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CONCEPT OF EFFECTIVE AND NON-EFFECTIVE INCLUSION COMPLEX FORMATION IN ISOTACHOPHORESIS

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SUMMARY

On the basis of experimental experiences, a simple mathematical model of 1:1 cyclodextrin–solute complex formation is proposed. A possible role of two limiting cyclodextrin–solute interactions, introduced as effective and non-effective complex formation, on the quality of isotachophoretic resolution has been studied and is discussed. Additional parameters, *e.g.*, the solute molecular weight, its effective charge and the molecular weight of the cyclodextrin used, are involved in the proposed model. Their influence on the isotachophoretic separation was estimated by comparing the computer simulated mobility ratio curves of cyclodextrin-complexed solutes obtained at various values of the corresponding parameters.

INTRODUCTION

The optimization of the electrolyte system for the resolution of given solutes involves in general the influence of many physical-chemical and operational parameters[1]. As follows from the theory of isotachophoresis (ITP), a maximum resolution for a given solute pair is obtained in the electrolyte system ensuring the maximum (minimum) solute effective mobility ratio in the mixed zone. According to definition, the effective mobility, U, of a migrating ion is determined predominantly by its molecular weight, M, and effective charge, Z. Using relevant correlative equations[2–4] expressing the direct proportionality between U and $Z/(M)^R$ where $0 < R \leq 1$, an effective mobility ratio can be expressed as a function of M and Z. This is useful especially for the description of the resolution changes in electrolyte systems containing a complex-forming discriminator where the solute molecular weights and effective charges are selectively altered by complexation.

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Several theoretical studies dealing with inclusion complex formation in ITP have been published[5–7]. Proposed theories of complex formation and derived mathematical equations were successfuly utilized for the evaluation of important physical-chemical constants of complexed solutes such as effective mobilities and stability constants. The aim of this paper is to present a relatively simple mathematical model of the cyclodextrin (CD) supported separation of structurally related solute pairs and isomers under ITP conditions. The whole concept originates in the description of the changes in solute molecular weights and the effective charges caused by the complex formation with CD. Using the mentioned correlative equations, corresponding changes in the mixed zone solute mobilities were derived. Final considerations about the resolution are based on a computer simulation of the mobility ratio of CD-complexed solutes in the mixed zone.

THEORETICAL

The ITP separation of model solutes is outlined schematically in Fig. 1. The compounds I, III (II, III) represent pairs of structurally related solutes with identical fundamental molecular skeletons differing only in the variable substituents A, B. The charge of the groups G is supposed to be identical. Compounds I and II form pairs of structural isomers (positional isomers or enantiomers) with equal molecular weights and identical G-group charge. Each solute is proposed to have two binding sites available for the complexation with CD. The binding site N is relatively distant and isolated from the central framework C, which represents the area of structural differentiation of the solutes. The CD molecule bonded at N is not able to interact effectively with substituents G, A (B) and alter the effective charge of the solute. The stability of the CD complexes formed is the same for all three solutes, irrespective of the structural and geometrical changes in the central framework C. Such a type of complex formation can be considered as non-effective. The second CD binding site E is closely connected with the central framework C. The CD bonded at E is able to interact with substituents A (B) and alter the effective charge of group G. The stabilities of the CD complexes formed depend on the type and/or geometrical orientation of the

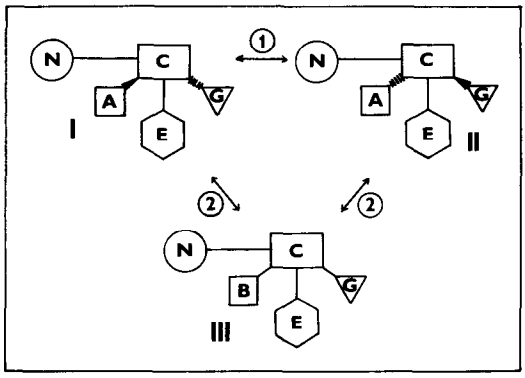

Fig. 1. Schematical outline of the model solutes studied. 1 = Enantiomers (positional isomers); 2 = structurally related compounds differing in substituents A and B; G = charged group; E = effective CD-binding site; N = non-effective CD-binding site; C = central framework of the molecule.

substituents; the differences in the stability constants may result in G-group effective charge differentiation. This type of structurally dependent complex formation may be considered as effective.

A possible role of the effective and non-effective CD-complex formation in ITP separation has been studied theoretically, using eqn. 1 as an initial correlation introduced according to Fujishita *et al.*[4]

$$U = a + b \cdot \frac{|Z|}{M} \tag{1}$$

where Z is the effective charge, M the solute molecular weight, U the effective mobility in the mixed zone and a, b are empirical coefficients.

Certain simplifications and limitations resulting from the proposed scheme of CD-complex formation and eqn. 1 must be taken into account. Sufficient linearity of eqn. 1 is expected in the range of solute $M/|Z|$ values from about 50 to 150 daltons. The extension of the validity of eqn. 1, as well as all considerations and conclusions based on its utilization, to higher and lower $M/|Z|$ values is rather unsafe. Assumed invariability of coefficients a, b for both complexed and uncomplexed solutes, as a necessary condition for further calculations, leads to ignoring all ionic interactions and hydration envelope changes influencing the quality of ITP separation. The proposed simplified scheme of complexation supposes attachment of only a single CD molecule either to the site N or E of the solute. Polymolecular inclusion complex formation is not considered.

Non-effective complex formation

Assumptions: $M_X \leqslant M_Y$[a] (X = I and Y = III for the pair of structurally related solutes, X = I and Y = II for the pair of positional isomers or enantiomers), $|Z_X| = |Z_Y| = Z$. The degree of CD-complex association, α, in the mixed zone is the same for all solutes ($0 < \alpha < 1$). Using eqn. 1, the mixed zone mobilities of uncomplexed ions can be expressed as:

$$\begin{aligned} U_X &= a + bZ/M_X \\ U_Y &= a + bZ/M_Y \end{aligned} \tag{2}$$

Likewise the effective mobilities of non-effectively complexed solutes can be expressed as

$$\begin{aligned} (U_X)_C &= a + bZ/\bar{M}_X \\ (U_Y)_C &= a + bZ/\bar{M}_Y \end{aligned} \tag{3}$$

where $\bar{M}_X = M_X + \alpha M_{CD}$ and $\bar{M}_Y = M_Y + \alpha M_{CD}$ (M_{CD} = relative molecular weight of CD used). Combining eqns. 2 and 3 we obtain:

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[a] There is no need to process $M_X \geqslant M_Y$ separately because it leads to the same conclusions.

$$(U_X)_C = U_X + bZ \Big/ \left(1 + \alpha \cdot \frac{M_{CD}}{M_X}\right) M_X - \frac{bZ}{M_X}$$
$$(U_Y)_C = U_Y + bZ \Big/ \left(1 + \alpha \cdot \frac{M_{CD}}{M_Y}\right) M_Y - \frac{bZ}{M_Y} \quad (4)$$

From eqn. 4 it follows directly that $(U)_C < U$ for each α from the interval [0;1]. This means that non-effective complex formation may result in solute retardation only.

Eqn. 4 can be utilized for the estimation of the interval for which expression 5 is valid.

$$\left(\frac{U_X}{U_Y}\right)_C > \frac{U_X}{U_Y} \quad (5)$$

Combining eqns. 4 and 5 we obtain:

$$\frac{U_X + bZ \Big/ \left(1 + \alpha \cdot \frac{M_{CD}}{M_X}\right) M_X - \frac{bZ}{M_X}}{U_Y + bZ \Big/ \left(1 + \alpha \cdot \frac{M_{CD}}{M_Y}\right) M_Y - \frac{bZ}{M_Y}} > \frac{U_X}{U_Y} \quad (6)$$

It can be proved mathematically that inequality 6 is regular only for negative, physically unreal α values. This means in practice that non-effective complex formation of structurally related solutes leads to a decrease in mobility ratio of separated solutes which is reflected in the deterioration of the separation quality.

The influence of changing α and M_{CD} values is illustrated in Fig. 2. The initial rapid decrease in the function $(U_X/U_Y)_C = f(\alpha)$ at lower α changes to a plateau for α values converging to one. Comparing curves 1, 2, it should be noted that the influence of the CD molecular weight on the decrease in the mobility ratio is only marginal, owing to the limiting range of M values for available CD types.

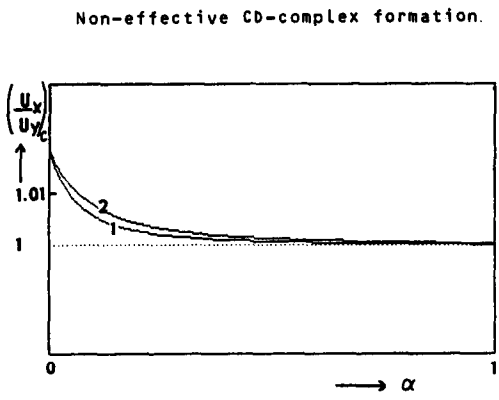

Fig. 2. Effect of the CD relative molecular weight, M_{CD}, on the decrease in the mobility ratio of non-effectively complexed solutes. $M_X = 150$, $M_Y = 200$, $Z = 0.5$, $a = 69.85$, $b = 1588$; $M_{CD} = 1430$ [corresponds to heptakis (2,3,6-tri-O-methyl)-β-cyclodextrin] (1) or 972.9 [corresponds to α-cyclodextrin] (2) Estimates for coefficients a, b were obtained according to ref. 4.

For a pair of geometrical isomers with equal molecular weights and effective charges, we obtain from eqn. 6:

$$\frac{U_X}{U_Y} = \left(\frac{U_X}{U_Y}\right)_C = 1 \tag{7}$$

It is obvious that a non-effective complex formation cannot contribute to the resolution of geometrical isomers in any way.

Effective complex formation

For the mixed zone mobilities of effectively complexed solutes, where the average effective charge and relative molecular weight of separated solutes depend on the selective complex formation ($\alpha_X \neq \alpha_Y$), it follows that

$$\begin{aligned}(U_X)_C &= a + b\,|\bar{Z}_X|/\bar{M}_X \\ (U_Y)_C &= a + b\,|\bar{Z}_Y|/\bar{M}_Y\end{aligned} \tag{8}$$

where $\bar{M}_X = M_X + \alpha_X M_{CD}$, $\bar{M}_Y = M_Y + \alpha_Y M_{CD}$, $\bar{Z}_X = Z + \alpha_X \Delta Z$ and $\bar{Z}_Y = Z + \alpha_Y \Delta Z$. The ΔZ value, defined as the difference between the effective charge of an uncomplexed and fully complexed solute, may be positive or negative and is supposed to be equal for all solutes studied. Combining eqns. 2 and 8 we obtain:

$$\begin{aligned}(U_X)_C &= U_X + b\,|Z|\left(1 + \alpha_X\left|\frac{\Delta Z}{Z}\right|\right) \Big/ M_X\left(1 + \alpha_X \cdot \frac{M_{CD}}{M_X}\right) - \frac{b\,|Z|}{M_X} \\ (U_Y)_C &= U_Y + b\,|Z|\left(1 + \alpha_Y\left|\frac{\Delta Z}{Z}\right|\right) \Big/ M_Y\left(1 + \alpha_Y \cdot \frac{M_{CD}}{M_Y}\right) - \frac{b\,|Z|}{M_Y}\end{aligned} \tag{9}$$

Using eqn. 9 it can be mathematically proved that $(U)_C > U$ only for $M_{CD} < M_{X(Y)}\left|\frac{\Delta Z}{Z}\right|$. This condition cannot be satisfied for experimentally possible M_{CD}, $M_{X(Y)}$, ΔZ and Z values. Therefore it may be concluded that the effective complex formation leads to a solute retardation only.

Two different cases must be distinguished when the role of effective CD-complex formation is discussed. For $U_X > U_Y$ and $\alpha_Y > \alpha_X$ the condition for improvement in the CD-based separation can be obtained by substituting eqn. 9 into eqn. 5:

$$\frac{\alpha_Y}{\alpha_X} > \underbrace{\frac{M_Y + \dfrac{b\,|Z|}{a}}{M_X + \dfrac{b\,|Z|}{a}}}_{A} \cdot \underbrace{\frac{1 - \dfrac{M_{CD}}{M_X}\left|\dfrac{Z}{\Delta Z}\right|}{1 - \dfrac{M_{CD}}{M_Y}\left|\dfrac{Z}{\Delta Z}\right|}}_{B} \cdot \underbrace{\frac{1 + \alpha_Y \cdot \dfrac{M_{CD}}{M_Y}}{1 + \alpha_X \cdot \dfrac{M_{CD}}{M_X}}}_{C} \tag{10}$$

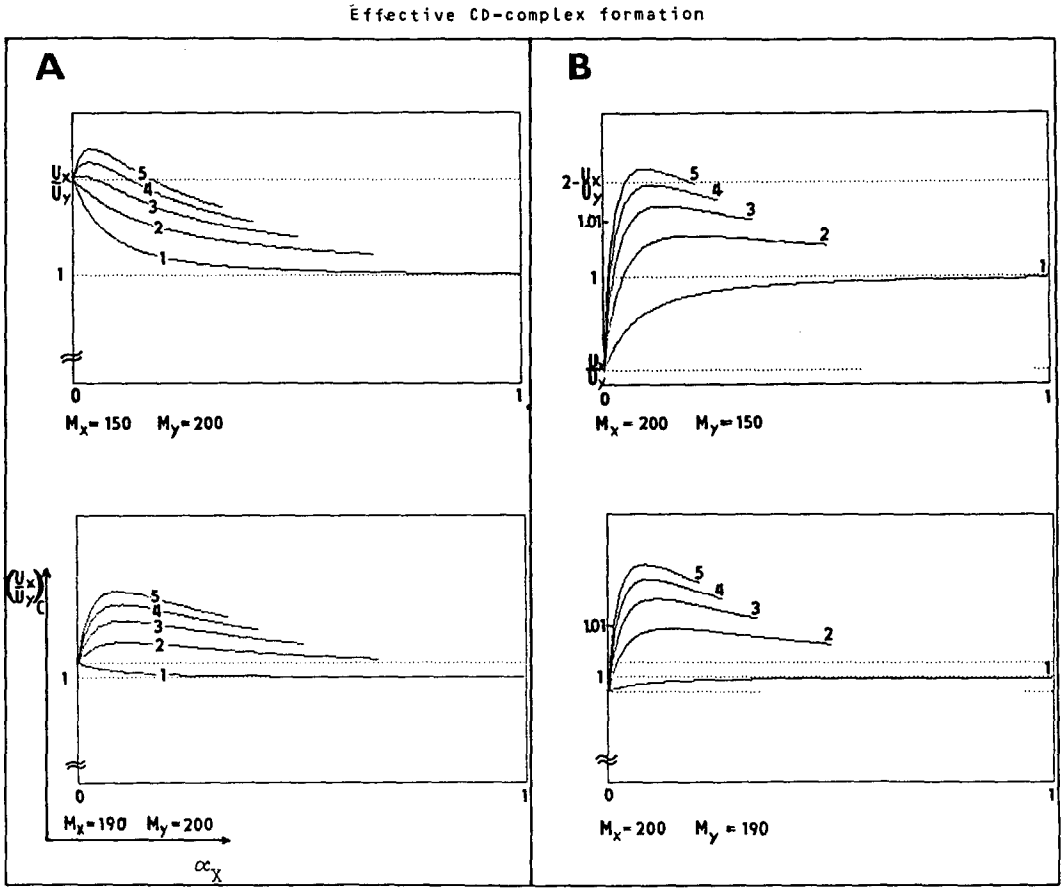

Fig. 3. Effect an increase in the solute molecular weight differences on the possibility of CD-based separation improvement. (A) $U_X > U_Y$, $\alpha_Y > \alpha_X$; $\alpha_Y = K\alpha_X$ where K = 1.0 (1), 1.5 (2), 2.0 (3), 2.5 (4) and 3.0 (5). (B) $U_X < U_Y$, $\alpha_Y > \alpha_X$; $\alpha_Y = K\alpha_X$ where K = 1.0 (1), 2.0 (2), 3.0 (3), 4.0 (4) and 5.0 (5). In all cases: Z = 0.5, M_{CD} = 1135, a = 69.85 and b = 1588.

It follows from eqn. 10 that a resolution improvement is expected for $\alpha_Y/\alpha_X > AB$. The additional requirement on the increase in α_Y/α_X results from the existence of the α-dependent term C. From the computer simulated mobility ratio curves illustrated in Fig. 3A, it should be noted that the effect of an increase in solute molecular weight, caused by CD-complex formation, results in a lowering of the ratio $(U_X/U_Y)_C$ at higher α_X, α_Y values and leads to a re-deterioration of the resolution. This effect must be compensated by an increase in the ratio α_Y/α_X in order to preserve the separation improvement.

Comparing eqn. 10 with Fig. 3A it is possible to estimate the influence of the parameters involved. The most significant is undoubtedly the effect of the solute molecular weight and the effective charge. The requirement of α_X, α_Y having different behaviours, necessary for the achievement of a separation improvement, grows rapidly

with increasing ratio M_Y/M_X and decreasing $|Z|$ value. The influence of M_{CD} and ΔZ is not so significant.

For $U_X < U_Y$ and $\alpha_Y > \alpha_X$ a general condition for a separation improvement is:

$$\left(\frac{U_X}{U_Y}\right)_C > 2 - \frac{U_X}{U_Y} \tag{11}$$

This criterion is, of course, much more demanding than the previous one mentioned for $U_X > U_Y$ and $\alpha_Y > \alpha_X$. Comparing the computer simulated dependences of $(U_X/U_Y)_C$ on α_X values for given solute characteristics (Fig 3B), it can be estimated that the ratio α_Y/α_X increases very quickly with increasing ratio M_X/M_Y. Therefore it is almost impossible to achieve an improved CD-based resolution for higher solute molecular weight differences.

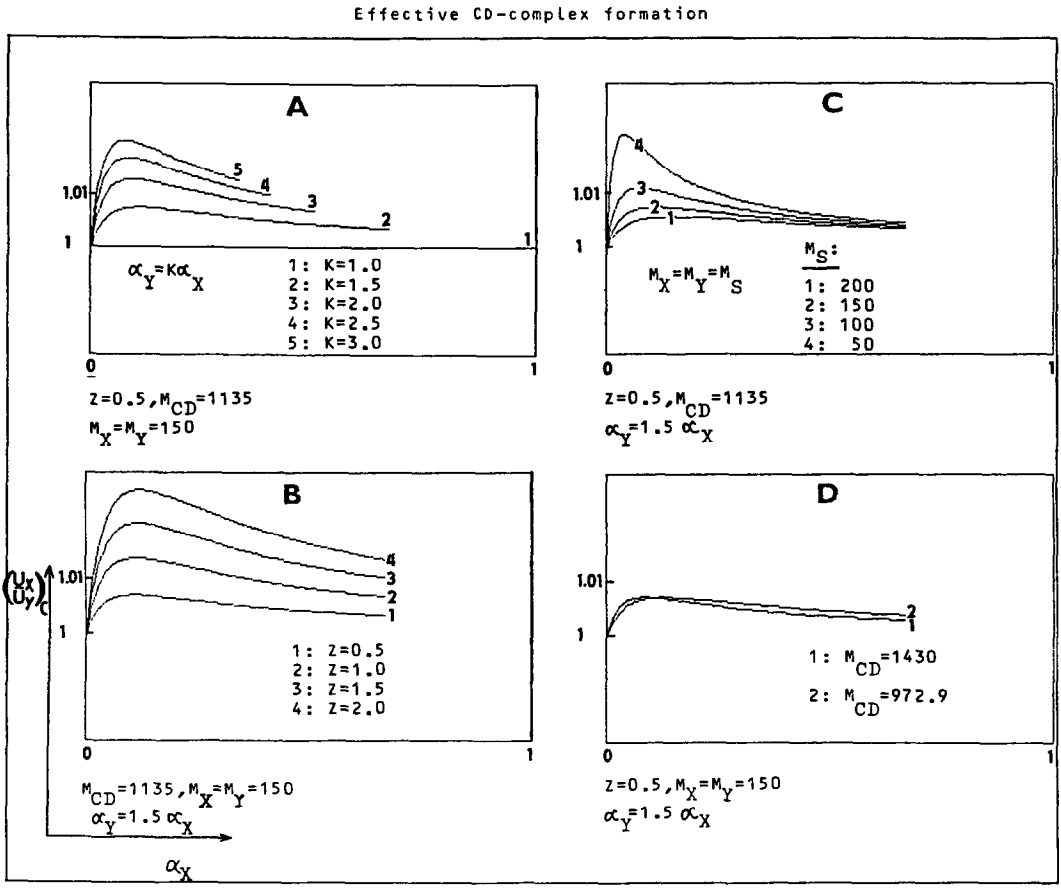

Fig. 4. Effect of changing ratio α_Y/α_X (A), effective charge, Z (B), solute molecular weight, M_S (C), and CD molecular weight, M_{CD} (D), on the quality of CD-based separation improvement.

The criterion for the CD-based separation improvement of structural isomers ($M_X = M_Y = M_S$), which is most important from the analytical point of view, is:

$$\left(\frac{U_X}{U_Y}\right)_C \neq 1 \tag{12}$$

Combining eqns. 9 and 12 it follows that:

$$\alpha_Y/\alpha_X \neq 1 \tag{13}$$

It is obvious that there is no additional requirement on the ratio α_Y/α_X resulting from the existence of an area of deteriorated resolution.

The influence of changing parameters characterizing separated solutes, complex-forming CD and the selectivity of complex formation can be simply estimated by a computer simulation of $(U_X/U_Y)_C = f(\alpha_X)$ for various α_Y, M_S, Z, ΔZ and M_{CD} values. From Fig. 4A it follows directly that higher selectivity of complex formation, characterized by increasing ratio α_Y/α_X, leads to an apparent increase in the maximum $(U_X/U_Y)_C$ value and to a separation improvement. It should be noted that the $(U_X/U_Y)_C$ maximum shifts towards lower α_X values. The same separation improvement can be observed by increasing the absolute value of the solute effective charge, but the position of the maximum shifts slightly towards higher α_X values (Fig. 4B). An increase in the solute molecular weights results on the contrary in a lowering of the CD-separation supporting effect and a decrease in the maximum mobility ratio value (Fig. 4C). A significant shift of the maximum towards higher α_X was observed. The influence of changing M_{CD} (Fig. 4D) and especially ΔZ is only marginal, owing to their limited numerical range.

CONCLUSIONS

The experiences with CD-modified electrolyte systems in ITP confirmed that the concept of effective and non-effective complex formation has meaning in the strategy of optimization of experimental conditions. In practice, unfortunately, the case of non-effective complex formation, connected with a loss in resolution, is not so rare[8,9]. Therefore it is highly important to compare the analytical results obtained in non-modified and CD-modified electrolyte systems, in order to exclude a possible disappearence of the resolution caused by non-effective complex formation.

The most important way of altering insufficient complex formation for a given solute pair is the replacement of one CD type which does not provide sufficient resolution by another one, the parameters of which are more suitable for a structural differentiation[8–13]. The set of CDs and their derivatives commercially available is sufficient for this purpose and makes it possible to achieve effectivity of CD-complex formation for a wide range of solutes.

Another factor altering significantly the effectivity of the CD-complex formation is the choice of suitable counter ion. As was suggested experimentally, competitive CD-complex formation with a "structurally suitable" counter ion decreases the degree of solute–CD complexation, distorts the resolution or even makes it impossible[8]. Therefore the use of weakly complexing counter ions is recommended.

The proposed model of effective complex formation indicates that the function $(U_X/U_Y)_C = f(\alpha_X)$ passes through a maximum. The character of this maximum depends on the parameters characterizing the solutes, the CD and the selectivity of the complexation. From computer simulated dependences, the maximum became flat and non-significant for similar α values, high solute molecular weights and low effective charges. This conclusion is in harmony with the generally known fact that the separation efficiency improves with increasing solute–pseudophase velocity differences and increasing structure-differentiating ability of the pseudophase[14]. As was shown for structurally related solutes with different molecular weights, the maximum completely disappears even for considerable α_X, α_Y differences and an improvement in separation was not achieved.

Due to a dependence of the α value on the concentration of CD in the mixed zone for a given CD stability constant, there must be an optimum CD concentration, where the separation improvement is maximal. This conclusion, which indicates that the concentration of CD in the leading electrolyte is an important optimization parameter, was confirmed experimentally and shown to be decisive especially for the resolution of enantiomers[8,12,13].

The possibility of predicting the optimum CD concentration for a given solute pair is interesting both from the theoretical point of view and for practical analytical purposes. The main problem of such a calculation results from the lack of data characterizing the stability of the CD complexes formed, necessary for the transformation of the values obtained into the mixed-zone CD concentrations. The mixed-zone CD concentration is equal to the initial analytical concentration of CD in the leading electrolyte because of the typical non-electrolyte behaviour of CD, the concentration of which is the same throughout the separation compartment and does not depend on the electrophoretic mass-distribution process.

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