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Capillary electrophoretic chiral separations using β-cyclodextrin as resolving agent

II. Bases: chiral selectivity as a function of pH and the concentration of β-cyclodextrin

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ABSTRACT

An equilibrium model, first developed to describe the pH and cyclodextrin (CD) concentration dependence of the electrophoretic mobilities as well as the separation selectivities observed during the capillary electrophoretic (CE) separation of the enantiomers of weak acids, is extended to cover the separation of the enantiomers of weak bases as well. Model parameters are derived from three series of CE experiments: (i) one with cyclodextrin-free background electrolytes of varying pH values to obtain the ionic mobilities and base dissociation constant of the weak base enantiomers, (ii) one with constant, low pH background electrolytes with varying concentrations of β-cyclodextrin to obtain the mobilities and formation constants for the protonated base enantiomer–β-cyclodextrin complexes, and (iii) one with constant, high pH background electrolytes with varying concentrations of β-cyclodextrin to obtain the formation constants for the nonprotonated base enantiomer–β-cyclodextrin complexes. Homatropine was used as a model substance to test the validity of the model and an excellent agreement has been found between the calculated and the measured mobility and selectivity values. Baseline separation of the enantiomers of homatropine could be achieved in less than 15 min using a pH 6.25 phosphate buffer, which contained 15 m*M* β-cyclodextrin.

INTRODUCTION

In a recent paper [1] we reviewed the latest literature dealing with the use of cyclodextrins as chiral resolving agents in the capillary electrophoretic separations of enantiomers, and developed a theoretical model, based on simultaneous multiple equilibria, to describe the solute migration times, the solute mobilities, as well as the separation selectivities observed during the separation of the enantiomers of weak acids. The model contains, as parameters, the acid dissociation constants and ionic mobilities of the weak acid enantiomers, the formation constants and ionic mobilities of the dissociated enantiomer–β-cyclodextrin complexes, and the formation constants of the non-dissociated enantiomer–β-cyclodextrin complexes. Three different classes of separation problems were identified: in Type I separations only the non-dissociated acid enantiomers complex selectively with β-cyclodextrin, in Type II separations only the dissociated acid enantiomers complex selectively with β-cyclodextrin, while in Type III separations both the non-dissociated and dissociated acid enantiomers complex selectively with β-cyclodextrin. It was shown that for Type I acids, such as ibuprofen and fenoprofen, the separation selectivities were at their highest values in low pH background electrolytes (where the acids were only slightly dissociated), an entirely *non-intui-*

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tive conclusion considering the coulombic nature of the CE separation process. Because we observed similar, often nonintuitive, migration and separation selectivity trends during the CE separation of the enantiomers of weak bases as well, the original theory developed for chiral weak acids is extended here to cover the separation of chiral weak bases as well. In Part I, the effects of the pH and the concentration of β-cyclodextrin in the background electrolyte will be studied experimentally using homatropine as a model substance, and the experimental results will be compared with the predictions of the theoretical model.

THEORY

The model

Let us assume that a background electrolyte contains a weak acid, HX, and its conjugate base, X^-, as the buffer components and β-cyclodextrin, CD, as the chiral resolving agent. The chiral weak base enantiomers to be separated from each other are R and S. Let the analytical concentration of the buffer be much higher than that of the CD and/or the analyte enantiomers, R and S. β-Cyclodextrin will complex with the buffer and the analyte, both in the non-dissociated and the dissociated form. If the buffer concentration is much higher than the CD concentration, and the CD concentration is much higher than the analyte concentration, then almost all the CD will be bonded in the CD–buffer complex, unless the formation constant for this complex is uncharacteristically small. Because the concentration of the chiral weak base analyte is very low with respect to the CD concentration, and because there is a sufficiently high excess of the uncomplexed buffer, to a first approximation, the analytical concentration of CD can be considered identical to the analytical concentration of the CD–buffer complexes. In addition, this analytical concentration remains constant and independent from the presence of the chiral weak base analyte. Therefore, to a first approximation, the equilibria which lead to the formation of the CD–buffer complexes may be omitted from the considerations, and, for the sake of simplicity, the terms CD and [CD] will be used instead of the proper but cumbersome CD–buffer and [CD–buffer] terms.

In an aqueous solution the weak base solute enantiomers, R and S, undergo base dissociation:

$$R + H_2O \rightleftarrows HR^+ + OH^- \quad (1)$$

$$S + H_2O \rightleftarrows HS^+ + OH^- \quad (2)$$

β-Cyclodextrin complexes with both the non-dissociated and dissociated analyte enantiomers:

$$R + CD \rightleftarrows RCD \quad (3)$$

$$S + CD \rightleftarrows SCD \quad (4)$$

$$HR^+ + CD \rightleftarrows HRCD^+ \quad (5)$$

$$HS^+ + CD \rightleftarrows HSCD^+ \quad (6)$$

The equilibrium expressions for these reactions are:

$$K_R = \frac{[HR^+][OH^-]}{[R]} \quad (7)$$

$$K_S = \frac{[HS^+][OH^-]}{[S]} \quad (8)$$

$$K_{RCD} = \frac{[RCD]}{[R][CD]} \quad (9)$$

$$K_{SCD} = \frac{[SCD]}{[S][CD]} \quad (10)$$

$$K_{HRCD^+} = \frac{[HRCD^+]}{[HR^+][CD]} \quad (11)$$

$$K_{HSCD^+} = \frac{[HSCD^+]}{[HS^+][CD]} \quad (12)$$

One can write mass balance equations for the R and the S related species using their analytical concentrations, c_R and c_S:

$$c_R = [R] + [HR^+] + [RCD] + [HRCD^+] \quad (13)$$

$$c_S = [S] + [HS^+] + [SCD] + [HSCD^+] \quad (14)$$

The mole fraction functions of the positively charged species HR^+, $HRCD^+$, HS^+, $HSCD^+$ become:

$$\alpha_{HR^+} = \frac{[HR^+]}{c_R} \quad (15)$$

$$\alpha_{HS^+} = \frac{[HS^+]}{c_S} \quad (16)$$

$$\alpha_{HRCD^+} = \frac{[HRCD^+]}{c_R} \quad (17)$$

$$\alpha_{HSCD^+} = \frac{[HSCD^+]}{c_S} \quad (18)$$

By combining eqns. 7–18 the mole fraction functions become:

$$\alpha_{HR^+} = \frac{\frac{K_R}{[OH^-]}}{1 + K_{RCD}[CD] + \frac{K_R}{[OH^-]} \cdot (1 + K_{HRCD^+}[CD])} \quad (19)$$

$$\alpha_{HS^+} = \frac{\frac{K_S}{[OH^-]}}{1 + K_{SCD}[CD] + \frac{K_S}{[OH^-]} \cdot (1 + K_{HSCD^+}[CD])} \quad (20)$$

$$\alpha_{HRCD^+} = \frac{K_{HRCD^+} \frac{K_R}{[OH^-]}[CD]}{1 + K_{RCD}[CD] + \frac{K_R}{[OH^-]} \cdot (1 + K_{HRCD^+}[CD])} \quad (21)$$

$$\alpha_{HSCD^+} = \frac{K_{HSCD^+} \frac{K_S}{[OH^-]}[CD]}{1 + K_{SCD}[CD] + \frac{K_S}{[OH^-]} \cdot (1 + K_{HSCD^+}[CD])} \quad (22)$$

The electroosmotic flow-corrected effective mobilities of the enantiomers are the linear combinations of the respective mole fractions and ionic mobilities [2]:

$$\mu_R^{eff} = \mu^0_{HR^+}\alpha_{HR^+} + \mu^0_{HRCD^+}\alpha_{HRCD^+} \quad (23)$$

$$\mu_S^{eff} = \mu^0_{HS^+}\alpha_{HS^+} + \mu^0_{HSCD^+}\alpha_{HSCD^+} \quad (24)$$

Combination of eqns. 19–24 results in:

$$\mu_R^{eff} = \mu^0_{HR^+} \cdot \frac{1 + \frac{\mu^0_{HRCD^+}}{\mu^0_{HR^+}} \cdot K_{HRCD^+}[CD]}{1 + K_{HRCD^+}[CD] + \frac{[OH^-]}{K_R} \cdot (1 + K_{RCD}[CD])} \quad (25)$$

$$\mu_S^{eff} = \mu^0_{HS^+} \cdot \frac{1 + \frac{\mu^0_{HSCD^+}}{\mu^0_{HS^+}} \cdot K_{HSCD^+}[CD]}{1 + K_{HSCD^+}[CD] + \frac{[OH^-]}{K_S} \cdot (1 + K_{SCD}[CD])} \quad (26)$$

Thus, the effective mobility of either enantiomer is a function of the ionic mobilities of both the free and the CD-complexed forms of that enantiomer, the base dissociation constants, the complex formation constants of both the ionic and the nondissociated forms of the enantiomers, as well as the hydroxide ion concentration and the CD concentration of the background electrolyte.

Separation selectivity, $A_{R/S}$, in CE can be expressed as the ratio of the effective mobilities [2]:

$$A_{R/S} = \frac{\mu_R^{eff}}{\mu_S^{eff}} \quad (27)$$

Substitution of eqns. 25 and 26 into eqn. 27 yields:

$$A_{R/S} = \frac{\mu^0_{HR^+}}{\mu^0_{HS^+}} \cdot \frac{1 + \frac{\mu^0_{HRCD^+}}{\mu^0_{HR^+}} \cdot K_{HRCD^+}[CD]}{1 + \frac{\mu^0_{HSCD^+}}{\mu^0_{HS^+}} \cdot K_{HSCD^+}[CD]} \cdot \frac{1 + K_{HSCD^+}[CD] + \frac{[OH^-]}{K_S} \cdot (1 + K_{SCD}[CD])}{1 + K_{HRCD^+}[CD] + \frac{[OH^-]}{K_R} \cdot (1 + K_{RCD}[CD])} \quad (28)$$

As long as the CD-free background electrolyte is an isotropic medium, the ionic mobilities of the two dissociated enantiomers are identical: $\mu^0_{HR+} = \mu^0_{HS+} = \mu^0_+$. The base dissociation constants of the two enantiomers are also identical: $K_R = K_S = K_b$. Thus, eqn. 28 is simplified to:

$$A_{R/S} = \frac{1 + \frac{\mu^0_{HRCD^+}}{\mu^0_+} \cdot K_{HRCD^+}[CD]}{1 + \frac{\mu^0_{HSCD^+}}{\mu^0_+} \cdot K_{HSCD^+}[CD]} \cdot \frac{1 + K_{HSCD^+}[CD] + \frac{[OH^-]}{K_b} \cdot (1 + K_{SCD}[CD])}{1 + K_{HRCD^+}[CD] + \frac{[OH^-]}{K_b} \cdot (1 + K_{RCD}[CD])} \quad (29)$$

Eqn. 29 indicates that the separation selectivity in a β-CD-based CE separation depends on both solute specific parameters and operator-dependent parameters. The solute specific parameters include the ionic mobilities of the free and the β-CD complexed enantiomers (μ^0_+, μ^0_{HRCD+} and μ^0_{HSCD+}), the base dissociation constant of the analyte (K_b), the complex formation constants of the ionic enantiomers (K_{HRCD+} and K_{HSCD+}), and the complex formation constants of the nondissociated enantiomers (K_{RCD} and K_{SCD}). The operator-dependent extensive parameters are the CD concentration and the hydroxide ion concentration (pH) of the background electrolyte.

Discussion of the model

Depending on whether (i) only the nonionic forms of the two enantiomers, (ii) only the ionic forms of the two enantiomers, or (iii) both forms of the two enantiomers interact differently with CD, there are three fundamentally different types of chiral CE separations.

Type I enantiomers. Type I enantiomers pose the simplest separation problem. Because only the non-ionic forms of the enantiomers interact differently with CD; K_{HRCD+} and K_{HSCD+} and μ^0_{HRCD+} and μ^0_{HSCD+} are identical:

$$K_{HRCD^+} = K_{HSCD^+} = K_{HBCD^+} \quad (30)$$

$$\mu^0_{HRCD^+} = \mu^0_{HSCD^+} = \mu^0_{HBCD^+} \quad (31)$$

Thus:

$$A_{R/S} = \frac{1 + \frac{\mu^0_{HBCD^+}}{\mu^0_+} \cdot K_{HBCD^+}[CD]}{1 + \frac{\mu^0_{HBCD^+}}{\mu^0_+} \cdot K_{HBCD^+}[CD]} \cdot \frac{1 + K_{HBCD^+}[CD] + \frac{[OH^-]}{K_b} \cdot (1 + K_{SCD}[CD])}{1 + K_{HBCD^+}[CD] + \frac{[OH^-]}{K_b} \cdot (1 + K_{RCD}[CD])} \quad (32)$$

The first term of eqn. 32 is reduced to unity, and the expression for separation selectivity becomes simply:

$$A_{R/S} = \frac{1 + K_{HBCD^+}[CD] + \frac{[OH^-]}{K_b} \cdot (1 + K_{SCD}[CD])}{1 + K_{HBCD^+}[CD] + \frac{[OH^-]}{K_b} \cdot (1 + K_{RCD}[CD])} \quad (33)$$

Therefore, the value of the separation selectivity factor will be different from unity, and its magnitude will depend on the values of K_{RCD}, K_{SCD}, K_{HBCD+}, K_b, [CD] and [OH^-]. If [CD] = 0, $A_{R/S}$ is unity, because there is no chiral separation without a resolving agent. $A_{R/S}$ increases with increasing hydroxide ion and β-cyclodextrin concentration. The theoretical maximum of separation selectivity (chiral selectivity, $A_{R/S} = K_{SCD}/K_{RCD}$) can be reached when the third term in both the numerator and the de-

nominator (the selective complexation term) becomes much larger than the second term in both the numerator and the denominator (the parasitic complexation term) as the concentrations of CD and OH^- are increased *ad infinitum*. This limiting value can be approximated when:

$$\frac{[OH^-]}{K_b} \cdot (1 + K_{SCD}[CD]) \geq 100(1 + K_{HBCD^+}[CD]) \quad (34)$$

i.e.:

$$K_{SCD} \geq \frac{100K_b - [OH^-]}{[OH^-][CD]} + \frac{100K_b}{[OH^-]} \cdot K_{HBCD^+} \quad (35)$$

If the hydroxide ion concentration is expressed as a multiple of the base dissociation constant,

$$[OH^-] = nK_b \quad (36)$$

(*i.e.* $pOH = pK_b - \log n$), then eqn. 35 becomes

$$K_{SCD} \geq \frac{100 - n}{n[CD]} + \frac{100}{n} \cdot K_{HBCD^+} \quad (37)$$

By taking [CD] = 15 m*M*, a value that can be safely maintained close to the ambient temperature solubility limit of β-CD, the highest pOH (lowest pH) values that lead to maximized chiral selectivities at the fastest migration rates can be calculated from eqn. 37. A few representative K_{SCD}, K_{HBCD+} and pOH combinations are listed in Table I. The minimum K_{SCD} requirement outlined in rows 1 and 2 of Table I is often fulfilled for Type I chiral bases resulting in a simple expression for separation selectivity:

$$A_{R/S} = \frac{1 + K_{SCD}[CD]}{1 + K_{RCD}[CD]} \quad (38)$$

These considerations explain the *non-intuitive* experimental observation that in the CE separation of some chiral weak bases, separation selectivity increases with increasing pH. Obviously, the parasitic complexation of the ionic enantiomers is reduced as, at higher pH values, the ionic species is gradually turned into the selectively complexing nonionic species. Optimization of such separations is very simple: the pH of the background electrolyte must be increased to increase selectivity until it becomes sufficiently large and produces the desired peak resolution with the available separation efficiency, resulting, automatically, in the shortest possible separation time.

Type II enantiomers. Type II enantiomer separations are more difficult to predict. Because only the dissociated forms of the enantiomers interact differently with CD, K_{RCD} and K_{SCD} are identical:

$$K_{RCD} = K_{SCD} = K_{BCD} \quad (39)$$

μ^0_{HRCD+} and μ^0_{HSCD+} may be equal or different, depending on the volume of the respective hydrated ionic enantiomer–CD complex. Thus, eqn. 29 is simplified only slightly:

$$A_{R/S} = \frac{1 + \frac{\mu^0_{HRCD^+}}{\mu^0_+} \cdot K_{HRCD^+}[CD]}{1 + \frac{\mu^0_{HSCD^+}}{\mu^0_+} \cdot K_{HSCD^+}[CD]} \cdot \frac{1 + K_{HSCD^+}[CD] + \frac{[OH^-]}{K_b} \cdot (1 + K_{BCD}[CD])}{1 + K_{HRCD^+}[CD] + \frac{[OH^-]}{K_b} \cdot (1 + K_{BCD}[CD])} \quad (40)$$

The R-enantiomer related values are in the numerator in the first term, and in the denominator in the second term of eqn. 40. Therefore, theoretically, $A_{R/S} < 1$, $A_{R/S} = 1$, and $A_{R/S} > 1$ are all possible, depending on the

TABLE I

REPRESENTATIVE COMPLEXATION CONSTANT AND MAXIMUM pOH VALUE PAIRS LEADING TO MAXIMUM SEPARATION SELECTIVITY FOR TYPE I AND TYPE II BASES

n	pOH	Type I base minimum K_{SCD} at [CD] = 15 m*M*	Type II base minimum K_{BCD} at [CD] = 15 m*M*
1000	$pK_b - 3$	$0.1K_{HBCD+} - 60$	$0.1K_{HSCD+} - 60$
100	$pK_b - 2$	K_{HBCD+}	K_{HSCD+}
10	$pK_b - 1$	$600 + 10K_{HBCD+}$	$600 + 10K_{HSCD+}$
1	pK_b	$6600 + 100K_{HBCD+}$	$6600 + 100K_{HSCD+}$

actual K and μ^0 values, and the hydroxide ion and the β-cyclodextrin concentrations, suggesting that the migration order of the enantiomers might be reversed by varying the concentration of the hydroxide ion.

For Type II enantiomers, because of the counteracting effects of the first and second terms in eqn. 40, separation selectivity can be maximized if one forces the second term to unity by increasing the value of the nonselective complexation term relative to the selective complexation term. This can be easily accomplished by increasing the concentration of the hydroxide ion, *i.e.* by going to higher pH values:

$$\frac{[OH^-]}{K_b} \cdot (1 + K_{BCD}[CD]) \geq 100(1 + K_{HSCD^+}[CD]) \quad (41)$$

or:

$$K_{BCD} \geq \frac{100K_b - [OH^-]}{[OH^-][CD]} + \frac{100K_b}{[OH^-]} \cdot K_{HSCD^+} \quad (42)$$

If, once again, the hydroxide ion concentration is expressed as a multiple of the base dissociation constant (*i.e.* $pOH = pK_b - \log n$), eqn. 42 becomes:

$$K_{BCD} \geq \frac{100 - n}{n[CD]} + \frac{100}{n} \cdot K_{HSCD^+} \quad (43)$$

Except for the subscripts, this relation is formally analogous to the one in eqn. 37. By taking [CD] = 15 mM as the limiting concentration, the highest pOH values that lead to maximized separation selectivities for Type II bases can be calculated from eqn. 43. A few representative K_{BCD}, K_{HSCD+} and pOH combinations are listed in Table I. Once again, the minimum K_{BCD} requirement shown in rows 1 and 2 of the last column of Table I is often easily fulfilled for Type II chiral bases resulting in a simple expression for separation selectivity:

$$A_{R/S} = \frac{1 + \frac{\mu^0_{HRCD^+}}{\mu^0_+} \cdot K_{HRCD^+}[CD]}{1 + \frac{\mu^0_{HSCD^+}}{\mu^0_+} \cdot K_{HSCD^+}[CD]} \quad (44)$$

These considerations explain the *non-intuitive* observation that in the CE separation of some chiral weak bases, selectivity increases with increasing pH. Optimization of the separation is still simple: the pH of the background electrolyte must be increased until the selectivity becomes sufficiently high, permitting the realization of the desired peak resolution, with the available separation efficiency, in the shortest period of time.

However, in the case of Type II bases, another alternative could also result in chiral resolution: one could suppress the last multiple in both the numerator and denominator of the second term in eqn. 40 by sufficiently lowering the pH of the background electrolyte. This would occur when

$$1 + K_{HSCD^+}[CD] \geq 100\frac{[OH^-]}{K_b} \cdot (1 + K_{BCD}[CD]) \quad (45)$$

that is, when

$$K_{HSCD^+} \geq \frac{100[OH^-] - K_b}{K_b[CD]} + \frac{100[OH^-]}{K_b} \cdot K_{BCD} \quad (46)$$

Using the $[OH^-] = nK_b$ approach, eqn. 46 becomes:

$$K_{HSCD^+} \geq \frac{100n - 1}{[CD]} + 100nK_{BCD} \quad (47)$$

By taking [CD] = 15 mM, the lowest pH values that lead to these alternative separation selectivity maxima can be calculated from eqn. 47. A few representative K_{BCD}, K_{HSCD+} and pH combinations are listed in Table I. Only the minimum K_{HSCD+} requirement shown in the last row of Table I is likely to occur in practice. In this case, the separation selectivity expression becomes:

$$A_{R/S} = \frac{1 + \frac{\mu^0_{HRCD^+}}{\mu^0_+} \cdot K_{HRCD^+}[CD]}{1 + \frac{\mu^0_{HSCD^+}}{\mu^0_+} \cdot K_{HSCD^+}[CD]} \cdot \frac{1 + K_{HSCD^+}[CD]}{1 + K_{HRCD^+}[CD]} \quad (48)$$

Although leading to higher mobilities, compared to the first approach, the use of low pH background electrolytes might be disadvantageous for

the separation selectivity of Type II weak bases. This is so, because the two terms in eqn. 48 counteract each other and result in separation selectivities that are lower than what could have been achieved in the high pH electrolytes (eqn. 44).

Type III enantiomers. It is very difficult to predict, a priori, what are the best conditions for maximization of the separation selectivity of Type III enantiomers. Since both the dissociated and the non-dissociated forms of the enantiomers interact differently with CD, eqn. 29 cannot be simplified and the "best" background electrolyte pH cannot be selected without the knowledge of the respective K and μ^0 values. A detailed pH study is indispensable if the optimum separation selectivity is to be determined. However, from a practical point of view, the use of a low pH electrolyte seems the most promising approach, because this would maximize the importance of the second term in eqn. 29 and result in as fast a separation as possible.

Determination of the model parameters

The model parameters (K_b, K_{RCD}, K_{SCD}, K_{HRCD+}, K_{HSCD+}, μ^0_{HR+}, μ^0_{HS+}, μ^0_{HRCD+}, μ^0_{HSCD+}), are generally not available from the literature for the particular chiral analyte studied. However, they can be determined by making a few simple assumptions and three sets of experiments, as follows.

In an isotropic, β-cyclodextrin-free background electrolyte both enantiomers have the same ionic mobilities and base dissociation constants. Therefore, both can be determined from electropherograms taken at different pH values, as widely discussed in the literature (*e.g.* ref. 3). When [CD] = 0, eqns. 25 and 26 simplify to eqn. 49:

$$\frac{\mu_+^{eff}}{\mu_+^0} = \frac{\mu_R^{eff}}{\mu_{R^+}^0} = \frac{\mu_S^{eff}}{\mu_{S^+}^0} = \frac{1}{1+\frac{[OH^-]}{K_R}} = \frac{1}{1+\frac{[OH^-]}{K_S}} = \frac{K_b}{K_b+[OH^-]} \quad (49)$$

$$\frac{1}{\mu_+^{eff}} = \frac{1}{\mu_+^0} + \frac{[OH^-]}{\mu_+^0 K_b} \quad (50)$$

from which the μ_+^0 and the K_b values can be determined by plotting $1/\mu_+^{eff}$ as a function of $[OH^-]$, where μ_+^{eff} is, naturally, corrected for the electroosmotic flow.

When $pOH = pK_b + 3$, $[R] \ll [HR^+]$ and $[S] \ll [HS^+]$, then eqns. 13 and 14 become:

$$c_R = [HR^+] + [HRCD^+] \quad (51)$$

$$c_S = [HS^+] + [HSCD^+] \quad (52)$$

and with these, eqns. 19–22 simplify to:

$$\alpha^*_{HR^+} = \frac{[HR^+]}{[HR^+]+[HRCD^+]} = \frac{1}{1+K_{HRCD^+}[CD]} \quad (53)$$

$$\alpha^*_{HS^+} = \frac{[HS^+]}{[HS^+]+[HSCD^+]} = \frac{1}{1+K_{HSCD^+}[CD]} \quad (54)$$

$$\alpha^*_{HRCD^+} = \frac{[HRCD^+]}{[HR^+]+[HRCD^+]} = \frac{K_{HRCD^+}[CD]}{1+K_{HRCD^+}[CD]} \quad (55)$$

$$\alpha^*_{HSCD^+} = \frac{[HSCD^+]}{[HS^+]+[HSCD^+]} = \frac{K_{HSCD^+}[CD]}{1+K_{HSCD^+}[CD]} \quad (56)$$

Substitution into eqns. 23 and 24 yields:

$$\mu_R^{eff} = \mu^0_{HR^+}\alpha^*_{HR^+} + \mu^0_{HRCD^+}\alpha^*_{HRCD^+} = \frac{\mu^0_{HR^+} + \mu^0_{HRCD^+}K_{HRCD^+}[CD]}{1+K_{HRCD^+}[CD]} \quad (57)$$

$$\mu_S^{eff} = \mu^0_{HS^+}\alpha^*_{HS^+} + \mu^0_{HSCD^+}\alpha^*_{HSCD^+} = \frac{\mu^0_{HS^+} + \mu^0_{HSCD^+}K_{HSCD^+}[CD]}{1+K_{HSCD^+}[CD]} \quad (58)$$

If $K_{HRCD+}[CD] \gg 1$ and $K_{HSCD+}[CD] \gg 1$, and the CD concentration is close to saturation (18 mM), that is, the enantiomer cations are sufficiently strongly complexed by CD ($K_{HRCD+} \geq$

500), then, considering that $\mu^0_{HR+} = \mu^0_{HS+} = \mu^0_+$, eqns. 57 and 58 can be rearranged to yield:

$$\mu_R^{eff} = \frac{\mu^0_+}{K_{HRCD^+}} \cdot \frac{1}{[CD]} + \mu^0_{HRCD^+} \quad (59)$$

$$\mu_S^{eff} = \frac{\mu^0_+}{K_{HSCD^+}} \cdot \frac{1}{[CD]} + \mu^0_{HSCD^+} \quad (60)$$

Both μ^0_{HRCD+} and K_{HRCD+} and μ^0_{HSCD+} and K_{HSCD+} can be determined by plotting μ_R^{eff} and μ_S^{eff} as a function of 1/[CD], because μ^0_+ is known from the previous calculations. With these values K_{RCD} and K_{SCD} can be obtained explicitly from eqns. 25 and 26 as:

$$K_{RCD} = \frac{K_b}{[OH^-][CD]\mu_R^{eff}} \left\{ (\mu^0_+ - \mu_R^{eff}) + K_{HRCD^+}[CD](\mu^0_{HRCD^+} - \mu_R^{eff}) - \frac{[OH^-]}{K_b} \cdot \mu_R^{eff} \right\} \quad (61)$$

$$K_{SCD} = \frac{K_b}{[OH^-][CD]\mu_S^{eff}} \left\{ (\mu^0_+ - \mu_S^{eff}) + K_{HSCD^+}[CD](\mu^0_{HSCD^+} - \mu_S^{eff}) - \frac{[OH^-]}{K_b} \cdot \mu_S^{eff} \right\} \quad (62)$$

If, on the other hand, $K_{HRCD+}[CD] \ll 1$ and $K_{HSCD+}[CD] \ll 1$, while the CD concentration is close to saturation (18 m*M*), that is, the enantiomer cations are very weakly complexed by CD ($K_{HRCD+} \leq 5$), then eqns. 57 and 58 can be rearranged to yield:

$$\mu_R^{eff} = \mu^0_+ + \mu^0_{HRCD^+} K_{HRCD^+}[CD] \quad (63)$$

$$\mu_S^{eff} = \mu^0_+ + \mu^0_{HSCD^+} K_{HSCD^+}[CD] \quad (64)$$

from which the multiples $\mu^0_{HRCD+}K_{HRCD+}$ and $\mu^0_{HSCD+}K_{HSCD+}$ can be determined. Assuming that $[OH^-] \geq 100K_b$, and that $K_{RCD}[CD] \gg 1$, substitution of these multiples into eqns. 25 and 26 results in eqns. 65 and 66,

$$\frac{\mu_R^{eff}[OH^-]}{K_b} = \frac{\mu^0_+}{K_{RCD}} \cdot \frac{1}{[CD]} + \frac{\mu^0_{HRCD^+} K_{HRCD^+}}{K_{RCD}} \quad (65)$$

$$\frac{\mu_S^{eff}[OH^-]}{K_b} = \frac{\mu^0_+}{K_{SCD}} \cdot \frac{1}{[CD]} + \frac{\mu^0_{HSCD^+} K_{HSCD^+}}{K_{SCD}} \quad (66)$$

which permit the determination of K_{RCD} and K_{SCD} from plots of the effective mobilities observed at low pOH as a function of 1/[CD].

If the complexation constants are intermediate values, $5 \leq K_{HRCD+} \leq 500$, then a least-square nonlinear parameter estimation method has to be used to extract the K_{HRCD+} and K_{HSCD+}, as well as the μ^0_{HRCD+} and μ^0_{HSCD+} values from the measured effective mobility values.

Once the ionic mobilities, the base dissociation constants, and the complex formation constants are known, the effective mobilities of the individual enantiomers and the separation selectivity of the given system can be calculated using eqns. 25, 26 and 29. The calculated values then can be compared with the measured values to test the validity of the model and predict the directions in which the background electrolyte parameters should be changed in order to achieve (or improve) the selectivity of a particular chiral CE separation.

EXPERIMENTAL

The electrophoretic experiments were completed with a P/ACE 2100 system, equipped with a variable-wavelength UV detector (Beckman, Fullerton, CA, USA). The UV detector was set at 210 nm. The electrode at the injection end of the capillary was kept at positive potential; the electrode at the detector end of the capillary was at ground potential.

Untreated, 25 μm I.D. × 150 μm O.D. fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) were used (39 cm from injector to detector, 45.8 cm total length). Before each and every series of measurements the capillaries were rinsed with deionized water and the background electrolyte (10 min and 15 min, respectively).

The samples were injected electrokinetically for 5 s at 10 kV potential. The sample concentrations were kept at 0.1 m*M*. In each run, a dilute solution of benzyl alcohol was coinjected

with the sample as a non-charged electroosmotic flow marker, providing us with the accurate corrected mobilities. All separations were completed at a thermostatting liquid bath temperature of 37°C.

The field strength used for the separations was varied between 150 and 660 V/cm, depending on the actual conductivity of the background electrolyte, in order to keep the power dissipation in the 80 to 100 mW range and insure linear potential *vs.* current plots (Ohm-plots).

β-Cyclodextrin was obtained from American Maize Products (Hammond, IN, USA). Reagent-grade phosphoric acid, lithium hydroxide, sodium hydroxide and racemic homatropine were obtained from Aldrich (Milwaukee, WI, USA), 250MHR PA hydroxyethyl cellulose (HEC) from Aqualon (Wilmington, DE, USA). All chemicals were used as received without further purification. All solutions were freshly prepared using deionized water from a Milli-Q unit (Millipore, Milford, MA, USA). The background electrolytes were prepared by adding 25 ml of a 140 m*M* H_3PO_4 stock solution, 25 ml of deionized water and 20 ml of a 1% (w/w) HEC stock solution to a 100 ml volumetric flask. Then the weighed amount of β-CD was added and the solution was stirred until the β-CD dissolved. Next, the flask was made up with deionized water almost to the mark, and the pH was adjusted to the desired value by adding up to a few hundred μl of saturated LiOH solution. Finally, the flask was made up to mark with deionized water. The background electrolyte was degassed prior to loading into the electrolyte reservoirs.

RESULTS AND DISCUSSIONS

First, the base dissociation constant, K_b, of homatropine was determined using background electrolytes which contained 0.2% (w/w) HEC and 35 m*M* phosphoric acid. The pH of the background electrolyte was varied in the 6 to 10.5 range by adding LiOH. The effective mobilities of homatropine, corrected for the electroosmotic flow, μ_+^{eff}, were determined in triplicate. The reciprocal effective mobilities were plotted as a function of the hydroxide ion concentration according to eqn. 50, for a power load of 85 mW and thermostatting liquid temperature of 37°C, and the K_b and μ_+^0 values were determined (Table II). In Fig. 1, the measured mobilities are shown by symbol ×, the least-square best-fit line is shown by the solid line. Because the background electrolytes used in these studies have a high ionic strength (in excess of 10 m*M*), accurate activity coefficient values cannot be calculated using the simple Debye–Hückel approach as suggested by Beckers *et al.* [3]. Yet, the experimentally found value, pK_b =

TABLE II

IONIC MOBILITY AND APPARENT EQUILIBRIUM CONSTANT DATA FOR HOMATROPINE

Conditions as in Figs. 1–4.

Parameter	Homatropine
μ_+^0 (10^{-5} cm^2/Vs)	23.40
K_b (M)	$3.47 \cdot 10^{-5}$
pK_b	4.46
pK_b (ref. 4)	4.1
$\mu^0_{HRCD+} = \mu^0_{HSCD+}$ (10^{-5} cm^2/Vs)	9.15
K^0_{HRCD+} (M^{-1})	87.8
K^0_{HSCD+} (M^{-1})	104.0
K_{RCD} (M^{-1})	1305
K_{SCD} (M^{-1})	1350

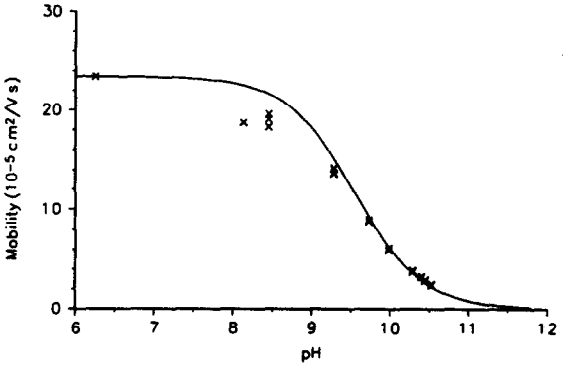

Fig. 1. The effective mobility *vs.* pH plot for homatropine in 35 m*M* H_3PO_4, 0.2% HEC background electrolyte (pH adjusted with saturated LiOH solution) at a thermostatting liquid temperature of 37°C and power load of 85 mW. Symbols: × = measured values; —— = least-square best-fit line.

4.46 is close to the infinite dilution $pK_b = 4.1$ value reported in the literature [4].

Second, the effects of the β-cyclodextrin concentration were tested in a high pOH (low pH) background electrolyte in which homatropine is almost completely dissociated (pH 6.25). When the effective mobilities were plotted against the reciprocal CD concentration according to eqns. 59 and 60, estimates could be obtained for μ^0_{HRCD+}, μ^0_{HSCD+}, K_{HRCD+} and K_{HSCD+} from the limiting slopes and the intercepts. These estimates were then used to find the parameters which assure the best fit between the measured values and the values calculated by eqns. 57 and 58. The μ^0_{HRCD+}, μ^0_{HSCD+}, K_{HRCD+} and K_{HSCD+} parameters obtained for homatropine are listed in Table II. The μ^0_{HRCD+} and the μ^0_{HSCD+} values are equal, and are about half as large as the μ^0_+ value, reflecting the size increase brought about by β-cyclodextrin. In Fig. 2, the measured values of the more mobile and the less mobile enantiomers of homatropine (symbols + and ×) are compared with the calculated effective mobilities (solid and dashed lines) obtained from eqns. 57 and 58 and the best-fit parameters listed in Table II. The agreement between the measured and calculated values is good.

Third, a series of mobility measurements were carried out in a pH 9.63 background electrolyte in which homatropine is only partially dissociated. The β-CD concentration was varied between 0 and 15 m*M*, all other conditions were kept the same as in Fig. 2, except for the pH. The complex formation constants of the nondissociated homatropine enantiomers (K_{RCD}, K_{SCD}) were calculated using the effective mobility values, eqns. 61 and 62, the respective parameters from Table II, and the actual [CD] and [OH^-] values. The result are listed in the last two lines of Table II. Interestingly, the complex formation constants of the nondissociated homatropine enantiomers (K_{RCD}, K_{SCD}) are about ten times larger than the respective complexation constants of the cations (K_{HRCD+}, K_{HSCD+}). In Fig. 3, the measured mobilities of homatropine

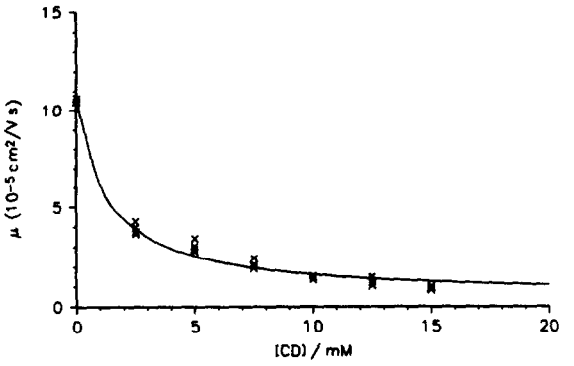

Fig. 3. Effective mobility of homatropine as a function of the β-cyclodextrin concentration in a pH 9.63 background electrolyte. Conditions as in Fig. 1. Symbols: × = measured values; —— = calculated values using the parameters in Table II.

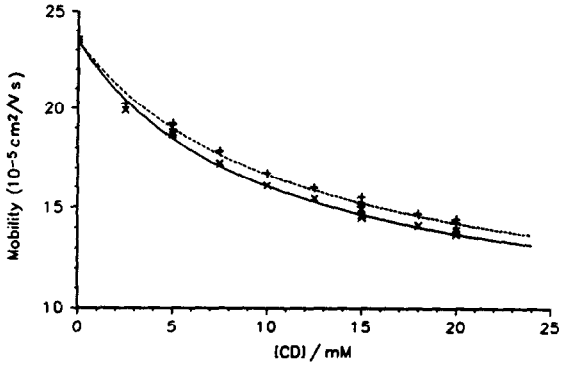

Fig. 2. Effective mobilities of the homatropine enantiomers as a function of the β-cyclodextrin concentration in a pH 6.25 background electrolyte. Conditions as in Fig. 1. Symbols: ×, + = measured values; lines = calculated values using the parameters in Table II.

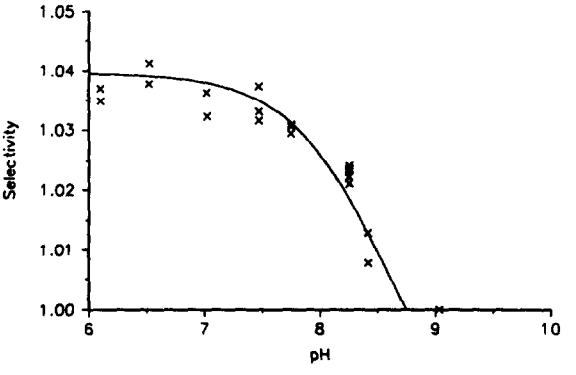

Fig. 4. Comparison of the measured and calculated separation selectivities for homatropine as a function of the pH. Conditions as in Fig. 1, except [CD] = 15 m*M*. Symbols: × = measured values; —— = calculated values using eqn. 29 and the parameters in Table II.

(symbol ×) are compared with the calculated effective mobilities (solid line) obtained from eqns. 25 and 26 using the best-fit parameters listed in Table II. The agreement between the measured and calculated values is good.

In order to further test the correctness of the estimated parameters, another series of measurements were made by keeping the β-CD concentration constant at 15 m*M* and varying the pH of the background electrolyte between 6 and 9. The measured separation selectivity values are shown by symbol × in Fig. 4, the values calculated by eqn. 29 using the parameters in Table II are shown as solid lines. The agreement is quite good. The quality of the fit between the calculated and the measured values can be tested in an orthogonal plane as well as shown in Fig. 5. The measured separation selectivity values obtained at pH 6.25 as a function of the β-cyclodextrin concentration (symbol ×) are compared here with the calculated values (solid line) obtained by eqn. 29 using the parameters in Table II. The agreement once again is good, indicating that the proposed model, indeed, correctly represents separation selectivity.

Using the parameter values in Table II and eqns. 25, 26 and 29, the electrophoretic mobility surface and the separation selectivity surface can be calculated as a function of both the pH and the β-cyclodextrin concentration as shown in Figs. 6–9. In Figs. 6 and 8 the mobility and the separation selectivity surfaces are shown up to 150 m*M* β-CD concentration. The same surfaces are shown in Figs. 7 and 9 up to the experimentally accessible 15 m*M* β-CD concentration. For the Type III solute, homatropine, mobility decreases rapidly as β-CD is added to the background electrolyte, and begins to level off above [CD] = 10 m*M* (Figs. 6 and 8). Along the pH axis, most of the mobility change occurs in the $8 < pH < 10$ range, in agreement with the $pK_b = 4.46$ value, where the mobility surface is shaped like a regular mole fraction function.

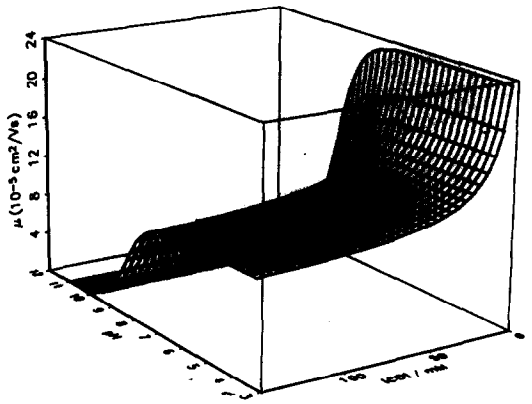

Fig. 6. Three-dimensional effective mobility surface for the more mobile enantiomer of homatropine as a function of the β-CD concentration and the pH, calculated using eqn. 25 and the parameters in Table II for the 0–150 m*M* β-CD range.

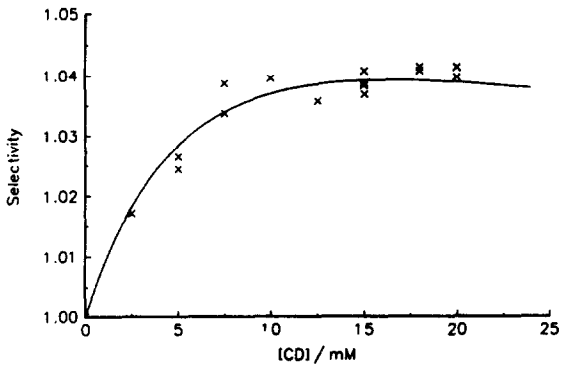

Fig. 5. Comparison of the measured and calculated separation selectivities for homatropine as a function of the β-CD concentration. Conditions as in Fig. 1, except pH 6.25. Symbols: × = measured values; —— = calculated values using eqn. 29 and the parameters in Table II.

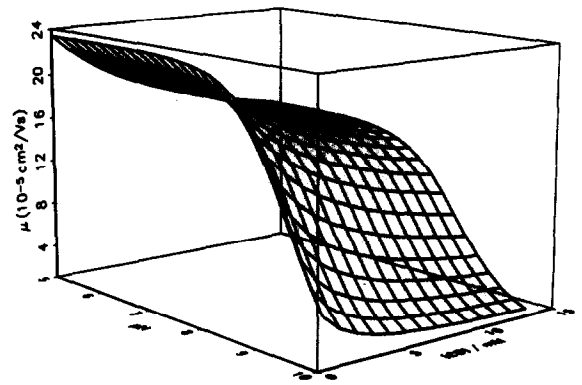

Fig. 7. Three-dimensional effective mobility surface for the more mobile enantiomer of homatropine as a function of the β-CD concentration and the pH, calculated using eqn. 25 and the parameters in Table II for the experimentally accessible 0–15 m*M* β-CD range.

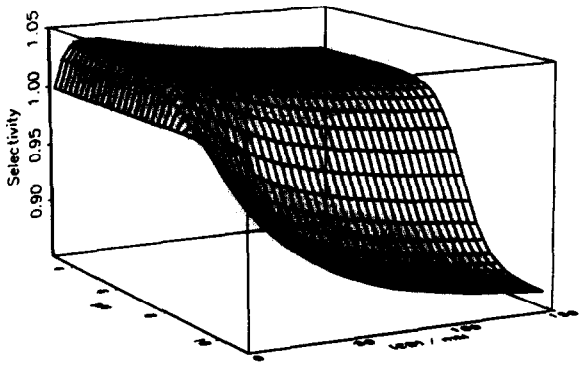

Fig. 8. Three-dimensional separation selectivity surface for homatropine as a function of the β-CD concentration and the pH, calculated using eqn. 25 and the parameters in Table II for the 0–150 m*M* β-CD range.

As shown in Fig. 8, the shape of the separation selectivity surface is much more complex. At any given pH in the acidic range, selectivity passes a maximum as the β-cyclodextrin concentration is increased. However, at low pH, this selectivity maximum is outside the experimentally accessible 0–15 m*M* range. As the pH is increased at a constant, sufficiently high β-CD concentration, the separation selectivity value falls below unity, indicating that the migration order of the two enantiomers is reversed. This is understandable when one considers that the second term in eqn. 29 approaches unity as the $[OH^-]/K_b$-multiplied terms in both the numerator and the denominator of eqn. 29 assume a large value with respect to both $(1 + K_{HSCD+}[CD])$ and $(1 + K_{HRCD+}[CD])$. This means that it is now the first term of eqn. 29, in which the R enantiomer-related parameters are in the numerator, that determines the value of the separation selectivity.

As shown in Fig. 9, at low pH values, separation selectivity increases monotonously as the β-CD concentration is varied within the experimentally accessible 0–15 m*M* range. This indicates that as far as separation selectivity is concerned it is beneficial to use a saturated β-CD solution. However, as the pH is increased beyond 7, selectivity becomes lower than at low pH, and both the β-CD concentration-dependent maximum and the reversal in the migration order occur in the 0–15 m*M* β-CD concentration range. This indicates that, from a practical point of view, it is better and safer to operate in the low pH range when the solute is a Type II or Type III base, because there is a fairly broad selectivity plateau between pH 2 and 7. Experimentally, the reversal of the migration order could not be demonstrated, because the separation efficiency of the system was too low at high pH due to greater mobility dispersion, ion-exchange effects [5] and limited peak capacity caused by the electroosmotic flow.

By considering the shapes of both the mobility and the selectivity surfaces of homatropine, a Type III solute, one may conclude that the best selectivity optimization strategy would call for β-CD concentrations that are close to the solubility limit (15 m*M* is safe) and background electrolyte pH values that are at or below pH 7, providing the highest selectivity in the shortest separation time.

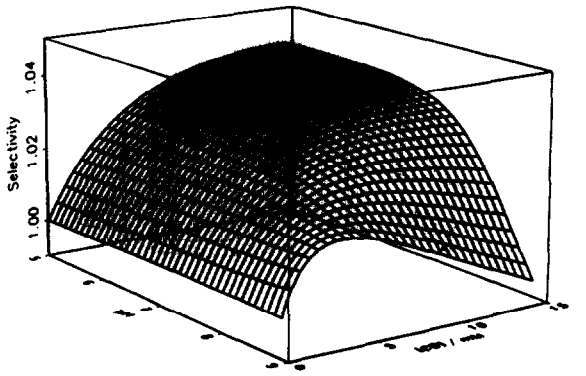

Fig. 9. Three-dimensional separation selectivity surface for homatropine as a function of the β-CD concentration and the pH, calculated using eqn. 25 and the parameters in Table II for the experimentally accessible 0–15 m*M* β-CD range.

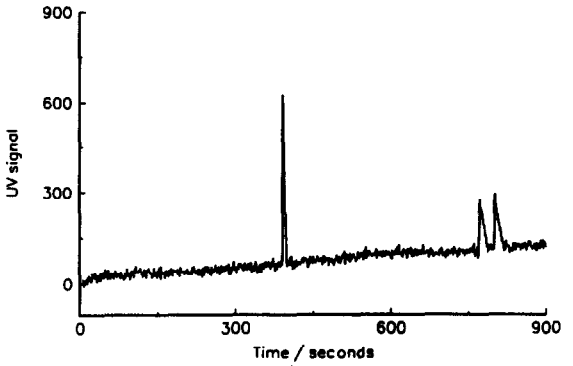

Fig. 10. CE separation of the benzyltrimethylammonium chloride (first peak) and enantiomers of homatropine. Conditions: 0.2% HEC, 35 m*M* H_3PO_4, 15 m*M* β-CD.

The electropherogram of a racemic mixture of homatropine is shown in Fig. 10. Baseline separation of the enantiomers can be achieved with 15 m*M* β-CD at pH 6.25 in about 15 min.

CONCLUSIONS

An equilibrium model has been developed to describe the electrophoretic mobilities of the enantiomers of chiral weak bases and the resulting separation selectivities, as a function of the pH and the β-cyclodextrin concentration of the background electrolyte. The parameters of the model can be readily derived from three sets of specifically designed separation experiments: the first at varying pH values without β-cyclodextrin in the background electrolyte, the second at low pH values with varying concentrations of β-cyclodextrin in the background electrolyte, and the third at high pH values with varying concentrations of β-cyclodextrin in the background electrolyte. The validity of the model has been demonstrated by comparing the measured and the calculated mobility and selectivity values for a test probe, homatropine. Homatropine behaves as Type III solute (defined under Theory) with β-cyclodextrin as the resolving agent, permitting rugged and fast separations in low pH background electrolytes which contain β-cyclodextrin at high concentrations. Further work is under way in our laboratory to extend the migration and separation selectivity model proposed here to other resolving agents as well [5].

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