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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

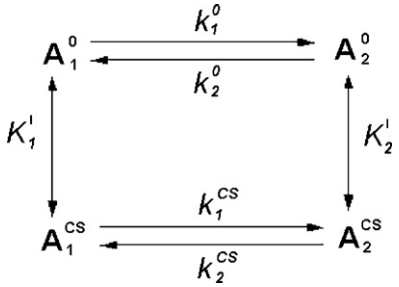

Fig. 1. Scheme of interactions equilibria involved in the separation system with two enantiomers of one analyte (A_1 and A_2) proposed by Bürkle et al. [25] for chromatographic enantioseparation techniques.

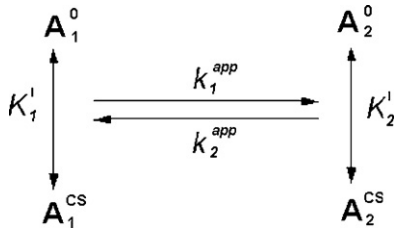

Fig. 2. Apparent separation-reaction scheme as arises from the true scheme (Fig. 1) after application of the principle of microscopic reversibility.

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.

the apparent rate constant, Eq. (7), is the only kinetic parameter that governs interconversion and, furthermore, is the same for both enantiomers. We defined a new parameter, the "global" rate constant:

$$k_{\mathrm{glob}} = k_i^0 + K_i' k_i^{\mathrm{CS}} = k_i^0 + K_i c_{\mathrm{CS}}^0 k_i^{\mathrm{CS}} \tag{9}$$

Due to the dependence of K_i' on concentration of the CS in CE, see Eqs. (4) and (9), the k_{glob} is a linear function of the parameter, where the intercept equals the $k_1^0 = k_2^0$ and the slope $K_1 k_1^{\mathrm{CS}} = K_2 k_2^{\mathrm{CS}}$ (notice that K_i, not K_i', stays here). The k_i^{CS} can be obtained by the evaluation of the distribution constant K_i, e.g., from ACE experiments [21,23]. In CE the concentration of CS can be easily varied and both the equilibrium constants, K_1 and K_2, the respective local rate constants, k_1^0, k_2^0, and k_1^{CS} and k_2^{CS}, can be obtained from the dependences of effective mobilities on CS concentration, $c_{\mathrm{CS}}^{\mathrm{tot}}$, or global rate constants on CS concentration in one series of measurements.

All the relations given above are obeyed in the systems with a single CS ("single-CS" enantioseparation systems). Nevertheless, commercially available CSs are often supplied as not well-defined mixtures of isomers with various positions of substituents or even with various degrees of substitution [15–20]. Also, mixtures of CSs are intentionally employed in order to achieve better enantioseparation conditions [2,8–14]. The question arises whether the same or a different model has to be adopted when the CS is a mixture of different chemical compounds ("multi-CS" enantioseparation systems) with different ability to form complexes (or to interact) with the enantiomeric analytes interconverting during the separation process.

Theoretically based methodology to optimize separation conditions has been developed for single-CS enantioseparation systems [3,22,18,38–42]. Some quantitative approaches have been also published describing dual-CS (namely dual-CD) systems under the conditions that: (i) the analyte's enantiomers interact with any of CSs in 1:1 ratio, (ii) independent complexation occurs (i.e., no mixed complexes are formed) and (iii) the two CS derivatives are well-defined compounds [2,9,43–46]. Additional presumptions that (iv) complexation reaction between the enantiomer and any CS in the mixture is much faster than the separation – and possibly interconversion – and that (v) the enantiomers are present in a concentration small enough not to considerably change the concentration of free CSs are not usually mentioned but should also be considered. The proposed relationships are, however, limited to dual-CS systems, do not describe a possible interconversion and do not allow making some general conclusions that will be discussed in this paper. Moreover, as a number of CSs in the mixture is not limited in the present model, it remains valid even if the condition (iii) is not fulfilled, i.e., not pure selectors are used or even composition of the mixture is not exactly known.

The present paper (divided into two parts) deals with the electromigration separation systems with multiple CSs. The first part (Part I) is focused on the theoretical description of the multi-CS system and its comparison with the single-CS model. The description is based on the continuity equations model. Migration and interconversion of the two enantiomers under an influence of a mixture of CSs are considered. The approach will be examined experimentally in Part II of the paper. The main goal of the Part II shall be to examine whether our formerly proposed "single-CS" approach to resolve interconversion separately in both chiral and achiral environments is applicable for the multi-CS systems too. The model will be used for computer simulation of the dynamics of on-column interconversion. Rate constants and thermodynamic parameters of interconversion will be determined.

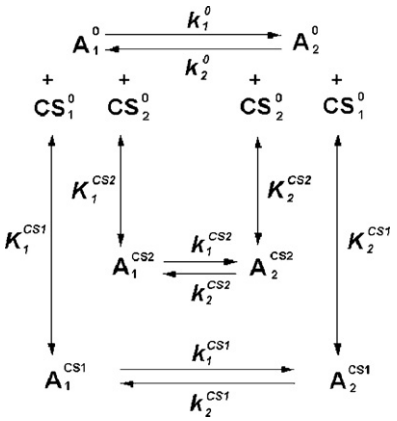

Fig. 3. Scheme of interactions equilibria involved in a separation system with two enantiomers of one analyte (A_1 and A_2) and two chiral selectors (CS_1 and CS_2). Generally, more than two CSs can be involved in the scheme.

2. Theory

2.1. Equilibria

If more than one CS is present in the enatioseparation system, the separation-reaction scheme originally proposed by Bürkle et al. [25] for one CS have to be modified, as shown in Fig. 3.

The presumptions of the model that we shall consider are:

(i) Complexation (association) reaction between the enantiomer and any of the CSs is much faster than separation and interconversion. This ensures that Eq. (10) remains continuously valid in the separation process regardless of the actual interconversion:

$$K_i^q = \frac{c_i^q}{c_i^0 c_q^0} \tag{10}$$

K_i^q is the equilibrium constant of the reaction between ith enantiomer and qth CS. c_i^0 and c_i^q are concentrations of the ith enantiomer in the free and complexed form, respectively, c_q^0 is the concentration of the free form of the qth CS. The subscript i attains 1 or 2, for the 1st or the 2nd enantiomer, respectively.

(ii) The analyte is present in a much lower concentration when compared to the concentration of CSs, so the concentration c_q^0 of the free form of the CS can be approximated by its total concentration c_q^{tot}:

$$c_q^0 \cong c_q^{\mathrm{tot}} \tag{11}$$

(iii) All analyte–CS interactions are considered in 1:1 ratio and fully independent on other interactions (i.e., no mixed complexes are formed).

As follows from Eq. (10), $c_i^q = c_i^0 K_i^q c_q^0$, and

$$c_i^{\mathrm{tot}} = c_i^0 + \sum_q c_i^q \tag{12}$$

Then Eq. (12) can be modified:

$$c_i^q = c_i^0 K_i^q c_q^0 \cong c_i^0 K_i^q c_q^{\mathrm{tot}}$$

$$c_i^{\mathrm{tot}} \cong c_i^0 \left(1 + \sum_q K_i^q c_q^{\mathrm{tot}}\right) \tag{13}$$

The total concentration of the qth CS, c_q^{tot}, can be expressed as

$$c_q^{\text{tot}} = c_{\text{CS}}^{\text{tot}} \chi_q \tag{14}$$

where $c_{\text{CS}}^{\text{tot}}$ is the analytical concentration of a mixture of all CSs and χ_q is the molar fraction of the qth CS in the mixture. Consequently,

$$c_i^{\text{tot}} = c_i^0 \left(1 + c_{\text{CS}}^{\text{tot}} \sum_q K_i^q \chi_q\right) \tag{15}$$

Comparing Eq. (15) with Eq. (2), the later being defined for the single-CS separation system, allows us to define the "overall" equilibrium constant K_i^{over}

$$K_i^{\text{over}} = \sum_q K_i^q \chi_q \tag{16}$$

2.2. Electrophoretic movement

When considering assumptions (i) to (iii) and, further, the constant electric field in the separation channel, the continuity equation governing electrophoretic movement can be formulated as

$$\frac{\partial c_i^{\text{tot}}}{\partial t} = -v_i^0 \frac{\partial c_i^0}{\partial x} - \sum_q v_i^q \frac{\partial c_i^q}{\partial x} = -\left(v_i^0 + c_{\text{CS}}^{\text{tot}} \sum_q v_i^q K_i^q \chi_q\right) \frac{\partial c_i^0}{\partial x} \tag{17}$$

where v_i^0 and v_i^{q} are velocities of the free and complexed forms of the ith enantiomer, respectively. Inserting Eqs. (15) and (16) gives

$$\frac{\partial c_i^{\text{tot}}}{\partial t} = -\frac{v_i^0 + c_{\text{CS}}^{\text{tot}} \sum_q v_i^q K_i^q \chi_q}{1 + c_{\text{CS}}^{\text{tot}} K_i^{\text{over}}} \frac{\partial c_i^{\text{tot}}}{\partial x} \tag{18}$$

which enables us to express the effective mobility μ_i of the enantiomer as

$$\mu_i = \frac{\mu_i^0 + c_{\text{CS}}^{\text{tot}} \sum_q \mu_i^q K_i^q \chi_q}{1 + c_{\text{CS}}^{\text{tot}} K_i^{\text{over}}} = \frac{\mu_i^0 + c_{\text{CS}}^{\text{tot}} K_i^{\text{over}} \mu_i^{\text{over}}}{1 + c_{\text{CS}}^{\text{tot}} K_i^{\text{over}}} \tag{19}$$

where μ_i^0 and μ_i^q are the mobilities of the free and complexed forms, respectively, of the enantiomer. A term $\mu_i^{\text{over}} = \left(\sum_q \mu_i^q K_i^q \chi_q / K_i^{\text{over}}\right)$ can be regarded as the overall mobility of the ith enantiomer when completely complexed with the entire mixture of CSs. Eq. (19) is formally identical with that proposed for single-CS systems (3) so, similarly, a series of determination of the effective mobilities μ_i in systems with various concentration $c_{\text{CS}}^{\text{tot}}$ of the mixture of CSs allows us to evaluate important parameters of both enantiomers: (i) the mobility μ_i^0 of the free form (in fact, $\mu_1^0 = \mu_2^0$), (ii) the overall mobility μ_i^{over} of the enantiomers when complexed with a mixture of CSs, and (iii) K_i^{over}, the overall equilibrium constant. There is no way how to resolve mobilities μ_i^q and equilibrium constants K_i^q of individual enantiomers. On the other hand, Eq. (19) allows us to predict behavior of analyte's enantiomers in multi-CS separation systems when using a mixture of CSs with a known composition.

In three special cases the overall mobility μ_i^{over} in multi-CS enantioseparation system obtains a simple meaning: (i) If all mobilities μ_i^q of the enantiomer complexed with any CS are the same, the overall mobility equals them, $\mu_i^{\text{over}} = \mu_i^q$, (ii) if all equilibrium constants K_i^q are the same, then $K_i^{\text{over}} = K_i^q$ and the overall mobility is a weighed sum of the individual mobilities, $\mu_i^{\text{over}} = \sum_q \chi_q \mu_i^q$, and (iii) similarly to single-CS systems, the overall mobility approaches the effective mobility of an enantiomer when $c_{\text{CS}}^{\text{tot}}$ tends to infinity.

2.3. Kinetics and thermodynamics of interconversion

The kinetic term of continuity equation for the ith and jth enantiomer is as follows:

$$\begin{aligned}\frac{\partial c_i^{\text{tot}}}{\partial t} &= -k_i^0 c_i^0 - \sum_q k_i^q c_i^q + k_j^0 c_j^0 + \sum_q k_j^q c_j^q \\ &= -\left(k_i^0 + c_{\text{CS}}^{\text{tot}} \sum_q k_i^q K_i^q \chi_q\right) c_i^0 + \left(k_j^0 + c_{\text{CS}}^{\text{tot}} \sum_q k_j^q K_j^q \chi_q\right) c_j^0\end{aligned} \tag{20}$$

Using Eq. (13) we obtain

$$\begin{aligned}\frac{\partial c_i^{\text{tot}}}{\partial t} &= -\frac{k_i^0 + c_{\text{CS}}^{\text{tot}} \sum_q k_i^q K_i^q \chi_q}{1 + c_{\text{CS}}^{\text{tot}} K_i^{\text{over}}} c_i^{\text{tot}} + \frac{k_j^0 + c_{\text{CS}}^{\text{tot}} \sum_q k_j^q K_j^q \chi_q}{1 + c_{\text{CS}}^{\text{tot}} K_j^{\text{over}}} c_j^{\text{tot}} \\ &= -k_i^{\text{app}} c_i^{\text{tot}} + k_j^{\text{app}} c_j^{\text{tot}}\end{aligned} \tag{21}$$

where the apparent rate constants are defined as

$$k_i^{\text{app}} = \frac{k_i^0 + c_{\text{CS}}^{\text{tot}} \sum_q k_i^q K_i^q \chi_q}{1 + c_{\text{CS}}^{\text{tot}} K_i^{\text{over}}} = \frac{k_i^0 + c_{\text{CS}}^{\text{tot}} K_i^{\text{over}} k_i^{\text{over}}}{1 + c_{\text{CS}}^{\text{tot}} K_i^{\text{over}}} \tag{22}$$

Comparison of Eqs. (22) and (7) defined for single-CS systems shows that, analogously, the interconversion of enantiomers in multi-CS system is described in the same way as there was only one CS with

$$k_i^{\text{over}} = \frac{\sum_q k_i^q K_i^q \chi_q}{K_i^{\text{over}}} \tag{23}$$

being the overall rate constant of interconversion in the chiral environment of a mixture of CSs. In contrast to single-CS systems, the overall rate constant is not a true (intrinsic) rate constant but rather should be considered as a limit apparent rate constant, k_i^{app}, when a concentration of CSs mixture approaches infinity. Similarly to the overall mobility (Eq. (19)): (i) the overall rate constant becomes the true rate constant if all rate constants of interconversion k_i^q of the enantiomer complexed with any CS are the same, $k_i^{\text{over}} = k_i^q$, and (ii) the overall rate constant is a weighed sum of the individual rate constants if all equilibrium constants K_i^q are the same, $k_i^{\text{over}} = \sum_q \chi_q k_i^q$.

As in the case of single-CS systems, Eq. (21) turns the "true" scheme of interconversion of enantiomers under separation, Fig. 3, into the apparent one, Fig. 2.

Outside the separation system, the interconversion reaches equilibrium at infinite time, so the time derivatives of concentrations are zero. Then, considering Eq. (20) along with the equality of rate constants in an achiral environment ($k_i^0 = k_j^0$) and equality of concentrations ($c_i^0 = c_j^0$) that should be attained in the achiral part of the system, and realizing that $\sum_q k_i^q K_i^q \chi_q = K_i^{\text{over}} k_i^{\text{over}}$ (Eq. (23)), it results:

$$k_1^0 = k_2^0 \tag{24}$$

$$k_1^{\text{over}} K_1^{\text{over}} = k_2^{\text{over}} K_2^{\text{over}} \tag{25}$$

Since all terms in Eqs. (24) and (25) are constants, the relationships should be obeyed universally, regardless of the actual separation and/or interconversion process. Eqs. (24) and (25) express the principle of microscopic reversibility as valid in the multi-CS systems. Further, as comes out from it:

$$k_1^0 + c_{\text{CS}}^{\text{tot}} K_1^{\text{over}} k_1^{\text{over}} = k_2^0 + c_{\text{CS}}^{\text{tot}} K_2^{\text{over}} k_2^{\text{over}} = k_{\text{glob}} \tag{26}$$

In practice, most of the above parameters connected with enantioseparation of interconverting enantiomers can be determined experimentally. The overall equilibrium constant, K_i^{over}, is accessible from the dependence of the effective mobility on c_{CS}^{tot} (Eq. (19)). The "global" rate constant, k_{glob}, can be determined, e.g., by computer simulation (numerical calculation of the dynamical model of electromigration) to get the best fit of the real and simulated electropherogram. Then, (i) the linear dependence of k_{glob} on c_{CS}^{tot} allows us to evaluate the "achiral" rate constants $k_1^0 = k_2^0$ as the intercept, (ii) the slope, $K_1^{over}k_1^{over} = K_2^{over}k_2^{over}$, provides the overall rate constants, k_i^{over}, after division by the corresponding K_i^{over}. Resolving individual rate constants k_1^q and k_2^q is impossible.

The Eyring plot of $\ln(k_i^0/T)$ vs. $1/T$ should be linear and provide the "achiral" thermodynamic activation parameters, i.e., that for the free form of the enantiomers, $\Delta G_i^{\#,0}$, $\Delta H_i^{\#,0}$, $\Delta S_i^{\#,0}$. On the contrary, the overall rate constant k_i^{over} is not a true (intrinsic) rate constant as it is a weighted sum of individual rate constants, see Eq. (23), and thus the validity of the corresponding Eyring plot is limited. Only certain effective "overall" activation parameters (i.e., Gibbs energy, enthalpy and entropy) can be obtained in a certain temperature range, while the true thermodynamic parameters of individual components of the CS mixture remain unresolved. Here it should be noticed that the apparent activation parameters commonly determined on the basis of the "apparent" Eyring plots of $\ln(k_i^{app}/T)$ vs. $1/T$ are not fully plausible either, as the apparent rate constant are not the true (intrinsic) rate constants but rather a weighted sum of rate constants in the chiral and achiral environment.

3. Conclusion and remarks

We introduced a model of separation of interconverting enantiomers in multi-CS CE enantioseparation systems. The model is based on three assumptions: (i) complexation reaction between the enantiomer and any CS in the mixture is much faster than the separation and interconversion, (ii) the enantiomers are present in a concentration small enough not to considerably change the concentration of free CSs, (iii) an analyte's enantiomer interact with any of CSs in 1:1 ratio and fully independently on other interactions (i.e., no mixed complexes are formed). Under these assumptions, the multi-CS enantioseparation system can be described in formally the same way as a single-CS system.

When generalizing the single-CS enantioseparation systems to the multi-CS, the parameters μ_i^0 and k_i^0 related to the achiral part of the systems remain in the model the same while those connected to the chiral part of the system are: overall equilibrium constant K_i^{over}, overall mobility μ_i^{over} and overall rate constant of interconversion k_i^{over}. Mathematical equations expressing the "overall" variables have been derived.

Alike in single-CS enatioseparation system, the principle of microscopic reversibility leads to apparent rate constants of interconversion. The definition of a global rate constant allows to distinguish between the local rate constants of interconversion in chiral and achiral parts of the system, i.e., k_i^0 and k_i^{over}, separately (as has been already described for single-CS systems in [37]). In contrast to single-CS systems, the overall rate constant k_i^{over} of interconversion of enantiomers in the chiral environment of a mixture of CSs is not a true (intrinsic) rate constant and should rather be considered as a limiting apparent rate constant for c_{CS}^{tot} tending to infinity. Consequently, the validity of "overall" Eyring plots is limited and also the related "overall" activation parameters should be considered as valid in a certain (experimental) temperature range only.

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