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Model of CE enantioseparation systems with a mixture of chiral selectors Part I. Theory of migration and interconversion☆

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ABSTRACT

Theory of equilibria, migration and dynamics of interconversion of a chiral analyte in electromigration enantioseparation systems involving a mixture of chiral selectors for the chiral recognition (separation) are proposed. The model assumes that each individual analyte–CS interaction is fast, fully independent on other interactions and the analyte can interact with CS in 1:1 ratio and that the analyte is present in the concentration small enough not to considerably change the concentration of free CSs. Under these presumptions, the system behaves as there was only one chiral selector with a certain overall equilibrium constant, overall mobility of analyte–selector complex (associate) and overall rate constant of interconversion in a chiral environment. We give the mathematical equations of the overall parameters. A special interest is devoted to the dynamics of interconversion. Interconversion in systems with mixture of chiral selectors is governed by two apparent rate constants of interconversion in the same way as in case of singe-selector systems. We propose the experimental design that allows to determine rates of interconversion in both chiral and achiral parts of the enantioseparation system separately. The approach is verified experimentally in the second part of the article.

1. Introduction

Enantioseparation methods have been widely established as an important tool for analysis and/or theoretical studies of chiral compounds. Besides chromatography, electromigration methods have been found suitable for enantioselective separations [1–4]. The enantioselective resolution is based on different affinity (interaction, association, complexation) of analyte's enantiomers to chiral selector (CS) present in the separation system and/or different migration velocity of such complexes [5,6]. Capillary electrophoresis (CE) is a very versatile technique because CSs can be easily altered, if necessary, to achieve the required separation. Also mixtures of CSs can be employed either to improve separation of the given enantiomers [2,7–14] or because the CS is produced as a mixture of isomers or derivatives with various degrees of substitution and various positions of substituents [1,5,15–20].

Behavior of CE enantioseparation systems with a single compound as CS ("single-CS" enantioseparation systems) is well understood. The interaction between the analyte and the CS is supposed to be fast enough to allow thermodynamic equilibrium to be established in any time of separation:

$$K_i = \frac{c_i^{CS}}{c_i^0 c_{CS}^0} \quad (1)$$

K_i is the equilibrium constant of the reaction between *i*th enantiomer and the CS, also called as affinity, association, binding, complexation, formation constant [21]. c_i^0 and c_i^{CS} are the concentrations of the *i*th enantiomer in the free and complexed form, respectively, c_{CS}^0 is the concentration of the free form of the CS. The subscript i attains 1 or 2 for the 1st or the 2nd enantiomer, respectively (for simplicity we consider a chiral compound with one stereogenic center, thus having two enantiomers). Generally, none of these concentrations are experimentally available, however, if the CS is in sufficient excess, the approximation $c_{CS}^0 \cong c_{CS}^{tot}$ can be accepted, where c_{CS}^{tot} is an overall (total, analytical) concentration of the CS used. Then the total concentration of an enantiomer c_i^{tot} in the system can be expressed as

$$c_i^{tot} = c_i^0(1 + c_{CS}^{tot} K_i) \quad (2)$$

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Based on Eq. (2) Wren and Rowe [22] derived the effective electrophoretic mobility of the ith enantiomer, μ_i:

$$\mu_i = \frac{\mu_i^0 + c_{CS}^{tot} K_i \mu_i^{CS}}{1 + c_{CS}^{tot} K_i} \quad (3)$$

Here, μ_i^0 and μ_i^{CS} are the mobilities of the enantiomer in the free form and complexed with the CS, respectively. Eq. (3) is obeyed in systems where a single CS interacts with the enantiomer in 1:1 ratio. Measurements of μ_i in a wide range of CS concentrations have been carried out to confirm its validity [22]. Further, based on Eq. (3), the equilibrium constant K_i of analyte–selector complexes can be determined, e.g., by affinity capillary electrophoresis (ACE) [21,23].

A thermodynamic measure of the affinity to form the complex is given by the difference in Gibbs energies, ΔG_i^{eq}, of the enantiomer in the achiral environment of the free background electrolyte solution and the chiral environment when complexed with CS: $\Delta G_i^{eq} = -RT \ln K_i$ (R is the gas constant, T is the absolute temperature and superscript eq denotes "equilibrium").

The difference in affinities of two enantiomers to a particular CS is given by: $\Delta_{1,2}\Delta G^{eq} = -RT \ln K_2/K_1 = -RT \ln \alpha$, where α is an "intrinsic" [5] (chromatographic) selectivity factor that need not have necessarily any link to experimental enantioresolution results in CE [6]. The apparent distribution constant is defined as

$$K_i' = K_i c_{CS}^{tot} \quad (4)$$

If the rate of interconversion of one enantiomer into the other is comparable to the timescale of the separation, the electropherogram has a particular elution profile, plateau that arises between the two separated peaks. The height of the plateau is closely related to the rate of interconversion. Reactive-separations have firstly been studied in chromatography. Kellner and Giddings were the first who started theoretical considerations on reactive-separation in GC and HPLC [24]. Later Bürkle et al. have proposed a scheme for enantioselective separations in GC and HPLC [25]. The scheme shown in Fig. 1 includes four constants of interconversion, two of the forward and backward decay in the achiral part of the system k_1^0 and k_2^0, and two in the chiral part of the system, k_1^{CS} and k_2^{CS}. In Ref. [37] we have already denoted the k_i^0 and k_i^{CS} the "local" rate constants since they describe the local (true, non-apparent) thermodynamics of interconversion in the achiral environment of the background electrolyte in CE and in the chiral environment of a CS, respectively. Besides these four constants of interconversion, the two apparent distribution constants (Eq. (4)) were defined. The authors [25] have referred to the principle of microscopic

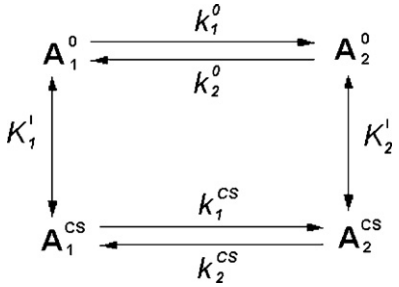

Fig. 1. Scheme of interactions equilibria involved in the separation system with two enantiomers of one analyte (A_1 and A_2) proposed by Bürkle et al. [25] for chromatographic enantioseparation techniques.

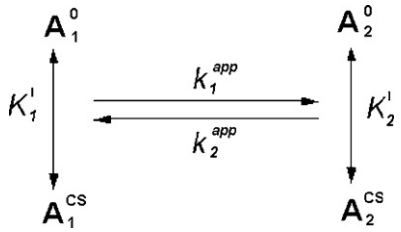

Fig. 2. Apparent separation-reaction scheme as arises from the true scheme (Fig. 1) after application of the principle of microscopic reversibility.

reversibility[1] and expressed relationships among the constants:

$$k_1^0 = k_2^0 \quad (5)$$

$$K_1' k_1^{CS} = K_2' k_2^{CS} \quad (6)$$

Based on Eqs. (5) and (6), the two apparent rate constants of interconversion are defined as

$$k_i^{app} = \frac{k_i^0 + K_i' k_i^{CS}}{1 + K_i'} = \frac{k_i^0 + c_{CS}^{tot} K_i k_i^{CS}}{1 + c_{CS}^{tot} K_i} \quad (7)$$

where subscript i represents either the 1st or the 2nd enantiomer. In this way, the originally proposed separation-reaction scheme of four rate constants of interconversion (Fig. 1) is transformed into a simpler one considering only one bi-directional reaction – interconversion of both enantiomers – regardless if actually present in a free form or in a complexed one (Fig. 2). The same simplified scheme, which has been proposed for chromatography, is supposed to hold for electromigration [26,27]. It has been used by the group of Schurig in all their papers dealing with kinetics of interconversion in chiral separations [28–33], and also by other authors [34–36]. On the basis of the theory of transition state, the corresponding apparent activation parameters, i.e., apparent free activation energy ($\Delta G_i^{\#,app}$), enthalpy ($\Delta H_i^{\#,app}$) and entropy ($\Delta S_i^{\#,app}$) are given by

$$k_i^{app} = \kappa \frac{k_B T}{h} \exp\left(-\frac{\Delta G_i^{\#,app}}{RT}\right) = \kappa \frac{k_B T}{h} \exp\left(-\frac{\Delta H_i^{\#,app}}{R}\frac{1}{T} + \frac{\Delta S_i^{\#,app}}{R}\right) \quad (8)$$

By plotting $\ln(k_i^{app}/T)$ versus $1/T$ the linear Eyring relation is obtained, the slope of which is equal to $-(\Delta H_i^{\#,app}/R)$ and the intercept equals $-\ln(h/k_B\kappa) + (\Delta S_i^{\#,app}/R)$. Here, k_B and h are the Boltzmann and Planck constants, respectively, and κ is the transmission factor ($\kappa = 0.5$ is considered for the reversible first order reaction of interconversion) [34,35].

Unfortunately, the information on the four local rate constants for both enantiomers in the chiral and achiral environments is inherently lost in the simplified "apparent" scheme. In order to overcome this problem, we recently proposed the novel approach allowing all the four rate constants of interconversion to be determined [37]. We showed that the numerator in the definition of

[1] The principle of microscopic reversibility is one of the basic principles of statistical mechanics (R.C. Tolman, 1938. The Principles of Statistical Mechanics. Oxford University Press, London, UK) stating that in a reversible reaction the mechanism in one direction is exactly the reverse of the mechanism in the opposite direction. As a direct consequence each mechanistic step must be in equilibrium when the whole system is in equilibrium. Bürkle et al. referred to this principle and expressed Eqs. (5) and (6) without further explanation. These Eqs. (5) and (6) can be derived from a kinetic equation of a reverse first order reaction of the enantiomerization in the same way as will be shown for multi-CS systems, Eqs. (20)–(25) in this paper.