation/protonation and complexation with multiple selectors take the shape of a highly interconnected multi-equilibrium scheme. This makes it difficult to express the effective mobility of the analyte in these systems, which are often encountered in electrophoretical separations, unless a generalized model is introduced. In the first part of this series, we presented the theory of electromigration of a multivalent weakly acidic/basic/amphoteric analyte undergoing complexation with a mixture of an arbitrary number of selectors. In this work we demonstrate the validity of this concept experimentally. The theory leads to three useful perspectives, each of which is closely related to the one originally formulated for simpler systems. If pH, IS and the selector mixture composition are all kept constant, the system is treated as if only a single analyte form interacted with a single selector. If the pH changes at constant IS and mixture composition, the already well-established models of a weakly acidic/basic analyte interacting with a single selector can be employed. Varying the mixture composition at constant IS and pH leads to a situation where virtually a single analyte form interacts with a mixture of selectors. We show how to switch between the three perspectives in practice and confirm that they can be employed interchangeably according to the specific needs by measurements performed in single- and dual-selector systems at a pH where the analyte is fully dissociated, partly dissociated or fully protonated. Weak monoprotic analyte (R-flurbiprofen) and two selectors (native β-cyclodextrin and monovalent positively charged 6-monodeoxy-6-monoamino-β-cyclodextrin) serve as a model system.

1. Introduction

Selectors are widely used in capillary electrophoresis (CE). A variety of them are available for CE; however, cyclodextrins (CDs) are the most popular, especially when used as chiral selectors [1–9]. Along with their increasing applications in analytical chemistry, theoretical models have been developed describing the electromigration of fully dissociated, neutral, or partly dissociated analytes interacting with one selector [10–23]. Several models dealing with dual- and multi-selector systems have also been published [24–40] (we recently summarized them in a review [41]). However none of these models takes into account the possible protonation equilibria of the analyte along with its simultaneous interaction with multiple selectors.

In Part I of this series, we presented the complete theory of the electromigration of multivalent weak acidic/basic analytes undergoing complexation with a mixture of selectors. The theory results in a generalized model of selector-assisted CE with 1:1 (analyte:selector) complexation stoichiometry (the overall multi-free-analyte-form multi-selector model, $M_A M_S$ model) [42]. In this model, L protonated/deprotonated states of the free (uncomplexed) analyte A_{i0} ($i = \{1, \ldots, L\}$) are considered to be present in a mixture of N selectors. Each of the free analyte forms interacts with each of the selectors S_j ($j = \{1, \ldots, N\}$). The interaction between the ith free analyte form and the jth selector is characterized by a complexation constant K'_{ij} and results in the formation of a complex with mobility μ_{ij}. The free analyte forms have individual mobilities μ_{i0}. Consequently, the effective mobility of the analyte μ_{eff} is:

$$\mu_{\mathrm{eff}} = \frac{\sum_{i=1}^{L} \chi_{i0}\mu_{i0} + \sum_{i=1}^{L}\left(\chi_{i0}\sum_{j=1}^{N} K'_{ij}\mu_{ij}\chi_{Sj}\right)\cdot c_{\mathrm{tot}}}{1 + \sum_{i=1}^{L}\left(\chi_{i0}\sum_{j=1}^{N} K'_{ij}\chi_{Sj}\right)\cdot c_{\mathrm{tot}}} \quad (1)$$

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where χ_{i0} are the molar fractions of the free analyte forms (related to the total amount of the free analyte), χ_{Sj} are the molar fractions of the selectors in the selector mixture and c_{tot} is the total concentration of the selector mixture. The complexation controls only the total amount of free analyte, but not its distribution into its various forms, A_{i0}. Thus the molar fractions χ_{i0} are determined entirely by the dissociation constants of the free analyte. The M_AM_S model (1) is valid under the following conditions: (i) only 1:1 complexation (analyte:selector) takes place; (ii) the kinetics of the complexation are much faster than those of the electrophoretic movement; (iii) the ionic strength (IS) of the BGE is constant (the complexation constants K'_{ij} are defined by the equilibrium concentrations, not the activities, and therefore depend on the ionic strength of the solution). The detailed derivation and validity conditions of the model are discussed in detail in Part I of this series [42]. Though not given explicitly in Eq. (1), the pH-dependence of the effective mobility of the analyte is implicitly present in the model through the molar fractions χ_{i0}. Notice, however, that the entire model works with the concentration-defined complexation and dissociation constants and thus the actual concentration of hydroxonium ions should be considered rather than the pH, which is dependent on the activity.

The M_AM_S model (1) is generally applicable to any system that fulfills the required conditions mentioned above. In simpler systems, the M_AM_S model reduces to one of the previously published models of electromigration. The model of Wren and Rowe [10] results when a single free analyte form interacts with a single selector (here further referred to as S_AS_S systems). When a single analyte form interacts with multiple selectors, the dual-selector model [40] and the multi-selector model [38,39] published by our group are obtained from the M_AM_S model (1). Finally, when there is only one selector but a monoacidic/monobasic analyte (and the dependence of χ_{i0} on $[H_3O^+]$ is expressed explicitly), the M_AM_S model results in the model of Williams and Vigh [18].

It has already been demonstrated that (i) an analyte which is a weak monovalent [15] or divalent [22] acid and interacts with one selector can be treated as if only one free analyte form were present; (ii) a mixture of selectors can analogically be regarded as a single selector [38–40]. A constant H_3O^+ concentration in the BGE is a prerequisite in the former case and a constant mixture composition is required in the latter case. The M_AM_S model investigated here indicates that the two approaches, (i) and (ii), remain valid even if a weak acidic/basic analyte interacts with multiple selectors. Any M_AM_S system can be viewed as (i) a single-selector system with multiple analyte forms, (ii) a single-analyte-form system with multiple selectors, or simply as a single-analyte-form/single-selector system; depending on whether $[H_3O^+]$ (and consequently also χ_{i0}), the composition of the selector mixture (represented by χ_{Sj}), or both are kept constant.

This work was carried out to demonstrate the validity of the M_AM_S model (1) experimentally. We chose the simplest possible (yet practically highly relevant) M_AM_S system: a weak monoacidic analyte interacting with a mixture of two cyclodextrins. First, we will simplify Eq. (1) by adapting it for two forms of the analyte and two selectors. Second, we will show how the system can be treated from the perspective of (i) multiple analyte forms and a single selector at a constant mixture composition; (ii) a single analyte form and multiple selectors at constant $[H_3O^+]$; and finally the simplest single-analyte-form/single-selector system at both constant mixture composition and constant $[H_3O^+]$. Finally, we will demonstrate the equivalence of the three approaches, which allows the analyst to choose the best one according to the requirements of the particular separation.

2. Materials and methods

2.1. Chemicals

All the chemicals were of analytical-grade purity. R-flurbiprofen, native β-cyclodextrin (β-CD), formic acid, cacodylic acid, lithium hydroxide monohydrate and nitromethane were purchased from Sigma–Aldrich (Prague, Czech Republic). 6-Monodeoxy-6-monoamino-β-cyclodextrin hydrochloride (A-β-CD) was purchased from CycloLab (Budapest, Hungary). Ortho-phosphoric acid was purchased from Lachema (Brno, Czech Republic). NaOH solutions used for rinsing the capillary were purchased from Agilent Technologies (Waldbronn, Germany). IUPAC buffers, pH 1.679, 4.005, and 7.000 (Radiometer, Copenhagen, Denmark), were used for calibration of the pH meter. The water used for preparation of all the solutions was purified by the Rowapur and Ultrapur water purification system (Watrex, San Francisco, USA).

2.2. Instrumentation

All the CE experiments were performed using an Agilent ^{3D}CE capillary electrophoresis instrument operated by ChemStation software (Agilent Technologies, Waldbronn, Germany). The instrument was equipped with a built-in photometric diode array detector (UV detector). The 50 μm id and 375 μm od fused-silica capillary was obtained from Polymicro Technologies (Phoenix, AZ, USA). The total length of the capillary and distance from the inlet to the UV detector were 50.3 cm and 41.8 cm, respectively. A pH meter (PHM 240 pH/ION Meter, Radiometer analytical) was employed to measure the pH of the BGEs.

2.3. Experimental conditions

The pH 2.02 stock buffer contained 96.0 mM ortho-phosphoric acid and 38.1 mM LiOH; the pH 4.01 stock buffer contained 70.0 mM formic acid and 48.0 mM LiOH; the pH 6.28 stock buffer contained 86.0 mM cacodylic acid and 48.0 mM LiOH. Stock solutions containing β-CD were prepared by dissolving the selector directly in the particular buffer. A-β-CD is a monovalent positively charged selector and was purchased as a salt. Therefore, in stock solutions of this selector, the concentrations of LiOH and of the relevant buffering constituent had to be decreased in order to keep the IS of the solution constant so that all the BGEs used in this work had IS of 48 mM according to the calculation by the PeakMaster software [43]. All the stock solutions of A-β-CD contained 10 mM of this selector. The concentrations of the buffer constituents were 76.0 mM ortho-phosphoric acid and 28.1 mM LiOH for the 2.02 pH buffer, 55.4 mM formic acid and 48.0 mM LiOH for the 4.01 pH buffer, and 68.1 mM cacodylic acid and 38.1 mM LiOH for the 6.28 pH buffer. The BGEs containing lower concentrations of the single selectors were prepared by diluting the stock solution of the particular selector and the particular pH with stock buffer of the same pH. The stock solutions of the dual-selector mixtures were prepared by mixing stock solutions of the single selectors (of the particular pH) in the required ratio to obtain the desired mixture composition. Consequently, the stock solution was diluted with pure buffer of the particular pH to obtain BGEs containing a lower concentration of the dual-selector mixture. See Table 1 for the concentration ranges used. All the solutions at the given pH level exhibited the same experimental pH regardless of the presence and amount of selector(s). This indicated that no significant interaction occurred between the selectors and the buffer constituents [44]. Because of the low solubility of R-flurbiprofen at low pH values, the stock solution of 4 mM R-flurbiprofen was prepared by dissolving the appropriate amount of the compound in a 4 mM solution of LiOH. The samples were