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Separation efficiency of dual-selector systems in capillary electrophoresis☆

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ABSTRACT

We introduce an easy but highly descriptive model of separation efficiency of dual-selector systems in capillary electrophoresis. The model expresses effective mobilities of analytes in dual-selector mixtures as a function of mixture composition and total concentration. The effective mobility follows the pattern familiar from single-selector systems, while complexation constant and mobility of the complex are replaced by the same but "overall" parameters and a total concentration of the mixture takes the role of a selector concentration. The overall parameters can be either calculated from the individual ones (an arbitrary mixture) or measured directly (a particular mixture). We inspected two model dual-selector systems consisting of heptakis(2,6-di-O-methyl)-β-CD and β-CD and of heptakis(2,6-di-O-methyl)-β-CD and 6-O-α-maltosyl-β-CD, and ibuprofen and flurbiprofen as model analytes (pH 8.2, non-enantioselective separation). Adopting any optimization strategy typically used in single-selector systems and finding an optimal mixture composition and total concentration is perhaps the prime benefit of the model. We demonstrate this approach on the selectivity parameter and show that the model is precise enough to be used in analytical practice. It also results that an electromigration order (reversal) of analytes can exhibit a rather curious dependency on the mixture composition and concentration. Last, the model can be used for better understanding of separation principles in dual-selector systems in general.

1. Introduction

In capillary electrophoresis (CE), interaction of analytes with selectors added into the background electrolyte (BGE) makes it possible to achieve enantioseparation or separation of neutral analytes as well as it is widely used to improve ordinary achiral separations. Additionally, combination of two selectors employed in a mixture (dual systems) proved advantageous when a single selector does not serve efficiently enough. Several mathematical models have been derived describing the mechanism of the separation. While these models provide help with finding optimal separation conditions in single selector systems [1–5], a lack of similarly systematic approach can still be identified when coming to dual systems.

In 1992, Wren and Rowe described electromigration behavior of chiral analytes interacting with one chiral selector as follows [6]:

$$\mu_{A,eff} = \frac{\mu_{A,f} + \mu_C K_C[S]}{1 + K_C[S]} \quad (1)$$

where $\mu_{A,eff}$ is the effective mobility of the analyte, $\mu_{A,f}$ the mobility of the free analyte (the effective mobility of the analyte in a BGE containing no selector), μ_C the mobility of the complex of the analyte with the selector, [S] is the equilibrium concentration of the selector and K_C is the apparent equilibrium complexation constant:

$$K_C = \frac{[C]}{[A][S]} \quad (2)$$

where [A] and [C] are the equilibrium concentrations of the free analyte and the complex of the analyte with the selector, respectively. The model is valid under 1:1 complexation stoichiometry and if the exchange between the complexed and free form of the analyte is much faster than electrophoretic movement. Even though this model originally aimed at chiral separations, it serves just as well for characterization of selector-assisted achiral separations [7,8]. Later published models were in their majority based on the approach by Wren and Rowe [7,9–15] (or a mathematically equivalent one [8,16–18]) extended with acido-base equilibria and

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various objective measures of the goodness of separation (mobility difference, selectivity, resolution) with respect to separation conditions, namely the selector concentration and possibly pH.

Dual separation systems (chiral [19–30] and achiral [31–35]) have been also described mathematically [2,3,36–38]. In many cases [19,21,23–26,36,38], authors extend the equation by Wren and Rowe (1) by the second selector, which finally results in equation (3):

$$\mu_{A,eff} = \frac{\mu_{A,f} + \mu_{C1}K_{C1}[S_1] + \mu_{C2}K_{C2}[S_2]}{1 + K_{C1}[S_1] + K_{C2}[S_2]} \quad (3)$$

where symbols have the same meaning as those in Eq. (1) and indexes 1 and 2 stays for the 1st and the 2nd selector, respectively. A different approach based on the chromatographic model developed originally for micellar electrokinetic chromatography separations [39] was used to describe separation of highly hydrophobic analytes by a dual selector system of neutral and charged cyclodextrin (CD) [31,33]. Several authors also described difference between effective mobilities of two enantiomers separated by a dual selector system, $\Delta\mu_d$, as a weighted sum of mobility differences generated by the first, $\Delta\mu_1$, and the second, $\Delta\mu_2$, selector [22,27,28,37]: $\Delta\mu_d = i\Delta\mu_1 + j\Delta\mu_2$. The (not quantitatively specified) coefficients i and j generally depend on the concentration of the selectors and their complexation constants. This approach can be utilized to judge qualitatively which affinity patterns and effects on analyte mobilities offer separation improvement (compared to single selectors) or lead to inversion of the electromigration order. Models have been also derived describing complexation of an analyte with more than two selectors [30,34,35].

The main drawback of the dual models is their higher complexity in comparison with the single models. With two independent variables (concentration of two selectors) it is more difficult to optimize the separation or even get an insight into the separation mechanism. Therefore, simplifications are often used which, however, result in mathematical models valid only for specific cases [21,31–33], or the models are used only for qualitative explanations of observed effects [19,22,28,38].

Recently, we have shown that Eq. (3) can be expressed in a form identical to that of complexation with a single selector (1) even when extended to an arbitrary number of constituents [40,41]:

$$\mu_{A,eff} = \frac{\mu_{A,f} + \mu_C^{over}K_C^{over}c_{tot}}{1 + K_C^{over}c_{tot}} \quad (4)$$

where $\mu_{A,eff}$ and $\mu_{A,f}$ have the same meaning as in Eq. (1), c_{tot} is the total molar concentration of the selector mixture (sum of molar concentrations of all present selectors) and K_C^{over} is the overall complexation constant:

$$K_C^{over} = \sum_i \chi_i K_i \quad (5)$$

and μ_C^{over} is the overall mobility of the complex:

$$\mu_C^{over} = \frac{\sum_i \chi_i \mu_i K_i}{\sum_i \chi_i K_i} = \frac{\sum_i \chi_i \mu_i K_i}{K_C^{over}} \quad (6)$$

Finally, χ_i in Eqs. (5) and (6) is the molar fraction of the ith selector in the mixture and K_i and μ_i are corresponding complexation constant and mobility of the complex, respectively. Note that the "overall mobility of the complex" actually does not refer to the mobility of any single specific compound in the solution, but should be understood as the limiting mobility of the analyte in BGE containing infinite concentration of the mixture of the selectors.

Equation (4) is valid under the following conditions: (i) the complexation is much "faster" than the electrophoretic movement, (ii) the analyte can interact with no more than one single selector at a time with 1:1 stoichiometry, (iii) consumption of each single selector by the complexation is negligible. The overall complexation parameters (overall complexation constant and overall mobility of the complex) can be either measured experimentally (in the same way as those of a single selector) or calculated (using Eqs. (5) and (6)) and can serve as input parameters for the already-well-developed single-selector models.

The objective of this work is both to verify our model experimentally and to demonstrate its potency to systematically characterize separation properties of dual-selector systems. We compare the calculated overall complexation parameters with the measured ones and use them to predict and measure the separation efficacy of various dual mixtures.

2. Materials and methods

2.1. Chemicals

All chemicals used, namely: (±)-ibuprofen, (±)-flurbiprofen, tris(hydroxymethyl)aminomethane (tris), N-[Tris(hydroxymethyl) methyl]glycine (tricine), nitromethane, heptakis(2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD), 6-O-α-maltosyl-β-cyclodextrin hydrate (Malt-β-CD) and β-cyclodextrin (β-CD); were purchased from Sigma-Aldrich (Prague, Czech Republic) and were of analytical-grade purity. Water used for preparation of all solutions was purified by Rowapur and Ultrapur water purification system (Watrex, San Francisco, USA).

2.2. Instrumentation

All experiments were performed using an Agilent ^{3D}CE capillary electrophoresis operated by ChemStation software (Agilent Technologies, Waldbronn, Germany). The instrument is equipped with a built-in photometric diode array detector (UV detector). Fused-silica capillary of 50 μm i.d. and 375 μm o.d. was provided by Polymicro Technologies (Phoenix, AZ, USA). The total length of the capillary and distance from inlet to UV detector were 52.1 and 43.6 cm, respectively. PHM 240 pH/ION Meter (Radiometer analytical, Lyon, France) was used for pH measurements.

2.3. Experimental conditions

The running buffer not containing any selector was composed of 50 mM tris and 50 mM tricine, pH of 8.2 (tris–tricine buffer). The stock solution of each single selector was prepared by dissolving the selector directly in the tris–tricine buffer to obtain the highest selector concentration used. BGEs containing a single selector at lower concentrations were prepared by diluting the stock solution of the particular selector with the tris–tricine buffer. The concentration ranges used were 0–8 mM, 0–10 mM and 0–5 mM for β-CD, DM-CD and Malt-CD, respectively. To prepare BGEs containing two different selectors, firstly stock solutions of the single selectors were mixed in required ratio to obtain the highest concentration of the desired mixture. Then the mixture was diluted with the pure tris–tricine buffer to obtain BGEs containing the mixture in lower concentrations. The concentration ranges used were 0–8 mM and 0–5 mM for dual system consisted of β-CD and DM-β-CD and of DM-β-CD and Malt-β-CD, respectively. All the BGEs used in this work had the same ionic strength of 26 mM according to the calculation by PeakMaster software [42]. Samples contained (±)-ibuprofen or (±)-flurbiprofen (0.4 mM and 0.2 mM, respectively), nitromethane serving as EOF marker (0.02%, v/v) and running buffer constituents. Samples did not contain any selector. All solutions were filtered using syringe filters, pore size 0.45 μm (Sigma-Aldrich, Prague, Czech Republic).