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Enantioseparation of 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate-derivatized amino acids by capillary zone electrophoresis using native and substituted β-cyclodextrins as chiral additives II. Evaluation of complexation constants

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Abstract

Enantioseparation of amino acids derivatized with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) reagent by capillary zone electrophoresis (CZE) is reported using native and four substituted β-cyclodextrins as chiral selectors added to the background electrolyte. The substituted selectors were (2-hydroxy)propyl-β-cyclodextrin, heptakis(2,6-di-*O*-methyl)-β-cyclodextrin, heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin and β-cyclodextrin polymer. The selectors were compared with respect to the enantioselectivity coefficients obtained at optimum selector concentrations. Complexation constants were evaluated for six amino acids from curve fitting procedures. For the complete set of amino acids approximate complexation constants were predicted assuming constant complex mobilities for amino acids of similar size. Limited correlation was found between strength of complexation and achieved enantioselectivity.

Keywords: Buffer composition; Enantiomer separation; Enantioselectivity; Complexation constants; Structure–enantioselectivity relationships; Amino acids; Cyclodextrins

1. Introduction

Enantioseparation of 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) derivatized amino acids by use of various derivatized β-cyclodextrins as chiral selectors has been reported in a recent paper [1]. Selector type and selector concentration best suited for the enantioseparation of the various AQC-amino acids have been reported discussing, in particular, separations in the presence of electroosmotic flow (EOF). These mobility and enantioselectivity coefficient data corrected for the EOF are the bases for the discussion in this paper, which focuses (i) on the determination of complexation constants between analytes and selectors by a curve fitting procedure and (ii) on aspects of structure–enantioselectivity relationships concluded from these curve-fitted complexation constants as well as from the approximately predicted ones.

All selectivity data given in this paper are effective selectivity coefficients, α^{eff}, defined here as the ratios of the effective mobilities, μ_2^{eff}/μ_1^{eff}, subscripts 1 and 2 indicating the first and second detected enantiomers. Effective mobilities are calculated from apparent mobilities by subtracting the contribution of

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the EOF, μ^{eof}, which is determined in each single run by an EOF-marker.

The complexation constants between analyte enantiomers and selector, K_1 and K_2, are obtained together with the mobilities of the analyte–selector complex, μ^{cplx}, (a) by curve-fitting the effective mobility data measured at various selector concentrations using Eq. (1) [2,3] and (b) by curve-fitting the corresponding α^{eff} data which can be measured with greater precision.

$$\mu^{eff} = \frac{\mu^{eff} + \mu^{cplx} K[S]}{1 + K[S]} \tag{1}$$

$$\alpha^{eff} = \frac{\mu^{free} + \mu_2^{cplx} K_2[S]}{\mu^{free} + \mu_1^{cplx} K_1[S]} \cdot \frac{1 + K_1[S]}{1 + K_2[S]} \tag{2}$$

μ^{eff} and μ^{free} being the effective electrophoretic mobilities of the enantiomers in the background electrolyte (BGE) in the presence and in the absence of the selector, respectively, and $[S]$ the selector concentration. The determination of K by curve fitting needs a series of at least 6 to 8 data points per analyte–selector pair. The K_1, K_2 and μ^{cplx} values of six amino acids and five cyclodextrin-based selectors have been determined within this paper by means of this procedure. The selectors are native β-cyclodextrin (β-CD), (2-hydroxy)propyl-β-cyclodextrin (HP-β-CD), heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CD), heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TM-β-CD) and β-cyclodextrin polymer.

For the other amino acids, approximate K values are predicted by means of Eq. (1) on the bases of assuming constant μ^{cplx} values for analytes similar in structure and size. For this purpose, only two mobility data are needed per analyte–selector pair, i.e., μ^{free} and μ^{eff} at one single concentration. Correlations between complexation selectivity (characterized by α^{eff} determined near optimum selector concentration) and analyte–selector affinity (measured by K_1) are discussed on the bases of the data obtained this way.

Finally, optimum selector concentrations (with respect to maximum α^{eff} values), $[S]^{opt,eff}$, are readily calculated for any analyte–selector pair by means of the Eq. (2) [2–4] starting from the predicted K data.

$$[S]^{opt,eff} = \frac{1}{\sqrt{K_1 K_2}} = \frac{1}{K} \qquad [\text{mol/l}] \tag{3}$$

2. Experimental

Viscosity measurements were made at four different selector concentrations by use of an automated microviscosimeter (AMV 200, A. Paar, Graz, Austria).

All other experimental data refer to reference [1]. The particular electrophoretic conditions were the following: Non-coated fused-silica capillary (Hewlett-Packard) of 48.5 cm total and 40 cm effective length, internal diameter 50 μm; applied voltage 30 kV; constant temperature of 20±0.1°C, detection wavelength 245 nm. The BGE consisted of an aqueous solution of 10 m*M* 1,3-bis[tris(hydroxymethyl)methylamino]propane (BTP) at pH 7.0 (adjusted with hydrochloric acid) and 5 m*M* selector, if not stated otherwise.

The EOF was measured by adding acetone, μ^{eof} usually ranged between 15 to $20 \cdot 10^{-5}$ $cm^2\ V^{-1}\ s^{-1}$ at the given pH of 7.

3. Results and discussion

Effective enantioselectivity coefficients, α^{eff}, evaluated for the set of AQC-amino acids with selector concentrations of 5 m*M* are given in Table 1, their corresponding effective mobilities are given in Table 2. These data refer to the apparent selectivity coefficient data reported by the authors in reference [1]. The selector concentrations of 5 m*M* are in most instances near the optimum selector concentration, $[S]^{opt,eff}$, as given in [1] with the exception of the permethylated cyclodextrins. On the bases of these data the extent of correlation is investigated between selectivity coefficients obtained by employing different selectors.

3.1. Correlation of selectivities obtained with different selectors

3.1.1. HP-β-CD vs. β-CD polymer

The α^{eff} values obtained by HP-β-CD and by the β-CD polymer are fairly linearly correlated and of

Table 1
Effective enantioselectivity coefficients, α^{eff}, of AQC-amino acids measured with native and differently substituted β-CDs

Amino acid	β-CD	β-CD polymer	HP-β-CD	DM-β-CD	TM-β-CD
Ala	1.012	1.037	1.028	1.022	1.015
Val	1.015	1.053	1.041	1.045	1.024
Leu	1.029	1.087	1.060	1.060	1.020
Ile[a]	1.015	1.058	1.043	1.052	1.028
		1.070		1.031	1.022
Met	1.017	1.055	1.043	1.035	1.013
Pro	1.004	1.028	1.029	1.00	1.00
Cys[b]	1.015	1.029	1.018	1.022	1.009
	1.00	1.011	1.00	1.018	1.015
Lys[c]	1.010	1.061	1.020	1.00	1.00
Ser	1.005	1.019	1.016	1.020	1.008
Thr	1.018	1.053	1.046	1.040	1.016
Asn	1.010	1.043	1.026	1.012	1.005
Gln	1.005	1.033	1.025	1.018	1.008
Phe	1.014	1.020	1.039	1.007	1.005
Trp	1.010	1.00	1.026	1.015	1.003
Tyr	1.012	1.037	1.036	1.011	1.00
His	1.00	1.00	1.00	1.00	1.00

Electrophoretic conditions: capillary: 40 cm effective length, 48.5 cm total length; BGE: aqueous solution of 10 m*M* BTP, pH 7.0, selector concentration of 5 m*M*, applied voltage: 30 kV.
[a] Two diastereomeric pairs of enantiomers.
[b] Two differently labelled pairs of enantiomers.
[c] Doubly AQC-labelled derivative.

comparable size (Fig. 1a). Apparently, the linking moieties between the cyclodextrin monomers in the polymer (i.e., a 2-hydroxypropyl group) and the 2-hydroxypropyl substituent on the monomer become very similarly involved during enantiorecognition. Exceptions are the aromatic amino acids which are more selectively complexed by the monomeric selector HP-β-CD. Probably, in the polymeric structure they cannot adopt optimum orientation for enantioselective interaction. Any expectation that the lower value of μ^{cplx} in the case of the polymer might be a source of significantly enhanced selectivities is not verified by the data shown in Fig. 1.

3.1.2. β-CD monomer vs. β-CD polymer and HP-β-CD

Linear correlation between the data of the native β-CD monomer and those of the polymer is still given as an overall trend (Fig. 1b), although the use of the native monomer yields a much lower "mean selectivity" (both selectors are used near optimum selector concentrations). Aromatic amino acids do not fit in linear correlations as long as the polymeric cyclodextrin is involved. In the case of the two monomeric (native and hydroxypropylated) cyclodextrins again better correlation is obtained (Fig. 1c). Correlation coefficients are 0.822 for the plot β-CD monomer/β-CD polymer, 0.835 for HP-β-CD/β-CD polymer and 0.884 for β-CD monomer/HP-β-CD, respectively.

3.1.3. HP-β-CD vs. DM-β-CD

Selectivity data of the hydroxypropylated and the dimethylated selectors are scarcely correlated. Some correlation is seen within the series of non-polar aliphatic amino acids, only (given by the open squares in Fig. 1d). The correlation coefficient is 0.680.

3.2. Evaluation of complexation constants by curve fitting

Prior to curve fitting, the μ^{eff} data measured at various selector concentrations were corrected for viscosity effects resulting from increase in selector concentrations. These correction factors are given in Table 3 and are seen to be independent of the type of selector used, as far as the monomeric selectors are

Table 2
Effective mobilities of AQC-amino acids in the cyclodextrin-free solution, μ^{free}, and of the first detected enantiomers, μ_1^{eff}, measured with native and differently substituted β-CDs at selector concentrations of 5 m*M*

Amino acid	μ^{free}	μ_1^{eff} ($\cdot 10^{-5}$ cm^2 V^{-1} s^{-1})				
		β-CD	β-CD polymer	HP-β-CD	DM-β-CD	TM-β-CD
Ala	17.3	14.0	11.0	12.2	13.3	16.8
Val	15.3	12.3	9.4	10.6	11.7	14.8
Leu	15.6	11.8	7.7	10.1	10.8	14.2
Ile[a]	15.7	11.6	8.3	9.9	11.3	13.9
			7.9		11.8	13.8
Met	16.4	12.2	8.6	10.6	11.8	15.0
Pro	17.6	14.7	13.7	13.8	15.2	16.5
Cys[b]	13.5	10.8	8.2	9.5	10.4	12.9
	13.6	11.0	8.7	10.0	10.7	13.0
Lys[c]	12.3	10.6	3.6	9.2	10.9	12.4
Ser	18.1	14.0	11.4	11.6	13.9	16.8
Thr	17.0	12.8	10.4	10.7	11.9	15.9
Asn	17.4	13.5	11.0	12.1	14.3	15.8
Gln	16.4	13.0	10.2	11.4	13.2	15.0
Phe	16.3	11.1	7.2	9.3	11.1	14.0
Trp	15.4	11.1	4.9	8.8	9.8	12.8
Tyr	17.3	11.0	6.5	9.2	10.6	13.4
His	6.3	5.1	3.4	5.0	5.1	5.1

Electrophoretic conditions as in Table 1.
[a] Two diastereomeric pairs of enantiomers.
[b] Two differently labelled pairs of enantiomers.
[c] Doubly AQC-labelled derivative.

considered. For selector concentrations in between, linear interpolation is performed. Graphs of the viscosity-corrected mobilities, $\mu^{eff}*$, plotted vs. the selector concentration and the fitting curves by means of Eq. (1) are shown for DM-β-CD and β-CD polymer in Fig. 2. The corresponding α^{eff} vs. selector concentration data are shown together with the simulated curves by use of Eq. (2) in Fig. 3. The K and μ^{cplx} values fitting these data in both types of plots are given for six amino acids in Table 4. The uncertainty in the evaluated K values is about ±15 to 20, of μ^{cplx} values about ±0.5 to 1. The data in Table 4 show that strength of complexation between AQC-amino acids and the permethylated selector is considerably lower than with all other selectors. The μ^{cplx} values evaluated were found to vary considerably comparing different monomeric selectors, and a clear dependence of μ^{cplx} on size of the amino acid side chain is found. The influence of the side chain polarity seems to be less decisive. Within the significance level of the assessed data, no difference could be found for μ^{cplx} values assigned to enantiomers.

3.3. Prediction of complexation constants and optimum selector concentrations

Approximate K values are predicted for those analyte–selector pairs for which mobility data were assessed at one selector concentration only. This prediction is made by using Eq. (1) and the effective mobility data μ^{free} and μ^{eff} given in Table 2. The prediction is based on two assumptions, i.e., (i) μ^{cplx} is equal for diastereomeric selector–analyte complexes; (ii) μ^{cplx} is constant for different AQC-derivatized amino acids of similar size. The μ^{cplx} values used for this prediction are given in the bottom lines of Table 5. With respect to assumption (i), it is known that mobilities of diastereomeric compounds might differ significantly in some instances [5,6]. It cannot be excluded that in the general case such differences exist also with diastereomeric host–guest complexes and even far from the pK_a value of the analyte. However, in our particular case no big differences have been found in the curve fitting data listed in Table 4. Thus, the

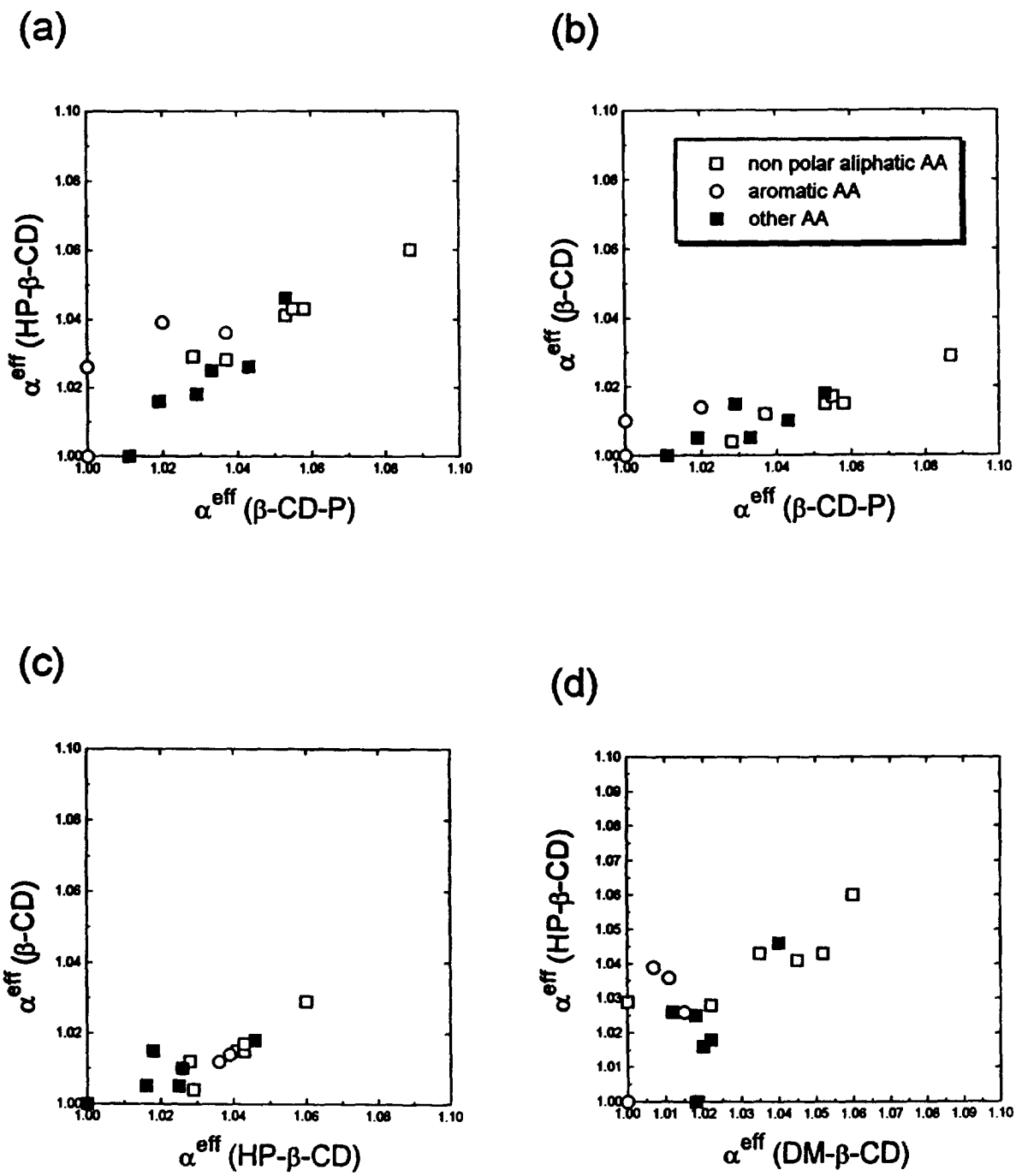

Fig. 1. Correlation of the effective enantioselectivity coefficients between various pairs of selectors. Experimental conditions as given in Section 2. Selector concentration of 5 m*M*. β-CD-P = β-cyclodextrin polymer; AA = amino acids; other abbreviations as in text (see Section 1).

Table 3
Correction factors for viscosities, η/η_0, of BGE solutions containing various concentrations of β-CD selectors

Selector concentration (m*M*)	η/η_0[a]	
	Momomeric CD selectors[b]	β-CD polymer
0	1	1
10	1.04	–
20	1.07	–
40	1.13	1.22

[a] η viscosity of the BGE solution; η_0 viscosity of the selector-free solution, $\eta_0 = 1.219$ mPa s.
[b] Native β-CD, HP-β-CD, DM-β-CD, TM-β-CD.

assumption (i)—which is made by most authors treating this problem [3,4]—seems to be reasonable, at least for the data set investigated here.

The predicted complexation constants for all analyte–selector pairs are given in Table 5. Comparing the predicted K data with those obtained by curve-fitting, considerable differences are seen in some instances. Analysis of the data show that the error in the predicted K values depend to a great extent on the scatter of the measured μ^{free} and μ^{eff} data and not only on the accuracy of the assumed values for μ^{cplx}. In spite of the uncertainty error in the assessed K data, these "predicted" values confirm the findings described above for the six amino acids as a more or less general trend within the total set of compounds.

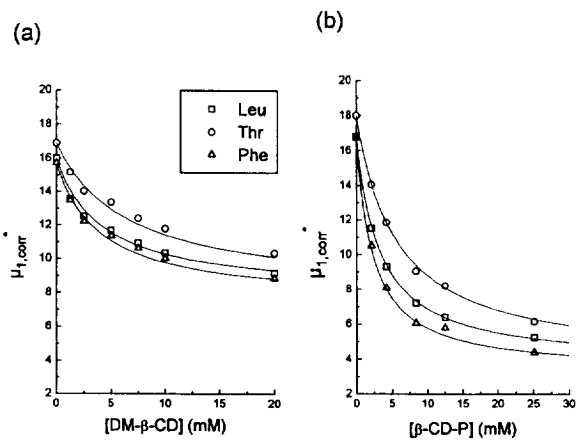

Fig. 2. Dependence of the viscosity-corrected effective mobilities of the first detected enantiomers of three selected AQC-amino acids on selector concentrations using (a) DM-β-CD and (b) β-CD polymer. The straight curves display the curve fitting line; parameters as in Table 4. Experimental conditions as in Section 2.

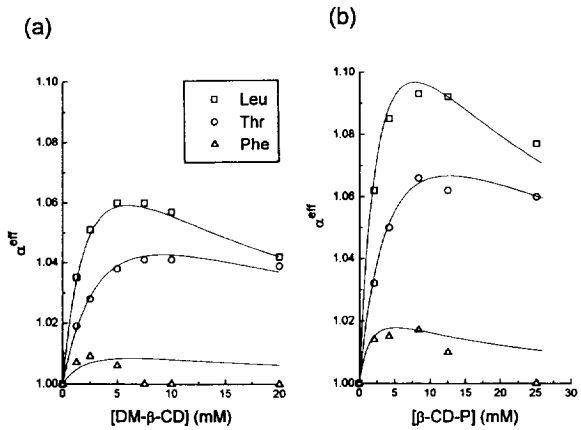

Fig. 3. Dependence of the effective enantioselectivity coefficients of three selected AQC-amino acids on selector concentrations using (a) DM-β-CD and (b) β-CD polymer. Straight lines display the curves corresponding to Eq. (2) using the parameters in Table 4. Experimental conditions as in Section 2.

(i) The binding strengths with native β-CD, DM-β-CD and HP-β-CD are comparable, whereas considerably lower values are assessed for the permethylated selector TM-β-CD. Considering the dependence of the complexation constants on type and size of the amino acid side chains, similar trends are observed when employing different selectors. Aromatic amino acids always show the highest complexation constants. For non-polar aliphatic amino acids a slight increase of K with chain length is observed.

(ii) Affinity and selectivity are rarely correlated, some exceptions are discussed below. Plots of effective enantioselectivity coefficients vs. complexation strength (K_1) are shown in Fig. 4a–d. These data refer to a selector concentration of 5 m*M* which is near the optimum concentration, $[S]^{opt,eff}$ of the shown selectors. In spite of bad overall correlations and regarding the series of non-polar aliphatic amino acids only, an increase of enantioselectivity with chain length and strength of complexation is observed in the order Pro, Ala, Val, Ile and Leu. Despite their high K values, enantioselectivities for aromatic amino acids are generally low with most selectors. Apparently, strong interactions between the aromatic moiety and the selector do not cause increased

Table 4
Complexation constants, K_1 and K_2 (l/mol), and mobilities of the analyte–selector complexes, μ_1^{cplx} and μ_2^{cplx} ($\cdot 10^{-5}$ cm^2 V^{-1} s^{-1}), from the first and second detected enantiomers, both achieved from curve fitting

Amino acid	β-CD		β-CD polymer		HP-β-CD		DM-β-CD		TM-β-CD	
	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2
Ala	230	190	–	–	200	180	190	170	45	40
Leu	250	210	310	240	340	250	230	180	40	32
Ser	260	220	–	–	210	200	160	150	40	35
Thr	300	250	190	160	220	180	170	130	45	35
Phe	300	280	445	435	320	280	250	240	45	40
Trp	270	260	–	–	340	290	450	430	55	52
	μ_1^{cplx}	μ_2^{cplx}	μ_1^{cplx}	μ_2^{cplx}	μ_1^{cplx}	μ_2^{cplx}	μ_1^{cplx}	μ_2^{cplx}	μ_1^{cplx}	μ_2^{cplx}
Ala	10.0	10.0	–	–	7.0	7.0	8.5	8.5	8.5	8.5
Leu	8.0	8.0	3.7	3.7	7.0	7.0	7.5	7.5	6.0	6.0
Ser	10.0	10.0	–	–	7.5	7.5	9.5	9.5	8.5	8.5
Thr	10.0	10.0	3.7	3.7	7.0	7.0	8.0	8.0	8.5	8.5
Phe	8.0	8.0	3.2	3.2	6.6	6.6	7.5	7.5	6.0	6.0
Trp	8.0	8.0	–	–	6.6	6.6	7.5	7.5	5.5	5.5

enantiodiscrimination. It is important to keep in mind this generally low degree of correlation for any structure–selectivity modelling.

The $[S]^{opt,eff}$ values corresponding to the "predicted" complexation constants are given in Table 6. With exception of the data obtained with TM-β-CD

Table 5
Predicted complexation constants of the first detected enantiomers, K_1 (l/mol), of AQC-amino acid enantiomers with differently substituted β-CDs

Amino acid	β-CD	β-CD polymer	HP-β-CD	DM-β-CD	TM-β-CD
Ala	165	205	195	180	55
Val	255	250	270	260	45
Leu	205	470	360	285	30
Ile[a]	225	390	395	230	45
		440		185	50
Met	375	380	325	330	45
Pro	125	95	115	80	30
Cys	654	280	315	455	25
Ser	205	210	285	175	30
Thr	305	225	340	340	30
Asn	225	210	210	115	45
Gln	225	225	230	150	45
Phe	335	540	515	295	60
Trp	280	1435	590	495	75
Tyr	410	775	635	435	100
	μ^{cplx} ($\cdot 10^{-5}$ cm^2 V^{-1} s^{-1}) [b]				
Aliphatic amino acids	10.0	3.7	7.0	9.0	8.5
Aromatic amino acids	8.0	3.2	6.6	7.5	6.0
Leu, Ile	8.0	3.7	7.0	7.5	6.0

Selector concentration 5 m*M*, other electrophoretic conditions as in Table 1.
[a] Two diastereomeric pairs of enantiomers.
[b] Values taken for the prediction of the given *K* data.

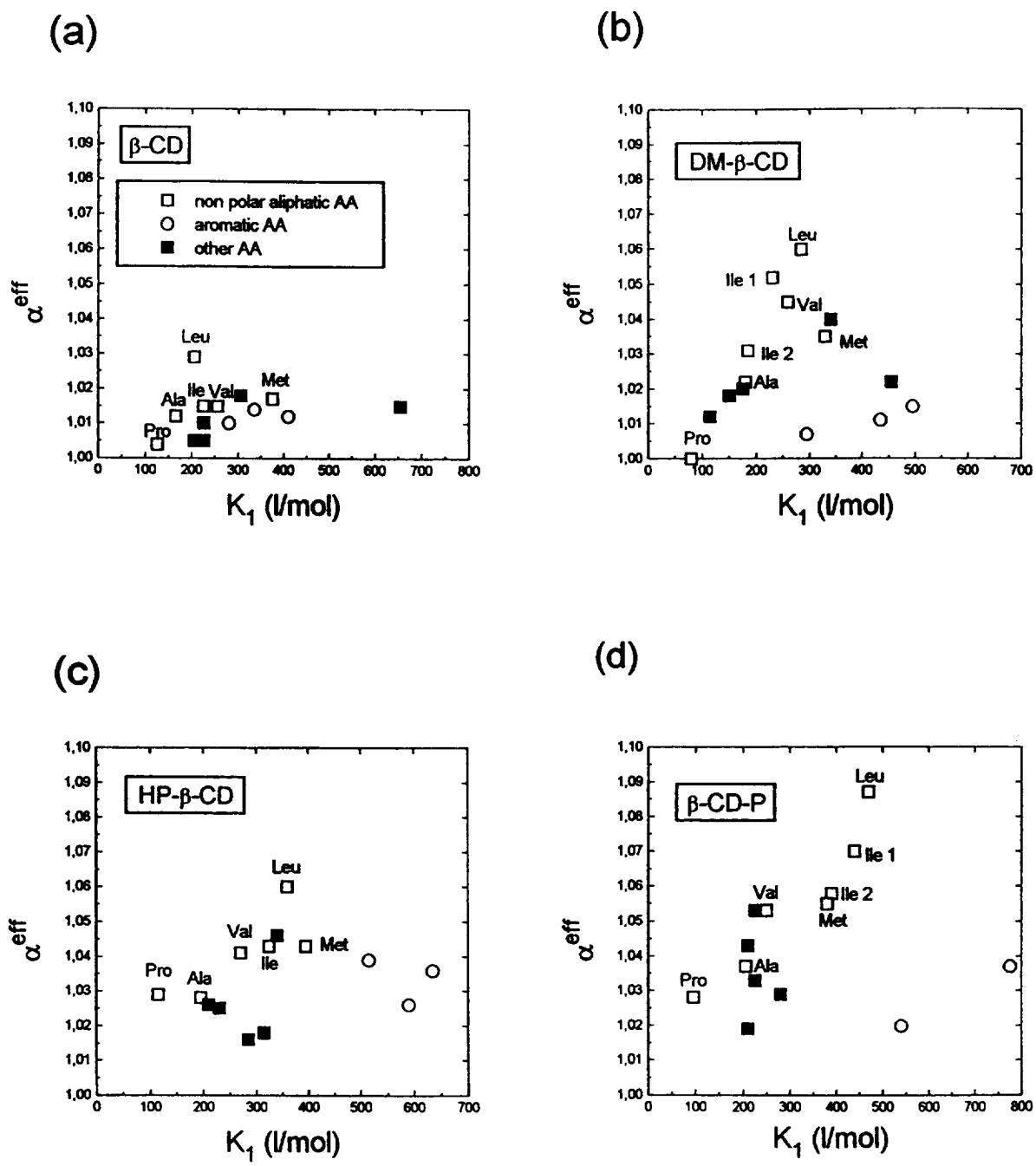

Fig. 4. Effective enantioselectivity coefficients of AQC-amino acids vs. predicted complexation constants of the first detected enantiomer using (a) β-CD, (b) DM-β-CD, (c) HP-β-CD and (d) β-CD polymer. Experimental conditions as in Section 2. Selector concentration of 5 m*M*.

Table 6
Optimum selector concentrations, $[S]^{opt,eff}$ (mM), calculated from the predicted values for the complexation constants

Amino acid	β-CD	β-CD polymer	HP-β-CD	DM-β-CD	TM-β-CD
Ala	6	5	6	6	20
Val	4	4	4	5	25
Leu	5	2	3	4	35
Ile[a]	5	3	3	5	25
		3		6	22
Met	2	3	3	3	25
Pro	8	12	10	13	35
Cys	2	4	3	3	45
Ser	5	5	4	6	33
Thr	4	5	3	3	41
Asn	5	5	5	9	23
Gln	5	5	5	7	23
Phe	3	2	2	4	18
Trp	4	1	2	2	13
Tyr	3	1	2	2	10

[a] Two diastereomeric pairs of enantiomers.

and the two weakly complexed amino acids Pro and the doubly labelled Lys, they generally range between 3 to 6 mM and near 2 to 3 for aromatic amino acids. The few cases where analytes have rather low K values [typically AQC-Pro and $(AQC)_2$-Lys], increased enantioselectivities are actually achieved when raising the selector concentration. Generally, however, selector concentrations above the optimum values do not effect a significant decrease in selectivities as shown by the typical shapes of the α^{eff} vs. $[S]$ curves.

Within this paper a simple predictive tool is evaluated which allows us to assess approximate values for complexation constants and optimum selector concentrations without needing extensive curve fitting procedures on the bases of assuming constant μ^{cplx} values for structurally similar analytes. Within the investigated set of analytes the error in the predicted data resulting from this approximation was in most instances not larger than that contributed by the scatter in the measured mobility data. The described procedure is thus not only suited for optimizing separation conditions in practical electrophoresis, but also for providing complexation constant data required for the discussion of affinity vs. selectivity relationships.

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