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MECCSIM, training software for micellar electrokinetic capillary chromatography

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Abstract

A training simulation program for micellar electrokinetic capillary chromatography was developed. The software provides a high speed of calculation, visual detail and model accuracy with a user-friendly interface. The instrument parameters that can be varied include capillary material, length, internal diameter and wall thickness, thermostating temperature, voltage and polarity. Hydrostatic injection is simulated using time and pressure as variables. The following properties of the buffer can be varied: pH, ionic strength, and sodium dodecylsulfate (SDS) concentration. The retention model is based on experimental values for δH° and δS°, obtained from the literature. In addition, the temperature and ionic strength dependence of the critical micelle concentration and the partial specific volume of the SDS micelle is taken into account. This allows the calculation of the temperature dependence of the partition coefficients and capacity factors and thus of the retention and migration behaviour. In addition, a number of dispersion factors are included in the model.

1. Introduction

Computerized simulations of analytical separation techniques can be used in different contexts: fundamental research, method development, training and demonstration. For most analytical techniques, the fundamentals no longer have secrets from the specialists in the field. For relatively new techniques, simulation from basic principles, such as the equation of continuity and other differential equations and charge and mass balances, can provide more insight. These programs do an exact calculation, without any assumptions or approximations. The system has to be defined very precisely, all data (constants, variables) and their mutual dependence must be available. As a result, they require calculation times in the range of at least minutes to hours, depending on the time and place resolution required. These are not suitable for training purposes.

At the other end of the spectrum we have the demonstration software, illustrating and visualizing in a qualitative way what the result of a separation will look like. A requirement for such training software is a high speed of calculation and a user-friendly interface. The price that is paid for such convenience is that a lesser degree of accuracy can be obtained. The kind of simulations that we have developed in recent years [1–3] have provided a high speed of calculation, visual detail and model accuracy with a user-

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friendly interface. The principle goal has been to simulate the results of the separation (such as a chromatogram or electropherogram) rather than the dynamics of it. Several assumptions as to the mutual independent behaviour, especially in the initial stages of separation, have consequently been necessary. The simulated results, however, do not deviate too much from the experimental results [3] and are certainly sufficient for training purposes.

Micellar electrokinetic chromatography is a relatively new technique, introduced in 1985 [4,5]. Within a relatively short time, a considerable body of literature has become available and the first textbook on the subject gives an impressive overview [6]. One of the more illustrative features of this book is the large number of illustrations of simulated electropherograms, used to illustrate the different aspects of migration and dispersion.

2. Description of the program

2.1. User interface

The basic structure of the program consists of the user interface of previously published training software for gas chromatography [1] and free capillary zone electrophoresis [2,3]. The program runs on any IBM-compatible PC under DOS, with a graphics monitor (CGA/EGA/VGA) and optionally a mouse. A numeric coprocessor is advisable to keep calculation times within 1 s. Several shortcut keys were added for fine tuning of selectivity parameters. Full-screen graphics alternates with current instrument settings along with the pull-down menus. Details on the availability of the program can be obtained from the first author on request.

2.2. Database

The data stored were thermodynamic properties δH° and δS° taken from the literature [6,7]. A supplementary program was written to edit the database (change values, add components). An additional possibility is to include also synthetic components with imaginary, "ideal" properties, e.g., a δH° value of -100 kJ mol^{-1} for an electroosmotic flow (EOF) marker.

2.3. Retention model

For the retention model, first the temperature rise due to heat dissipation in the capillary is calculated with an iteration [2]. The conductivity of the buffer has contributions from the buffer anion, the buffer cation, sodium, dodecyl sulfate, micelles and hydrogen and hydroxide ions. The ionic strength I is also calculated. A complication is that the mobilities of all ions will depend on the ionic strength of the buffer and also on the temperature. Then the micellar specific volume V (mg l^{-1}) and critical micelle concentration c_{cmc} are corrected for this temperature T and ionic strength I using a (mutually independent) linear fit to experimental data from different publications (e.g., [7,8]):

$$V = V_0 + \frac{\delta V}{\delta T}(T - 298) + \frac{\delta V}{\delta I} \cdot I \qquad (1)$$

$$c_{cmc} = c_{cmc,0} + \frac{\delta c_{cmc}}{\delta T}(T - 298) + \frac{\delta c_{cmc}}{\delta I} \cdot I \qquad (2)$$

Both the micellar specific volume V_0 and the critical micelle concentration $c_{cmc,0}$ under reference conditions of zero ionic strength and 25°C and all four differential coefficients can be changed by the user of the program. Further details are given in Table 1.

From the analytical concentration of SDS, c_{sds}, and its molar mass, M_{sds}, the phase ratio β is now calculated:

$$\beta = \frac{10^{-3} M_{sds} V (c_{sds} - c_{cmc})}{1 - 10^{-3} M_{sds} V (c_{sds} - c_{cmc})} \qquad (3)$$

For those cases where $c_{sds} < c_{cmc}$ and thus $\beta < 0$, there are no micelles and β is taken as zero.

The electroosmotic mobility, u_{eof}, in SDS solutions depends on pH. It is fitted from experimental data [9], with the following relationship:

$$\mu_{eof} = [80 - 0.0015\,(12 - \mathrm{pH})^5] \cdot 10^{-9} \qquad (4)$$

where it is presumed that under these conditions

Table 1
Relevant "constants" in the migration and dispersion model

Symbol	Dimensions		Default
	Units	Range	
c_{cmc}	mmol l^{-1}	1–8	3
$\frac{1}{c_{cmc}} \cdot \frac{\delta c_{cmc}}{\delta T}$	K^{-1}	−0.05 to +0.05	+0.033
V	ml g^{-1}	0.1–3	0.81
$\frac{1}{V} \cdot \frac{\delta V}{\delta T}$	K^{-1}	−0.05 to +0.05	+0.001
$\frac{1}{c_{cmc}} \cdot \frac{\delta c_{cmc}}{\delta I}$	l mol^{-1}	−9 to +9	+6.25
$\frac{1}{V} \cdot \frac{\delta V}{\delta I}$	l mol^{-1}	−9 to +9	+0.2
d_i	m	10^{-9}–10^{-7}	10^{-8}
D_{aq}	$m^2 s^{-1}$	$5 \cdot 10^{-11}$–$2 \cdot 10^{-9}$	10^{-9}
D_{mc}	$m^2 s^{-1}$	$5 \cdot 10^{-11}$–$2 \cdot 10^{-9}$	10^{-9}
k_d	s^{-1}	10–10 000	10 000
σ_{ep}	–	0–0.01	0.01

μ_{eof} does not depend on ionic strength or c_{sds}. The temperature dependence of electroosmosis works mainly through the viscosity of the liquid near the capillary wall, η, as can be seen from the equation for the zeta potential, ζ. This value can now also be calculated, using the dielectric constant ε:

$$\zeta = -\frac{\mu_{eof}}{\eta\varepsilon} \tag{5}$$

In contrast to the previously developed simulator for HPCE, here the ζ potential is not an independent variable.

Then, the net electroosmotic velocity v_{eof} is calculated from the electroosmotic mobility and the field strength E:

$$v_{eof} = \mu_{eof} E \tag{6}$$

Positive velocities are directed towards the detector. The same can be done for the net micellar velocity, v_{mic}, where it is assumed that the micellar mobility, μ_{mic}, has a constant value of $-61.9 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}:

$$v_{mic} = (\mu_{mic} + \mu_{eof})E \tag{7}$$

For each of the separands, the Gibbs free energy change $\delta G°$ is calculated from T, $\delta H°$ and $\delta S°$ values in the database:

$$\delta G° = \delta H° - T\delta S° \tag{8}$$

which in turn is converted into the temperature-dependent distribution coefficient K:

$$K = \exp\left(-\frac{\delta G°}{RT}\right) \tag{9}$$

With the phase ratio β, this yields the capacity factor, k', for each separand:

$$k' = \beta K \tag{10}$$

The field of application of micellar electrokinetic capillary chromatography (MECC) is mainly the analysis of non-ionic compounds, but in order to extend the possibilities of the simulation program to the more general case, ionic compounds can also be simulated. Here data on the pH dependence of the distribution coefficient as a function of pH are usually not available. A recent publication [10] reported on computer-assisted modelling of MECC of ionizable compounds. Distribution coefficients of ionized and non-ionized forms were determined. Their ratio ranged between 2 and 34. In the model we assume that the distribution coefficient refers mostly to the uncharged compound and that the distribution coefficient of the charged form is ten times smaller. Now the effective charge of the ion z is calculated from pK and pH, as described previously [2]. For ionizable monovalent separands, Eq. 10 is modified to

$$k' = (1 - 0.9|z|)\beta K \tag{11}$$

For each separand, the net velocity v_{net} is determined by its distribution between aqueous and micellar phase:

$$v_{net} = \frac{k'}{1 + k'} \cdot v_{mic} + \frac{1}{1 + k'} \cdot v_{eof} \tag{12}$$

Only if this net velocity is positive will the separand reach the detector and the retention time, t_R, can be calculated using the capillary length to the detector, L_d:

$$t_R = L_d / v_{net} \quad (13)$$

Also calculated in this way are the retention times of the marker, $t_{R,eof}$ (where $v_{net} = v_{eof}$), and of the micelle, $t_{R,mc}$ (where $v_{net} = v_{mic}$).

2.4. Dispersion model

A number of dispersion factors in MECC are similar to those in HPCE. The off-column dispersion factors used in this study are therefore identical with those given previously [2,3]. An overview of dispersion factors on-column in MECC has been given [5,6]. The following contributions can be distinguished: longitudinal diffusion (with diffusion coefficients D_{aq} and D_{mc}), sorption and desorption kinetics (with rate constant k_d), intermicelle diffusion (with D_{aq}), electrophoretic dispersion (with σ_{ep}) and thermal gradient effects. A number of interesting publications have been especially devoted to these effects [11,12]. In spite of the fact that under practical working conditions some of the effects mentioned are negligible, all are included in the present model. The purpose was to illustrate which parameters determine efficiency and to what extent. For an overview of the dispersion model incorporated into the present simulator, we refer to the relevant literature (Table 5.6 in Ref. [6]).

Table 1 lists the most important parameters included in the dispersion terms. The values of the constants in the dispersion terms show a considerable degree of uncertainty when consulting the different literature sources. Therefore, these "constants" can be changed, upon which they are automatically saved, so that subsequent simulation sessions start with the parameters so updated. This makes it possible to see in detail what would happen if a different detergent with other properties were to be used. The only limitation is that the thermodynamic properties δH° and δS° remain the same for that particular separand. On the other hand, δH° and δS° values can be temporarily changed to see the result of a change in separand properties, independent of the buffer system chosen.

3. Results and discussion

3.1. Temperature effects

As in MECC the temperature in the capillary is important, even more so than in CE, several workers have investigated this aspect. A linear relationship between the temperature rise and the power dissipation (W m^{-1}) was theoretically predicted [2,3] and was experimentally verified using thermochromic solutions [13]. Experiments for determining this temperature rise from MECC [7] did not yield the same result, although insufficient details of the capillary dimensions were provided in both publications. We assume that in both cases the O.D. was 375 μm and I.D. 75 μm. A temperature rise of 0.33°C m W^{-1} is then simulated for a quartz capillary, compared with 0.3°C m W^{-1} in Ref. [13] and 3°C m W^{-1} in Ref. [7].

3.2. Homologous series

The separation of a series of alkylbenzenes was simulated using data from Ref. [8] (see Fig. 1). In actual practice the migration time of the micelle can be determined by an iteration procedure, applied to such a homologous series [14]. When using different homologous series, a different value of t_{mc} was found [8], indicating that the micelle marker used (Sudan 3) was not fully micellarized. When calculating the capacity factor for Sudan 3, using the t_{mc} thus obtained, values in the range 100–200 were found [8]. In the simulation, a synthetic, temperature-independent marker for the micelle migration time was introduced, having $\delta H^\circ = 0$ and $\delta S^\circ = 100$ J mol^{-1} K^{-1}. The resulting capacity factor for this component was 1100, but it can still be distinguished from t_{mc}, where naturally k' approaches infinity.

3.3. Method development

Provided that sufficiently reliable data are available, the MECCSIM program can be used for method development. A typical application is shown in Fig. 2 for the separation of a mixture of

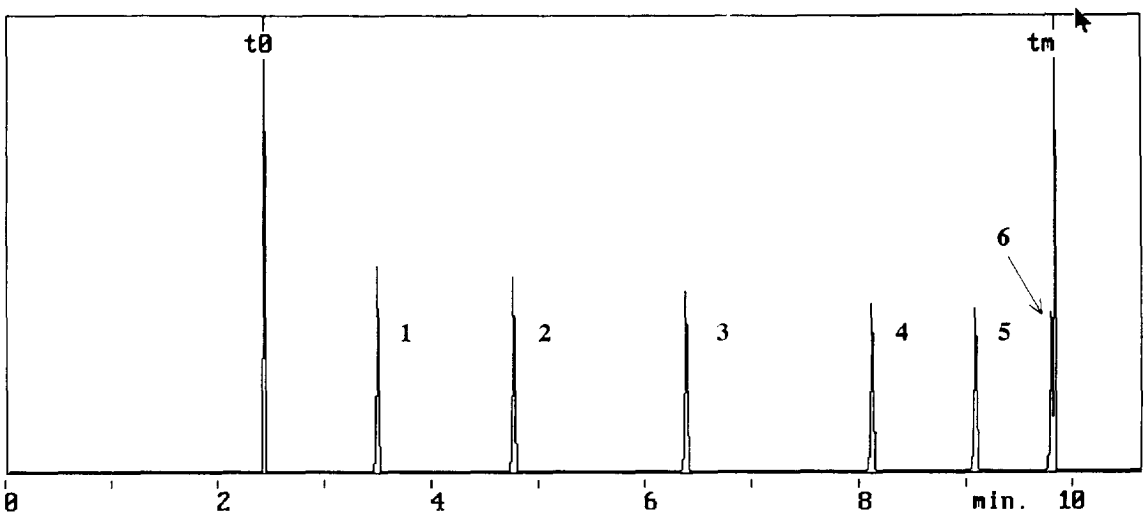

Fig. 1. Simulated chromatogram of a homologous series of alkylbenzenes [8] at 25°C, 20 kV, pH 8.5, 50 mmol l^{-1} SDS in a 50 μm I.D./375 μm O.D. quartz capillary with a length to the detector of 455 mm and 501 mm total length. Peaks: 1 = benzene; 2 = toluene; 3 = ethylbenzene; 4 = propylbenzene; 5 = butylbenzene; 6 = a synthetic component, with $\delta H° = 0$ and $\delta S° = 100$ J mol^{-1} K^{-1}.

six cold medicines [7]. As can be seen, the distribution coefficients result in a very broad range of capacity factors (0.16–124 in this case), so that almost the full retention window has to be used for simultaneous determination. This puts obvious constraints on the buffering system.

3.4. Comparison of input data

Literature values for thermodynamic properties of $\delta H°$ and $\delta S°$ in the water–SDS system under buffered MECC conditions are scarce. Influences of buffer type, ionic strength and pH

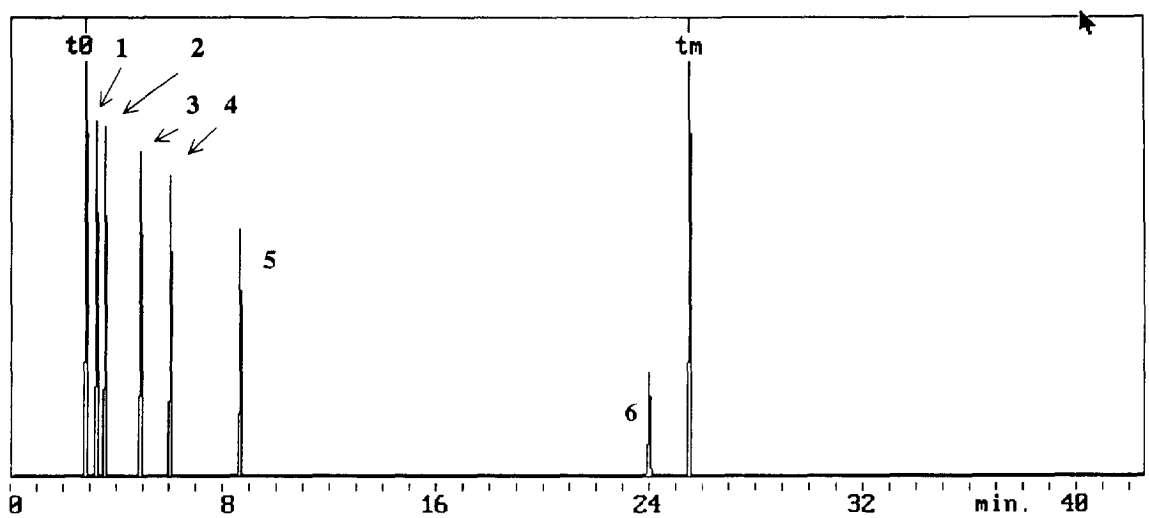

Fig. 2. Simulated chromatogram of a standard mixture of cold medicines [7] at 30°C, 20 kV, pH 7.0, 50 mmol l^{-1} SDS in a 75 μm I.D./375 μm O.D. quartz capillary of length 500 mm Peaks: 1 = acetaminophen; 2 = caffeine; 3 = guaifenisin; 4 = ethenzamide; 5 = isopropylantipyrine; 6 = trimethoquinol.

on the partition coefficient and thus on these thermodynamic properties can never be excluded. Two databases were made, based on results from two sources [7,8]. In the first publication [7], several buffers were used, in some cases leading to significantly but not extremely different values for $\delta H°$ and $\delta S°$. The c_{cmc} was also different in three different pH 7.0 buffers.

In the second publication, only one pH 8.5 buffer was used. The following solutes were measured by both: resorcinol, phenol, nitrobenzene and toluene. The results for $\delta H°$ and $\delta S°$ for these four solutes from the two publications differed considerably (see Table 2). Some values determined by micellar liquid chromatography (MLC) [15] were also included for comparison.

The $\delta G°$ and K values were also different, although the electropherograms based on the data from the first two sources were similar (see Fig. 3a and b). There can be two explanations. One might stress that values in different buffers may not be compared, but that does not explain the similarity between the two simulated chromatograms from entirely different $\delta H°$ and $\delta S°$ values. In the second publication [8], small relative standard deviations for $\delta H°$ and $\delta S°$ are claimed (3–5%); the systematic difference with [7] could be caused by the buffer.

Another conclusion might be that MECC is not a very reliable way of determining $\delta H°$ and $\delta S°$, where we must not overlook the fact that in order to obtain $\delta H°$ and $\delta S°$ from t_R one needs equally reliable values of t_{eof}, t_{mc}, V, c_{cmc} and c_{sds} under the experimental conditions concerned. Also, the temperature in these measurements is changed over a relatively small interval on the kelvin scale.

3.5. Kinetic limitations

Using the MECC simulator, separations can be highly optimized in terms of analysis time, using all the variables available. In this way one can go far beyond present limitations of equipment design. For example, the dispersive effect of heat production can be kept to a minimum when using a 5-μm capillary. If the wall of the capillary is also very thin, there will be only a small temperature increase at the high power dissipations usually encountered in MECC. Under these circumstances (analysis times in seconds, not minutes), kinetic limitations are seen to play a key role in dispersion, provided that the detector has a time constant <0.1 s, also unlikely at present. The effect of changing k_d on this kinetic limitation can now easily be visualized.

In practice, heat development will be an important limitation in speeding up analyses. With respect to thermostating, two limiting cases

Table 2
Experimental results from different sources [7,8,15] for thermodynamic properties $\delta H°$ (kJ mol^{-1}), $\delta S°$ (J mol^{-1} K^{-1}) and $\delta G°$ (kJ mol^{-1}) at 40°C for some solutes in SDS

Parameter	Ref.	Resorcinol	Phenol	Nitrobenzene	Toluene
$\delta H°$	[7]	−12.5	−11.1	−9.7	−7.6
	[8]	−6.1	−5.8	−5.0	−3.8
	[15]		−9.3		−2.2
$\delta S°$	[7]	−17.4	−5.4	+7.4	+21.1
	[8]	+7.5	+13.6	+23.8	+34.2
	[15]		−12.5		+25.9
$\delta G°$	[7]	−7.05	−9.41	−12.0	−14.2
	[8]	−8.45	−10.1	−12.4	−14.5
	[15]		−5.4		−10.3
K	[7]	15.0	37.2	101	234
	[8]	25.7	48.5	117	263
	[15]		8.0		52

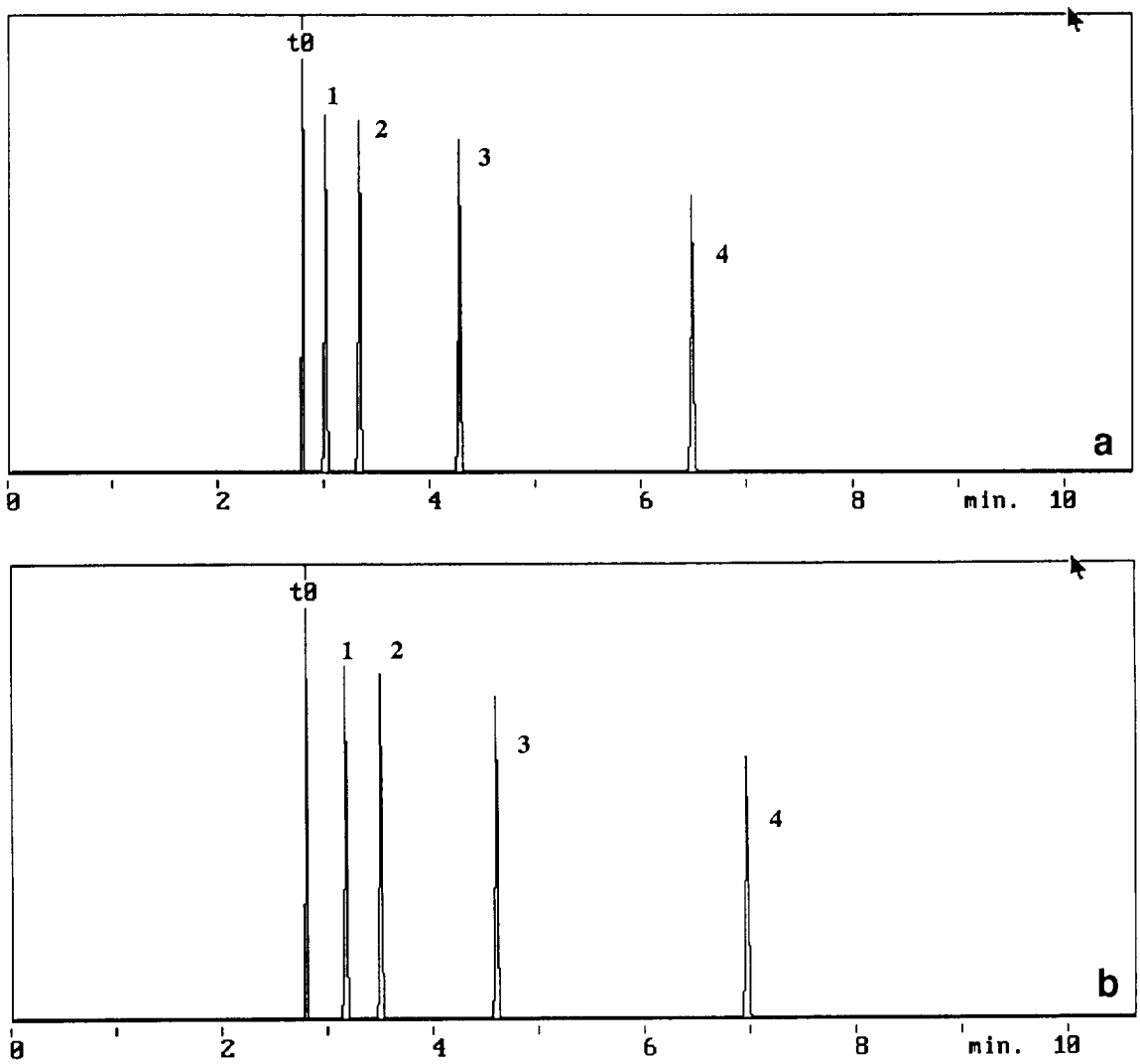

Fig. 3. Simulated chromatogram at 40°C, 20 kV, pH 7.0, 50 mmol l^{-1} SDS in a 75 μm I.D./375 μm O.D. quartz capillary of length 500 mm. Peaks: 1 = resorcinol; 2 = phenol; 3 = nitrobenzene; 4 = toluene. Based on data from (a) Ref. [7] and (b) Ref. [8].

are distinguished in the simulation program: in one, perfect liquid cooling (no temperature gradient outside the capillary) is assumed; in the other, the capillary is hanging in still air (large temperature gradient outside capillary), given by Knox's equation [16]. The cooling situation in practice will often be ill-defined but certainly between these limiting cases.

The former, idealized case is illustrated in Fig. 4 for the separation of xylenols. Under these conditions of high power dissipation (61 W m^{-1}), the temperature in the 300 mm × 75 μm I.D. capillary is increased from 30 to 62°C. This is about as far as one can go in speeding up the analysis with perfect liquid cooling. For the other limiting case where the capillary is hanging in still air, only 1.4 W m^{-1} would result in the same 32°C temperature rise. As the power dissipation is proportional to E^2, the voltage should be 6.6 times lower for air cooling in order to obtain the same temperature rise, thus illustrating the need for a good cooling mechanism.

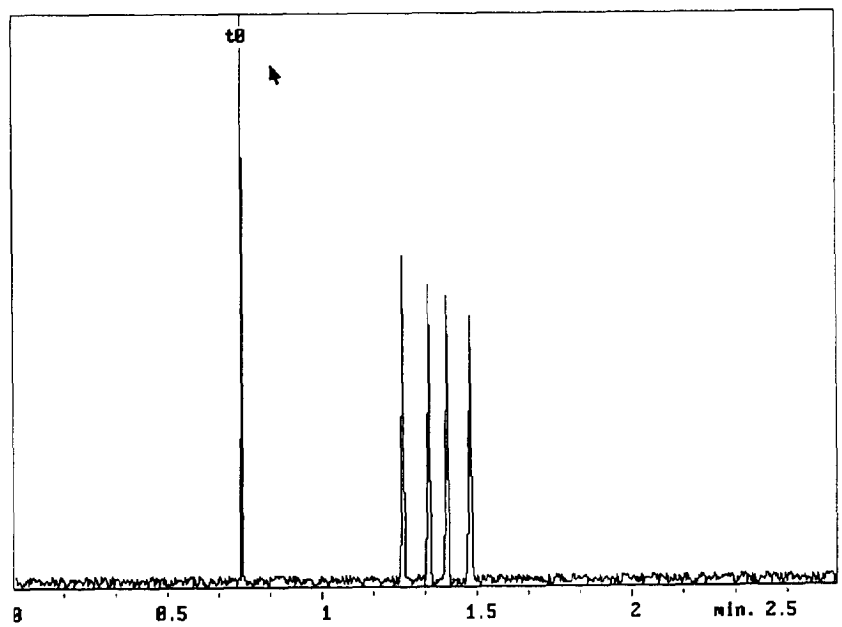

Fig. 4. Simulated chromatogram at 30°C, 30 kV, pH 6.0, 50 mmol l^{-1} SDS in a 75 μm I.D./375 μm O.D. quartz capillary of length 300 mm. Peaks from left to right: 2,6-xylenol, 2,3-xylenol, 3,4-xylenol and 2,4-xylenol.

4. Conclusions

The simulation model makes it possible to illustrate the influence of a large number of sample, buffer and equipment parameters on chromatograms obtained by MECC. The possibility of changing the "constants" in the migration and dispersion model makes it possible for the simulation program to learn from the input of new experimental data and thus to refine the theoretical model. Synthetic mixture can be used to visualize the many factors involved in retention and dispersion so that the examples given in the Vindevogel and Sandra's book [6] can be generated by the user in a very flexible manner.

The use of MECCSIM in method development, however, is still limited by the lack of sufficiently reliable data of $\delta H°$ and $\delta S°$, or alternatively k', values under different but well defined conditions.

Symbols and abbreviations

c_{mic}	critical micelle concentration (mol l^{-1})
c_{sds}	analytical concentration of SDS (mol l^{-1})
d_i	intermicelle distance (m)
D_{aq}	diffusion coefficient in the aqueous phase (m^2 s^{-1})
D_{mc}	diffusion coefficient in the micelle phase (m^2 s^{-1})
E	field strength (V m^{-1})
I	ionic strength of the buffer (mol l^{-1})
k'	capacity factor
k_d	(de)sorption rate constant (s^{-1})
K	distribution coefficient
L_d	capillary length to the detector (m)
M_{sds}	molar mass of SDS (g mol^{-1})
R	gas constant (8.314 J mol^{-1} K^{-1})
T	average temperature in the capillary (K)
t_R	retention time (s)
$t_{R,eof}$	retention time of EOF marker (s)
$t_{R,mc}$	retention time of the micelles (s)
v_{eof}	net electroosmotic velocity (m s^{-1})
v_{mic}	net micellar velocity (m s^{-1})
V	specific volume of micelle (ml g^{-1})
z	effective charge of ion
β	phase ratio
$\delta G°$	change in Gibbs free energy (J mol^{-1})
$\delta H°$	change in Free energy (J mol^{-1})

δS° change in entropy (J mol $^{-1}$ K $^{-1}$)
ε dielectric constant of the buffer ($0.708 \cdot 10^{-9}$ F m $^{-1}$)
η buffer viscosity near capillary wall (10^{-2} N s m $^{-2}$)
μ_{eof} electroosmotic mobility (m^{2} V $^{-1}$ s $^{-1}$)
μ_{mc} micellar mobility (m^{2} V $^{-1}$ s $^{-1}$)
σ_{ep} relative standard deviation of μ_{mc}
ζ zeta potential of the capillary wall (V)

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