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Thermodynamics of chiral selectivity in capillary electrophoresis: separation of ibuprofen enantiomers with β-cyclodextrin

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Abstract

The effect of temperature on the electrophoretic chiral separation of ibuprofen with β-CD was investigated. Background electrolytes with sodium acetate or formate were chosen because of their constant pK within 0.03 units in the temperature range 25–50°C. Ibuprofen has a temperature independent pK value of 4.36, and a mobility of $23.3 \cdot 10^{-9}$ m^2/Vs at 25°C. The mobility has a temperature coefficient of 2.0%/°C. At that same temperature, formation constants K_1 for the uncharged enantiomers are 9955 and 10294 M^{-1} respectively. The formation constant K_2 for the charged form is 5256 M^{-1} for both isomers. For these chiral formation constants, ΔH values are around −50 kJ/mol, whereas ΔS values are around −90 J/mol/K. © 1997 Elsevier Science B.V.

Keywords: Enantiomer separation; Thermodynamics; Selectivity; Ibuprofen; Cyclodextrins

1. Introduction

The effect of temperature on electrophoretic separations is well known because it influences, in principle, many of the parameters, variables and constants involved in the separation, such as mobilities and pK values of both analyte and buffer ions [1–3]. In chiral separations in CE, several additional formation constants between analyte and chiral selector, and their temperature dependence are involved as well. Detailed knowledge of the magnitude of these effects will lead to a better understanding. As a result, temperature may in some instances be also used as a tool for fine-tuning resolution, provided that the separation compartment can be sufficiently thermostated in order to ensure a homogeneous temperature throughout the analysis time. The present paper focuses on the chiral separation of ibuprofen, using β-cyclodextrin as chiral selector. The interaction model uses pK values and mobilities of analytes and analyte–CD complexes and the formation constants of these complexes, such as previously described in the literature [4]. In that reference, the interaction between ibuprofen and β-cyclodextrin was determined under the condition of 100 mM ionic strength and 37°C. The present paper focuses on the chiral separation of ibuprofen, using β-CD as chiral selector at 10 mM ionic strength and as a function of temperature. Resulting stability constants were fitted into van't Hoff plots and the corresponding thermodynamic properties, ΔH and ΔS were calculated.

2. Experimental

Experiments were performed using P/ACE 2500 equipment (Beckman, Fullerton, USA) with fused-

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silica capillaries of different lengths. Detection wavelength was 214 nm. All experiments were performed at 25, 32, 40 and 50°C.

Background electrolytes (BGE) were prepared using analytical grade reagents from Merck or Sigma. Ibuprofen racemate was obtained as 200 mg tablets from a local pharmacy, dissolved in water, filtrated and diluted to a final concentration of approximately 0.4 mmol/l. Pressure injection time was 2.0 s. Mobilities were measured with reference to the EOF dip, unless noted otherwise.

The mobilities and pK values of ibuprofen were determined at +20.0 kV in a 476/400 mm, 75 μm I.D. uncoated capillary with BGE's consisting of 10 mM NaOH, adjusted to pH values in the range 3–5 with acetic or formic acid. The driving current was in the range 20–40 μA, depending on pH and temperature.

Formation constants of β-CD with the uncharged analyte (K_1) and with the charged analyte (K_2) were determined by measuring the effective mobility of ibuprofen as a function of the CD concentration in the range 0–15 mmol/l at pH 4.20 (in 10.0 mM sodium–acetic acid) and pH 6.55 (in 10.0 mM sodium–MES) respectively. These experiments were done at +20.0 kV in a 470/400 mm, 50 μm I.D. uncoated capillary. Chiral resolution and δK_1 values were obtained from experiments at −25.0 kV in a 300/370 mm, 50 μm I.D. coated capillary, with a BGE of 10.0-mM sodium–acetate of pH 4.47.

3. Results and discussion

3.1. Effect of temperature on BGE conductivity and pK

Mobilities generally have a temperature coefficient of ca. 2%/°C, that amounts to a factor 1.64 between 25 and 50. Experimentally, a factor of 1.52 was found for the pH 4.98 BGE, a factor 2.0 for the pH 3.06 BGE as indicated by the driving current.

The choice of background electrolyte buffering co-ion was determined by the availability of temperature dependence data of their pK value. For formic and acetic acid, $\partial pK/\partial T$ values were taken from literature [3], they were less than 0.0005 per degree at 25°C. For this reason, it is assumed that the pH of the BGE is independent of temperature under the experimental conditions used in the lower pH range. For the experiments performed at pH 6.55, MES was used as a counter-ion. The temperature dependence of the pK of MES is considerable ($-0.02/K$) but this BGE was only used for experiments to determine K_2, where the pH is irrelevant as long as pH≥pK+2 for anions.

3.2. Effect of temperature on EOF

Electroosmosis is caused by a negative-potential of the capillary wall, according to the equation:

$$\mu_{eof} = -\zeta\epsilon/\eta \quad (1)$$

in which μ_{eof} is the electroosmotic mobility, ϵ the dielectric constant and η the dynamic viscosity of the solvent in the electric double layer. There is no reason to expect that $\epsilon\zeta$ depends on temperature. This would mean that the temperature dependence of μ_{eof} can be modeled with the temperature dependence of viscosity, which amounts to 2%/°C. This is confirmed by the experimental results: for each of the BGE's, the temperature coefficient of μ_{eof} was determined. The average value was 1.9%/°C with a standard deviation of 0.3%/°C. As expected, the EOF strongly increases with BGE pH. Values of μ_{eof} at pH 4.98 are also tabulated in Table 1. In the pH and temperature range mentioned, the values for the electroosmotic mobility were successfully fitted to the following two-parameter model:

$$\mu_{eof} = -10.29 + 5.562 \cdot pH - 0.5455 \cdot T + 0.2503 \cdot T \cdot pH \quad (2)$$

where μ_{eof} is in 10^{-9} m^2/Vs and T in °C. This

Table 1
The effect of temperature on the electrophoretic parameters of EOF (at pH 4.98) and ibuprofen at ionic strength 10.0 mmol/l

Temperature	$-\mu_{eof}$	$-\mu_0$	S.D.	pK	S.D.
25°C	35.35	20.91	0.7	4.37	0.02
32°C	40.23	23.10	1.6	4.36	0.02
40°C	45.85	27.18	1.9	4.39	0.02
50°C	51.63	31.95	2.3	4.40	0.02
%/°C	1.85	2.0	–	–	–

Mobilities are given in 10^{-9} m^2/Vs.

relation can be quite useful but not necessarily valid at higher pH values or in other buffer systems, especially at different ionic strengths.

3.3. Effect of temperature on ibuprofen pK and mobility

Inverse absolute values of the effective mobilities of ibuprofen were plotted vs the $[H^+]$ concentration (see Fig. 1). The intercept corresponds to $1/\mu_0$, whereas the slope of this curve equals $10^{pK}/\mu_0$. The highest $[H^+]$ concentration yielded outlying values, which were disgarded. The remaining 4 points, each determined in duplicate, yielded good correlations. The results are summarized in Table 1. The p*K* of ibuprofen has an average value of 4.38 (S.D. = 0.02) which was independent of temperature in the range measured. The value was compared with the following literature values: 4.48 at 100 m*M* ionic strength and 37°C [4], or 5.10 at zero ionic strength and 25°C [5]. No temperature dependence of p*K* ibuprofen was found in the literature.

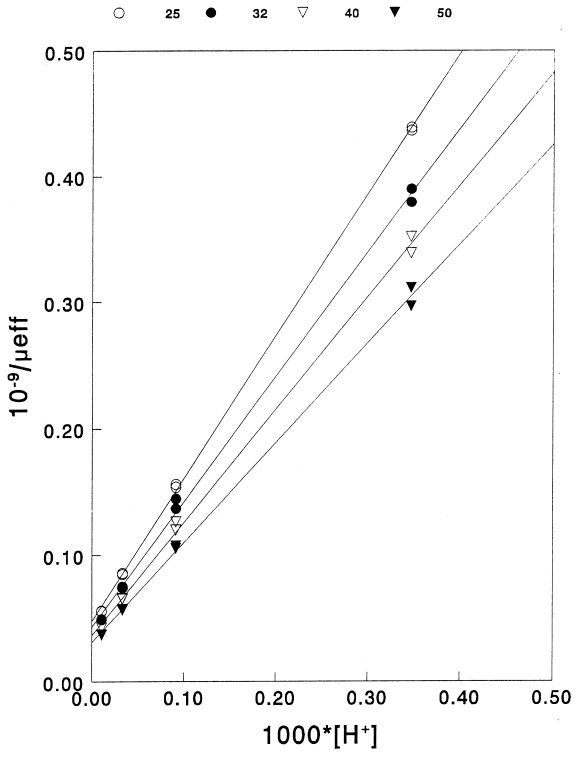

Fig. 1. Inverse absolute value of the effective mobility of ibuprofen as a function of the $[H^+]$ concentration at four different temperatures. Intercept and slope yield μ_0 and p*K* values respectively.

The mobility μ_0 is $20.91 \cdot 10^9$ m^2/Vs at 25°C and has a temperature coefficient of 2.0%/°C. A literature value of $21.32 \cdot 10^{-9}$ m^2/Vs was found at 100 m*M* ionic strength and 37°C [4]. When applying correction for temperature and ionic strength according to an empirical correction model [6], the difference between these mobility values is well within standard deviation. The standard deviation of μ_0 thus measured was rather high, due to the fact that is was obtained with extrapolation of the reciprocal value.

3.4. Determination of K_2 of ibuprofen–β-CD at different temperatures

First, the K_2 was determined at pH 6.55, where only interaction between the fully charged ibuprofen and β-CD takes place. According to the literature [4], the interaction involved is desionoselective (type I), so that no resolution is expected under these conditions. In Fig. 2, the effective mobility of ibuprofen μ_{eff} is plotted vs the β-CD concentration C_s. Mobility decreases strongly at low CD concentrations and saturation is visible above 10 m*M*, indicating a high K_2 value. The values were fitted according to the formula:

$$\mu_{eff} = (\mu_0 + \mu_c K_2 C_s)/(1 + K_2 C_s) \quad (3)$$

where μ_c is the mobility of the ibuprofen–β-CD complex. Coefficients of correlation ranged between 0.9990 and 0.9998. Values of μ_0, μ_c and K_2 obtained from the fit are listed in Table 2. As can be seen, μ_0 values obtained here are systematically different from those measured without CD in the pH range 3–5. The latter values were obtained through extrapolation of the data in Fig. 1, the former were directly measured at $C_s = 0$. For this reason it was decided to use the μ_0 values in Table 2 for subsequent calculations. The mobilities of the ibuprofen–β-CD complex range around half of the value of the free analyte, slightly increasing at elevated temperatures. The K_2 values, listed in Table 2 are somewhat higher than previously published values for ibuprofen at 37°C and ionic strength 100 m*M* [4].

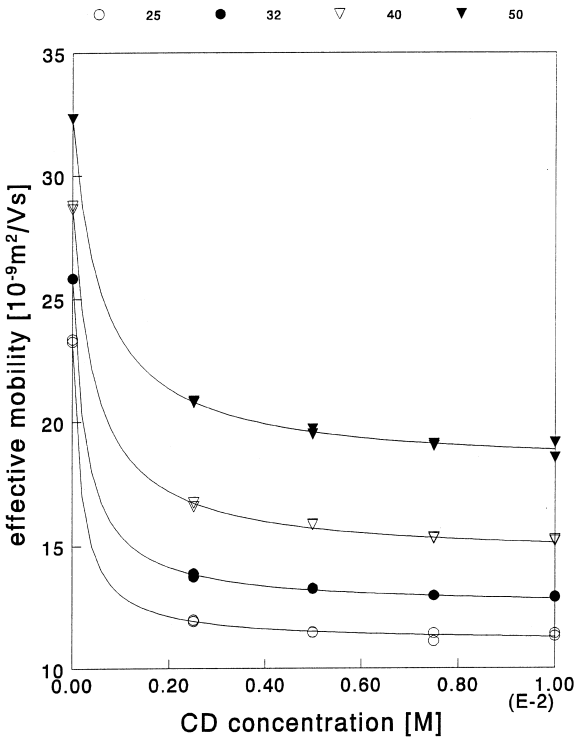

Fig. 2. Effective mobility of ibuprofen as a function of β-CD and temperature at pH 6.55 and ionic strength 10 mmol/l. For curve fit of K_2 see Section 3.4.

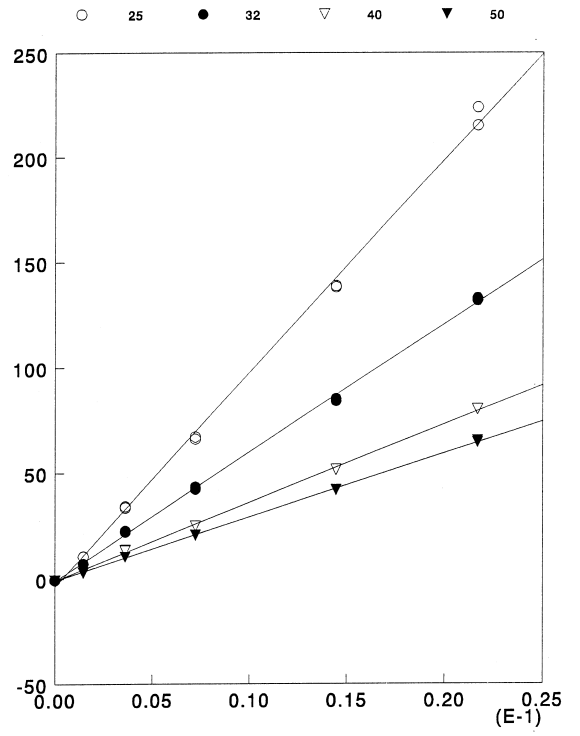

Fig. 3. Determination of K_1 at four different temperatures using linear regression, see Section 3.5 for axis information.

3.5. Determination of average K_1 of ibuprofen–β-CD at different temperatures

The interaction between uncharged ibuprofen and β-CD was determined in a BGE of 0.01 *M* sodium/acetate at pH 4.20, where both charged and uncharged forms of the analyte are present. Effective mobilities were again measured at different β-CD concentrations and temperatures in an uncoated capillary with mesityloxide as a neutral EOF marker for reference. Under these conditions, there was no resolution, in spite of the fact that we have a desionoselective interaction [4]. The equation for the effective mobilities under these conditions was linearized to the following [7]:

$$\mu_0/\mu_{\text{eff}} + K_2.C_s.\mu_c/\mu_{\text{eff}} - 1 - K_2C_s - [H_3O^+]/K_a = K_1.C_s.[H_3O^+]/K_a \quad (4)$$

so that plotting the left-hand side vs. $C_s \cdot [H_3O^+]/K_a$ yields a straight line with a slope equal to K_1. Results plotted this way are shown in Fig. 3.

Table 2
The effect of temperature on the chiral interaction parameters between ibuprofen and β-CD at ionic strength 10.0 mmol/l

Temperature	$-\mu_0$	S.D.	$-\mu_C$	S.D.	K_2	S.D.	K_1	S.D.	δK_1	S.D.
25°C	23.30	0.1	11.05	0.1	5256	740	10124	142	339	5
32°C	25.83	0.1	12.48	0.1	3550	253	6089	58	213	22
40°C	28.72	0.1	14.48	0.1	2139	176	3692	33	112	3
50°C	32.30	0.1	18.07	0.1	1675	145	3011	21	78	2
%/°C	1.5	–	2.0	–	–	–	–	–	–	–

Mobilities are given in 10^{-9} m^2/Vs, *K*'s in M^{-1}.

Coefficients of correlation were in all cases at least 0.999. Average K_1 values and their standard deviations are also listed in Table 2. Comparison with literature values at 37°C [4] indicate that our values for K_1 are higher, as was the case with K_2.

3.6. The effect of temperature in selectivity

The formation constants between the non-charged ibuprofen and β-CD are high and unequal for both optical isomers [4], so we have a desionoselective interaction. In order to obtain chiral resolution, electroosmosis was suppressed by working in a coated capillary with negative voltage [4]. In that case, there is no EOF marker for use as mobility reference. Therefore, we calculated the residual electroosmotic mobility from the average experimental migration time and the average effective mobility of ibuprofen, calculated from the data in Table 2. Using this information, we then calculated individual values of K_1 for both optical isomers from their experimental migration times, assuming equal values of K_2 for both isomers. The difference between the individual values of K_1 for both optical isomers is listed as δK_1 in Table 2. The values of δK_1 are well outside the standard deviation of the average K_1 values determined previously. It can be seen that not only K_1 and K_2 but also δK_1 decreases monotonously with increasing temperature.

3.7. Thermodynamic model for K_1 and K_2

Temperature dependence of equilibrium constants is usually modeled using a free energy (ΔG) relationship of the form [8]:

$$K_i = \exp(-\Delta G_i / RT) \quad (5)$$

with R the gas constant (8.314 J/mol/K) and T the absolute temperature. Using basic thermodynamics, this can be rewritten using enthalpy (ΔH) and entropy (ΔS) changes associated with the formation of the analyte-selector complex:

$$K_i = \exp(-\Delta H_i / RT + \Delta S_i / R) \quad (6)$$

Experimentally, both ΔH and ΔS can be obtained from a so-called Van't Hoff plot: the logarithmic of K_i vs. $1/T$. This is only valid under the assumption that both ΔH and ΔS are independent of temperature. Although there seem to be indications that this is not always the case for β-CD interactions [8], the data was processed under this assumption. The results are shown in Fig. 4. The negative sign of ΔH indicates a decrease of enthalpy, due to the release of high energy water out of the cyclodextrin cavity. The negative sign of ΔS indicates a decrease of entropy, due to complex formation, which consequently results in a decrease of the degree of freedom of the components involved in the interaction. As expected, the dominant force for analyte binding arises from enthalpy changes ($|\Delta H| \approx 2x|T\Delta S|$). The same was concluded in Ref. [10]. From our results, it was not possible to assign enantioselectivity to either $\Delta\Delta H$ or $\Delta\Delta S$ since the error in both ΔH and ΔS is higher than $\Delta\Delta H$ or $\Delta\Delta S$.

Probably the main source of sytematic errors arises from the temperature difference between the non-thermostated part (first 4 cm.) and the thermo-

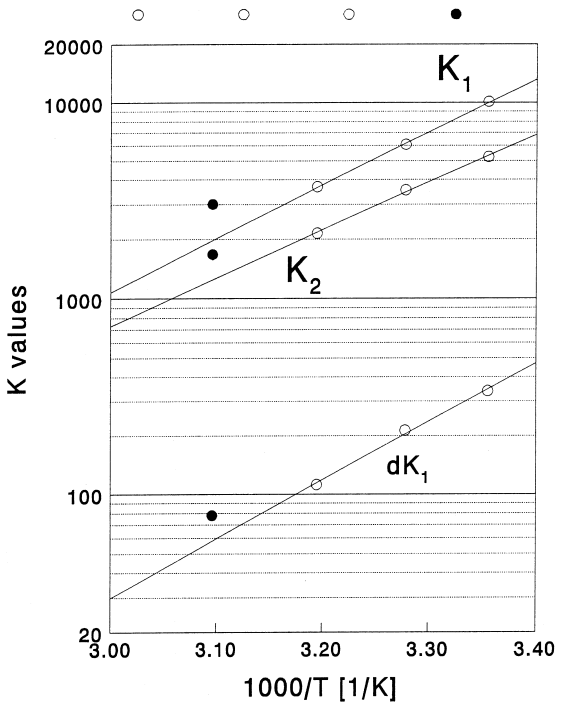

Fig. 4. Van't Hoff plots of the β-CD formation constants K_1 (and dK_1) and K_2 with uncharged and charged ibuprofen respectively. Outliers at 50°C (the leftmost, solid points) were not included in calculating ΔH and ΔS.

stated part of the capillary. This is clearly observed for the data points at 50°C, but a systematic error in the K_c-determination at lower temperatures cannot be excluded. However, this systematic error will be largest at 50°C and almost absent at 25°C. The leftmost point in both graphs (corresponding to 50°C) is considered an outlier.

The random error of ΔH and ΔS depends, among others, on the number of data points used, i.e. the number of different temperatures applied for the determination of the formation constants. Since the K_c-determination at 50°C is considered an outlier, only 3 data points are left. For obvious reasons, these data points are chosen in a relatively small temperature range: 298 K–323 K. Therefore, incertainties in ΔH and ΔS are relatively high, especially for ΔS since this parameter is obtained through extrapolation. Overall, accuracy and precision can be improved by increasing the number of temperatures and the number of experiments, and in insuring that the mobilities are measured exclusively in the thermostated part of the capillary. The latter can be achieved by the pressure mobilization method presented by Williams et al. [12]. Values of ΔH and ΔS were calculated, together with their standard deviations and tabulated in Table 3. No literature data on ibuprofen were available. Our values were somewhat higher than literature values for other analytes, possibly due to the fact that ibuprofen has very high stability constants with β-CD.

Using circular dichroism spectropolarimetry, Han et al. [9] determined ΔH and ΔS for interaction between β-CD and 8 barbital's: values were around -20 kJ/mol and -10 J/mol/K respectively. In a liquid chromatography study with β-CD as chiral stationary phase, Lipkowitz et al. [10] studied the enantioseparation of methyl mandelate. Their values for ΔH were around -30 kJ/mol, but $\Delta\Delta S$ values were 4 J/mol/K, 10 times higher than our values for ibuprofen. Effects of temperature on chiral resolution were also measured qualitatively in CE by Guttman et al. [11], who observed a decrease in both resolution and analysis time when increasing the temperature.

Using all data gathered, mobilities and selectivities can be calculated for any combination of parameters. One example is shown in Fig. 5a, a contour plot of selectivity vs. temperature and β-CD concentration at pH 4.47. As expected, selectivity increases with increasing β-CD concentration and decreasing temperature. When constructing the same contour plot for a higher pH value, for example pH 5.00, the 3D surface is shifted down as far as 0.01 selectivity units, making enantioseparation virtually impossible.

So far there seems to be no reason to increase operating temperature above 25°C, unless one takes into account analysis time as well. Consider for example a fixed selectivity of 1.01. In order to visualize a constant selectivity, the information contained in the 3D plot of Fig. 5a is reduced to 2 dimensions (T and Cs) in the form of a horizontal cross section of the 3D figure. Such a cross-section is shown as a dotted line in Fig. 5b. As expected, the CD-concentration, necessary to obtain a certain selectivity, increases strongly with increased temperatures. Next we calculated the effective mobility (Eq. 27, Ref. [4]) of the slowest migrating isomer. The solid line in Fig. 5b shows the migration time required to obtain a fixed selectivity of 1.01, applying a 300/370-mm coated capillary at -25 kV, assuming $\mu_{EOF}=0$. Now it is visualized that although temperature increase has an adverse effect on the amount of β-CD required, it might favor analysis time. Temperature optimization can lead to a gain in the speed of analyses, which might be favorable if the costs of the chiral selector are low (e.g. β-CD). The optimum temperature is very much dependent on the required selectivity. Increasing the required selectivity will result in a decrease in the optimum temperature.

Table 3
Thermodynamic parameters ΔH and ΔS for chiral interaction of ibuprofen and β-CD

	Value at 25°C (M^{-1})	ΔH (kJ/mol)	S.D.	ΔS (J/mol/K)	S.D.
$K_{1,1}$	9955	−52.08	1.6	−98.22	5.3
$K_{1,2}$	10294	−52.25	1.4	−98.49	4.7
K_2	5256	−46.58	2.3	−84.89	7.5

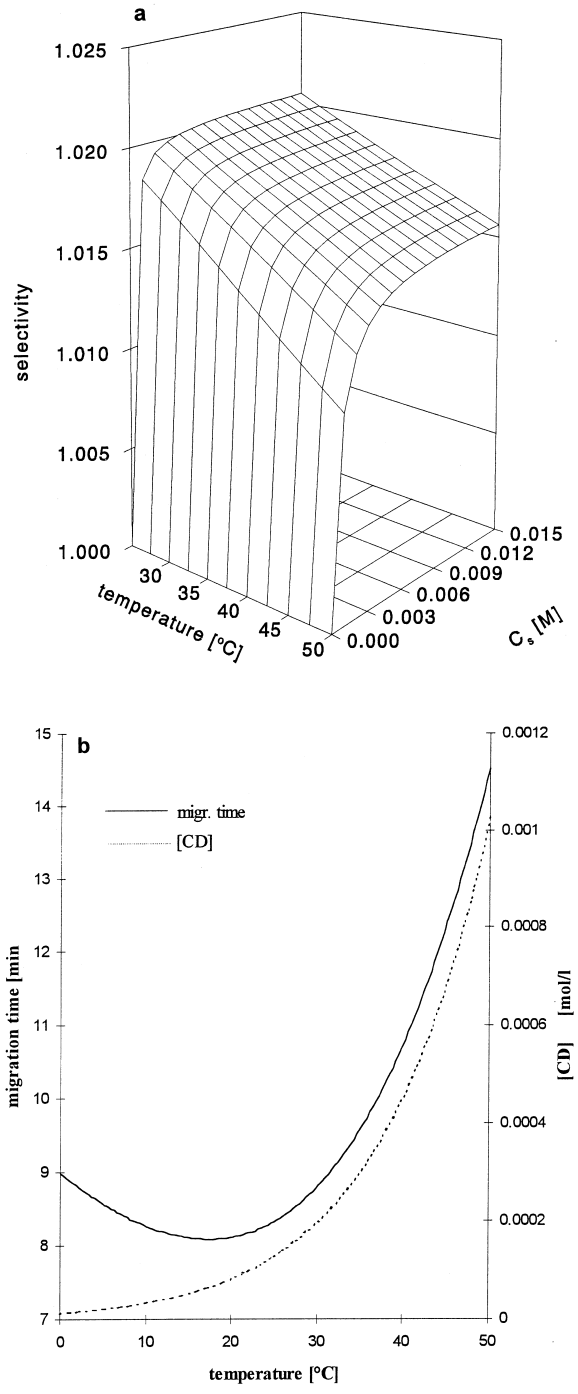

Fig. 5. a. Contour plot for selectivity of enantioseparation of ibuprofen as a function of temperature and β-CD concentration at pH 4.47. Mesh size is 2.5°C and 0.001 *M* respectively. b. Concentration of β-CD and analysis time required for a selectivity of 1.01, as a function of temperature, at pH 4.47. See Section 3.7 for further details.

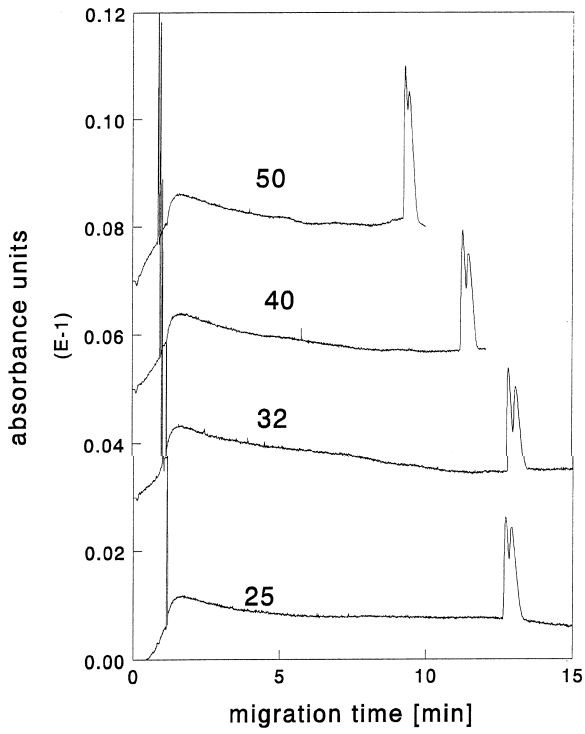

Fig. 6. Separation of a racemic mixture of ibuprofen in 0.01 *M* sodium/acetate, pH 4.47 with 2.5 m*M* β-CD in a coated capillary at different temperatures (indicated on the graphs in °C).

Summarizing, when finally choosing a set of separation parameters, through method development at room temperature, it seems certainly worthwhile to subsequently try different temperatures, as illustrated in the experimental electropherograms of Fig. 6. This is especially easy since it requires simple reprogramming of the analysis sequence in automated equipment.

4. Conclusions

In the presence of electroosmosis, increasing temperature leads to a shorter migration time of the EOF marker, which can be simply modeled with a coefficient of 2%/°C. If mobilities of anions and cations have approximately the same temperature coefficient, it follows that when increasing temperature, peaks in a mixture of cations and anions are spread over a larger time window. This may be

favorable, except for fast anions, because they will not reach the detector. Naturally the former only applies to uncoated capillaries at positive inlet voltages.

At the same time, BGE conductivity will increase by the same factor, so that one should verify if thermal dispersion plays a role. Another point of attention is a possible change of pH of the BGE with temperature.

Specifically for the chiral separation parameters, it was observed that K values decrease with increasing temperature, with negative values for both free energy and entropy changes. This means that when optimizing a chiral separation, using the present model [4], a different operating temperature may lead to different optimized conditions: selectivity will generally be lower at elevated temperatures. On the other hand, temperature effects all mobilities as well. What finally counts in optimizing analytical separation techniques such as CE is the combination of resolution and analysis time. Isoselectivity plots such as Fig. 5b can be most helpful in this respect. Most of these aspects can in principle be modeled by extending the present equations, e.g. Eq. (3) with Eq. (4). Admittedly, this is only feasible if all data (μ, K, ΔH and ΔS) are available, which will seldom be the case.

In conclusion one can observe that in principle temperature effects can be predicted by extending existing models. In addition, changing the temperature may sometimes be used to fine-tune separations, also in chiral CE applications.

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