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Chemometric evaluation of the band broadening in micellar electrokinetic chromatography of peptides

M. Thorsteinsdóttir[a,*], D. Westerlund[a], G. Andersson[b], P. Kaufmann[b]

[a]*Analytical Pharmaceutical Chemistry, Faculty of Pharmacy, Uppsala University Biomedical Centre, P.O. Box 574, 751 23 Uppsala, Sweden*

[b]*Scotia Lipid Teknik AB, Box 6686, 113 84 Stockholm, Sweden*

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Abstract

Very high efficiencies were obtained for enkephalin-related peptides with low distribution to anionic micelles of taurodeoxycholate (TDC), while the efficiencies decreased drastically for peptides with a strong association to the micelles. This phenomenon was evaluated by studying the influence of TDC concentration, injected plug length, applied voltage, temperature, ionic strength of the background electrolyte and composition of injected solution on the band broadening. A chemometric approach, using a fractional factorial design for the screening experiment and response surface modelling was applied to evaluate the effect of the experimental factors on the peak width of the peptides. Partial least square regression of the peak widths at different TDC concentrations revealed two different phenomena in the system. One in which electrophoretic migration in the aqueous phase dominated, giving narrow peak widths, and a second dominated by micellar solubilization, resulting in peak widths four-times broader for the peptides, probably due to slow sorption–desorption kinetics.

Keywords: Band broadening; Micellar electrokinetic chromatography; Chemometrics; Taurodeoxycholate micelles; Response surface modelling; Peptides

1. Introduction

The main contributions to band broadening during separation in micellar electrokinetic chromatography (MEKC) stem from longitudinal diffusion, sorption–desorption kinetics, intermicelle mass transfer, the radial temperature gradient, micelle microheterogeneity, injection and detection. Terabe et al. [1] studied these band broadening phenomena during separation of neutral solutes in MEKC using sodium dodecyl sulfate (SDS) as micellar agent. Their results showed that longitudinal diffusion was the dominant factor when the velocity was slow, while sorption–desorption kinetics and heterogeneity of the micelles became significant at higher voltages. Intermicelle mass transfer and the temperature gradient were not significant under the experimental conditions used. Sepaniak and Cole [2] found that dispersion due to resistance to mass transfer in the background electrolyte (BGE) and temperature gradients within the capillary were most significant with SDS as surfactant. Davis [3] reported that changes in partition coefficients due to Joule heating resulted in band broadening. Wallingford and Ewing [4] showed that

*Corresponding author.

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strong electrostatic interactions with the charged head group of the surfactant SDS can cause slow solute–micelle exchange kinetics and lead to band broadening. The extra-capillary parameters, such as injection size, detector bandwidth and detector rise time set demands on the instrumentation. Since detection occurs on-capillary different zones move past the detector at different velocities. Because of the difference in residence time in the detector region, observed peak widths do not directly reflect the actual spreading of the zone in the capillary and solutes with low mobilities become broader [5]; a normalization by dividing the peak area by the migration time is generally done. Huang et al. [6] found the length of the injection zone as the most significant contributor to the band broadening when the injection electrolyte was identical to the running electrolyte. By selecting proper injection buffers relative to the BGE it is possible to compress the injection zone, a process known as stacking [7].

The aim of this work was to evaluate why the efficiency for peptides with low association to taurodeoxycholate micelles was much higher than for those with strong association with the micelles. The experimental factors that cause band broadening during the separation were studied by a chemometric approach.

2. Experimental

2.1. Chemicals

The BGE consisted of analytical-reagent grade phosphoric acid and analytical-reagent grade sodium hydroxide, Titrisol from Merck (Darmstadt, Germany) diluted with water purified in a Milli-Q Water system (Millipore, Bedford, MA, USA), to give pH 3.00. γ-Aminopropyltriethoxysilane was from Dynamit-Nobel (Baden, Germany), toluene from Aldrich (Milwaukee, WI, USA), acetone and hydrochloric acid 37% from Merck. The surfactant used was taurodeoxycholic acid (TDC) from Sigma (St. Louis, MO, USA). The enkephalin-related peptides were from Chemicon (Stockholm, Sweden).

2.2. Apparatus

Experiments were carried out on a Hewlett-Packard ^{3D}capillary electrophoresis system (Hewlett-Packard, Waldbronn, Germany), with a capillary electrophoresis (CE) unit with diode-array detection and a ChemStation for system control, data collection and data analysis. Absorption detection was carried out at 200 nm. Separation was performed on amino-silylated fused-silica capillaries [8], of 48.5 cm (effective length of 40 cm)×50 μm I.D. Samples were injected by pressure and the injected amounts were less then 100 pg of each peptide.

2.3. Experimental design

Evaluation of the band-broadening of enkephalin-related peptides in MEKC was done by statistical experimental design. Significant factors for peak width were determined by screening experiments including six factors. A reduced (2^{6-2}) factorial design at two levels with resolution IV was selected. The experimental domain was defined as the applied voltage between 7 and 20 kV; the TDC concentration was from 4 to 15 m*M*; the ionic strength of the phosphate buffer was from 0.005 to 0.06 *M*; the plug length was between 1.3 (2 s 40 mbar) to 24 (37 s 40 mbar) mm; the samples were dissolved in a buffer with 10-times lower ionic strength than the BGE in order to achieve stacking, or in a buffer with the same ionic strength as in the BGE; and the composition of the injected solution was with or without micelles (see Table 1). The plug length has to be seen as an operational value, since the effective plug length is dependent on the viscosity of the electrolyte. We have assumed that the viscosity is that of water, but the viscosity of the electrolyte will probably increase at higher TDC concentrations, resulting in a lower effective plug length than calculated by the Hagen–Poiseuille equation. The experiments were carried out in a random order to avoid systematic long-term influence of a particular factor. The selected responses were the width of the peak at half height for peptides that had low-, moderate- and high-association to the micelles, respectively. Four factors; the TDC concentration, injection plug length, ionic strength of the background electrolyte and ionic strength of the injected solution were further evaluated by a central composite face (2^4+4+8) design. In central composite face (CCF) design, the axial points are placed on the face

Table 1
Experimental domain used in the screening with fractional factorial design

Variable parameter	Experimental domain		
	(−) Level	(0) Level	(+) Level
Applied voltage (kV)	7	13.5	20
TDC (m*M*)	4	9.5	15
Ionic strength (*I*)	0.005	0.03	0.06
Plug length (mm)	1.3	12.7	24
Composition of injected solution	A	B	B
Stacking	C	D	D

A: With same concentration of TDC in the sample solution as in the BGE, B: without TDC in the sample solution, C: the sample solution had the same ionic strength as the BGE, D: the sample solution had 10 times lower ionic strength than the BGE.

of the cube, that is, the factor is set to a low and a high level, respectively while the other factors are kept at the centre level [9]. The experimental domain was defined according to Table 2. The injected plug length was decreased such that the high level was set at the centre point from the screening design. The injected solution contained the same concentrations of TDC as in the BGE and the applied voltage was kept constant at 15 kV. After the CCF design a new experimental screening was performed at each of three TDC concentrations, 0.9, 4.5 and 15 m*M*. Five variables were studied by a fractional factorial design ($2^{5-2}+3$) with resolution III. Three variables; the injected plug length, the stacking and applied voltage were further evaluated by a central composite design by augmenting the factorial design with central and axial points. The programs used in the model building process were CODEX (SumIT System, Solna, Sweden) and MATLAB (MathWorks, Natick, MA, USA).

Table 2
Experimental domain used in the CCF design

Variable parameter	Experimental domain		
	(−) Level	(0) Level	(+) Level
Plug length (mm)	2.0	6.8	12
Stacking[a]	0.1	0.55	1.0
TDC (m*M*)	4	9.5	15
Ionic strength	0.005	0.03	0.06

[a] 0.1=10 times lower ionic strength in the injected solution than the BGE, 1.0=the same ionic strength in the injected solution as in the BGE, voltage: 15 kV, the injected solution contained TDC in the same concentration as the BGE.

3. Results and discussion

3.1. Partial least square (PLS) regression modelling over a large range of TDC concentrations

The experimental domain is given in Table 1 as described earlier. The experiments were carried out for two sets of sample solutions, one containing the peptides, TGG, DTLE, DMME, LE and LEA and the other DTME and ME, see Table 3. The isoelectric point (p*I*) for the peptides were 6.06 except for the one containing arginine which had p*I*=9.75 [8]. The peak width at half of the peak height was corrected for the migration time and spatial width according to Huang et al. [6]:

$$w_s = (L_d/t_m)w_t - w_d \quad (1)$$

where w_s is the spatial width of the sample in units of length, L_d is the effective capillary length, t_m is the migration time, w_t is the recorded temporal

Table 3
Characteristics of the enkephalin-related peptides

Peptide	Amino acid sequence	M_r	p*I*
TGG	Tyr–Gly–Gly	295	6.06
DTLE	Gly–Gly–Phe–Leu	393	6.06
DTME	Gly–Gly–Phe–Met	411	6.06
DMME	Tyr–Gly–Gly–Phe	443	6.06
LE	Tyr–Gly–Gly–Phe–Leu	556	6.06
ME	Tyr–Gly–Gly–Phe–Met	574	6.06
LEA	Tyr–Gly–Gly–Phe–Leu–Arg	712	9.75

widths in time units and w_d is the spatial width of the detector window.

Only the peak widths of the peptides, TGG and LEA were used as responses in the preliminary screening, since under certain experimental conditions the other peptides co-migrated with the system peak corresponding to the electroosmotic flow (EOF). This is illustrated in Fig. 1; the system peak became deformed due to co-migration of the peptides, DTLE, DMME and LE. The Figure also shows an enormous difference in peak width between the other two peptides. The peptide TGG, which had low association to the micelles migrated before the EOF, with much higher efficiency than the peptide LEA, migrating after the system peak. The peptide LEA had higher net charge than the other peptides and was under most conditions totally associated to the micelles, having the same mobility as naphthalene, which was assumed to be fully distributed to the micelles (data not shown). The reason behind the asymmetric peak shape of the LEA peptide under most experimental conditions was probably either due to adsorption to the capillary wall, or to electrodispersion effects.

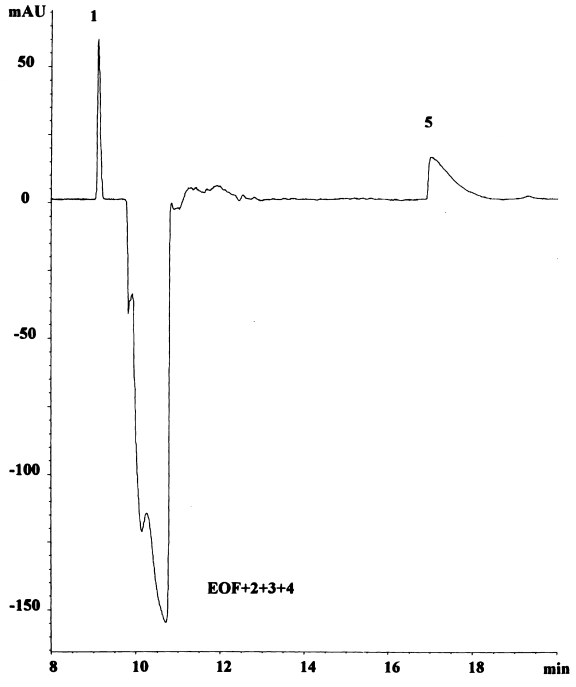

Fig. 1. Electropherogram from the preliminary screening indicating the deformation of the system peak. BGE: phosphate buffer, pH 3.00 ($I=0.06$) with 15 m*M* TDC, injected plug length 24 mm, applied voltage 15 kV, injection solution with 15 m*M* TDC and with 10 times lower ionic strength than the BGE. 1=TGG, 2=DTLE, 3=DMME, 4=LE, 5=LEA.

The results from the preliminary screening of six experimental variables in 17 experiments are shown in Table 4. The explained variance was low, especially for the peak width of LEA ($R^2=0.50$). The average responses of all experiments were different from the responses at the center points indicating a curvature in the model and that quadratic terms were needed to obtain an adequate model. The concentration of TDC had significant negative effect on the spatial peak width of TGG ($w_{s(TGG)}$), increasing TDC concentration gave higher efficiency, but did not influence the spatial peak width of LEA ($w_{s(LEA)}$).

The ionic strength of the buffer had a significant positive effect on $w_{s(LEA)}$ while $w_{s(TGG)}$ was not affected. Injected plug length had significant positive effect on both peak widths. Stacking had a negative significant effect on $w_{s(TGG)}$, i.e., resulting in higher efficiencies for peptides migrating before the system peak. The applied voltage and composition of the injected solution had no significant influence on the peak widths. However, preliminary studies showed that the addition of TDC micelles to the injected solution was an important parameter regarding the peak shape of peptides migrating close to the system peak. This is shown in Fig. 2, where the peak shape

Table 4
Results from the preliminary screening

	Responses	
Response	w_{TGG}	w_{LEA}
R^2	0.70	0.50
$\overline{w_s}$(model)	0.540	1.03
$\overline{w_s}$(centre point)	0.254	2.11
Regression coefficients		
Applied voltage	0.101	0.100
TDC	**−0.224**	0.085
Ionic strength	0.071	**0.293**
Plug length	**0.665**	**0.584**
Comp[a]	−0.087	0.117
Stacking	**−0.399**	−0.107

w_s = Spatial peak width at the half of the peak height (see Eq. (1)). Bold donates significant factors.
[a] See Table 1.

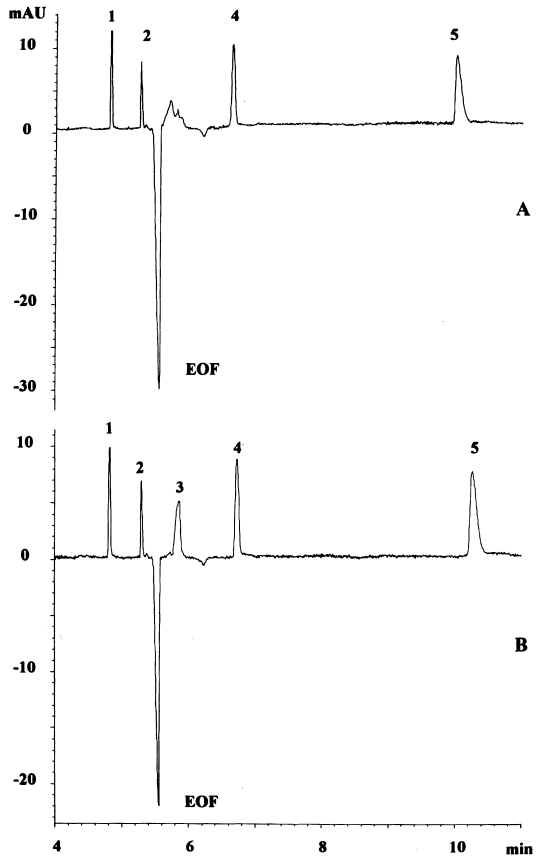

Fig. 2. The influence of sample composition on peak shape. BGE: phosphate buffer, pH 3.00 ($I=0.035$) with 10 m*M* TDC, applied voltage 10 kV. (A) Injected solution: phosphate buffer, pH 3.00 ($I=0.035$). (B) Injected solution: phosphate buffer, pH 3.00 ($I=0.035$), with 10 m*M* TDC. Peptide numbers as in Fig. 1.

of the peptide DMME, migrating close after the system peak, was distorted when the injected solution was without micelles (Fig. 2A). However, when TDC was added to the injected solution the peptide was detected (Fig. 2B), albeit at a low efficiency. The four significant variables; injected plug length, stacking, TDC concentration and ionic strength of the buffer were studied further via a central composite face design (CCF), see Table 2. The plug length was decreased so that the highest level was set equal to the center point from the preliminary screening. The applied voltage was kept at 15 kV and the injected solution was at the same concentration of TDC as in the BGE. The results from the experiments are shown in Table 5; this data matrix was

Table 5
Results from PLS regression in the CCF design

Response	R^2	RMSECV	$\overline{w_s}$(centre point) [a]	S.D.[a]
$w_{s(TGG)}$	0.53	0.14	0.26	0.0077
$w_{s(LE)}$	0.17	0.30	0.67	0.0091
$w_{s(LEA)}$	0.14	0.32	1.40	0.12

[a] $n=4$.

expanded with square and quadratic terms before the PLS regression analysis. The peptide, TGG, migrating before the system peak in all experiments gave the highest explained variance ($R^2=0.53$), while the explained variance was very low for the other peptides. The peptide LE migrated in some experiments before and in others after the system peak. LEA migrated after the system peak in all experiments except three, where it co-migrated with the system peak. The prediction ability of the model was very poor with high root mean squares error (RMSECV), which was estimated by leave-one-out cross validation [10]. The peak width at the center point was five-times higher for LEA than for TGG. The experimental error estimated by four repeated runs at the center point had a relative standard error of 2.7% for $w_{s(TGG)}$, 1.4% for $w_{s(LE)}$ and 8.6% for $w_{s(LEA)}$, see Table 5. The score vectors for the 1st and 2nd PLS component from the PLS analysis of the $w_{s(LEA)}$ are plotted against each other in Fig. 3. The experiments form two subgroups, one at the center point and at the face of the cube and another corresponding to the fractional factorial design. This

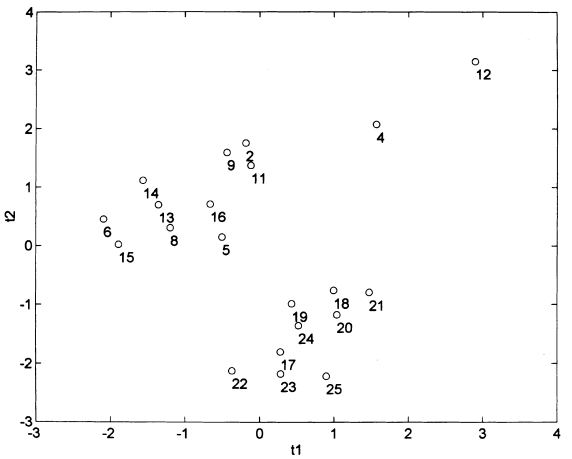

Fig. 3. Score plot for the peak width of LEA from the CCF design.

indicates that the experimental domain was too large and complicated to be explained by the postulated model. The range of the experimental factors were therefore narrowed and the experiments were performed at three different levels of TDC concentration instead, as described below.

3.2. PLS regression modelling at different TDC concentrations

Band broadening of the peptides was studied at three different TDC concentrations; below, just over and much higher, respectively, than the critical micelle concentration (CMC) value (4 m*M*). Five experimental factors, injected plug length, stacking of injected solution, applied voltage, temperature and ionic strength of the buffer, were studied via fractional factorial design at all three TDC concentrations. The experimental design together with the results from the PLS regression analysis are shown in Table 6. The variance explained was over 90% at all TDC concentrations. The most important variables for the peak widths at all three TDC concentrations were the injected plug length, the applied voltage and stacking of the injected solution. The peak width of LEA [$w_{t(LEA)}$] was, however, unaffected by stacking at any TDC concentration. The peptide, TGG, had no association to the micelles at 4.5 m*M* TDC, migrating before the system peak at all experiments. However, at 15 m*M* TDC the peptide had a low association to the micelles and stacking, did in this case, not have significant effect on the peak width [$w_{t(TGG)}$]. It has been shown in another study that the distribution of the enkephalins increased linearly with increasing concentration of TDC [8]. The peptide LE had some degree of association with the micelles at 4.5 m*M* TDC and stacking had then no effect on the peak width [$w_{t(LE)}$]. At 15 m*M* TDC neither the injected plug length nor stacking had an influence on $w_{t(LE)}$. Temperature and ionic strength of the buffer had no significant effect on peak widths at any TDC concentration and were thereafter set to 25°C and 0.035, respectively. This indicated that thermal gradients did not contribute to band broadening in this study.

The three significant variables, injected plug

Table 6
Experimental plan and results from the fractional factorial design at different TDC concentrations

	Variable parameters					Responses						
	Plug length	Stacking	Voltage	Temperature	Ionic strength	TDC (m*M*)						
Low:	1.9	0.1	10	20	0.01							
High:	6.5	1	25	40	0.06	0.9			4.5		15	
Exp						w_{TGG}	w_{LE}	w_{LEA}	w_{TGG}	w_{LE}	w_{TGG}	w_{LE}
1	1.9	0.1	10	40	0.06	0.061	0.071	0.050	0.037	0.052	0.064	0.360
2	6.5	0.1	10	20	0.06	0.078	0.097	0.12	0.051	0.075	0.101	0.517
3	1.9	1	10	20	0.01	0.073	0.085	0.080	0.042	0.071	0.096	0.397
4	6.5	1	10	40	0.01	0.150	0.187	0.150	0.096	0.103	0.135	0.337
5	1.9	0.1	25	40	0.01	0.021	0.027	0.030	0.017	0.023	0.031	0.116
6	6.5	0.1	25	20	0.01	0.033	0.045	0.050	0.023	0.034	0.052	0.250
7	1.9	1	25	20	0.06	0.026	0.031	0.030	0.017	0.027	0.038	0.130
8	6.5	1	25	40	0.06	0.058	0.067	0.050	0.035	0.032	0.042	0.124
9	4.2	0.55	17.5	30	0.035	0.058	0.071	0.065	0.039	0.071	0.050	0.222
Regression coefficients												
Plug length						**0.406**	**0.433**	**0.502**	**0.436**	**0.294**	**0.327**	0.180
Stacking						**0.336**	**0.310**	0.167	**0.294**	0.203	0.204	−0.205
Voltage						**−0.663**	**−0.646**	**−0.670**	**−0.638**	**−0.769**	**−0.756**	**−0.799**
Temperature						0.233	0.221	−0.0015	0.244	0.011	−0.050	−0.286
Ionic strength						−0.155	−0.182	−0.167	−0.176	−0.186	−0.223	0.023

Bold donates significant parameters, w = peak width at half peak height.

length, stacking and applied voltage were further studied by central composite design. Only eight more experiments were needed, six at the axial points and two at the centre point. The data matrix was expanded with square and cross terms before PLS regression analysis, the results from the analysis are shown in Table 7. The explained variance was much higher than in the CCF design (see Table 5) and the prediction ability of the model had improved. Electropherograms from the three different TDC concentrations at the center point are shown in Fig. 4. At 0.9 m*M* TDC no micelles were formed in the system and the TDC monomers were adsorbed to the positive charge surface of the aminopropyl capillary, giving an EOF towards the cathode. All peptides migrated before the EOF with LEA migrating first (see Fig. 4A). The migration pattern changed at 4.5 m*M* TDC, LEA migrated last, but under most experimental conditions it co-migrated with the system peak, only in a few cases after the EOF. Therefore, the peak width of LEA was not used in the PLS regression analysis due to many missing values. The other peptides were migrating before the system peak in all experiments at this TDC concentration, see Fig. 4B. When the TDC concentration was increased to 15 m*M* both LE and LEA where migrating after the system peak, while TGG migrated before the system peak at all experiments (see Fig. 4C). The peptide, LEA, was under most conditions, especially when the sample solution had ten-times lower ionic strength than the BGE, very difficult to detect. This is illustrated in Fig. 5, the signal-to-noise ratio was greatly improved by using an injection solution with higher ionic strength than in the BGE. This is partly due to decreasing migration time, which may be explained as follows. The micelles will quickly migrate into a sample plug with

Table 7
Results from PLS regression in the CCC design

	TDC (m*M*)		
	0.9	4.5	15
Response	R^2	R^2	R^2
w_{TGG}	0.85 (2 PC)	0.63 (1 PC)	0.93 (2 PC)
w_{LE}	0.75 (2 PC)	0.66 (1 PC)	0.92 (2 PC)
w_{LEA}	0.85 (2 PC)	–	–

–: Not used in the modelling due to many missing values.

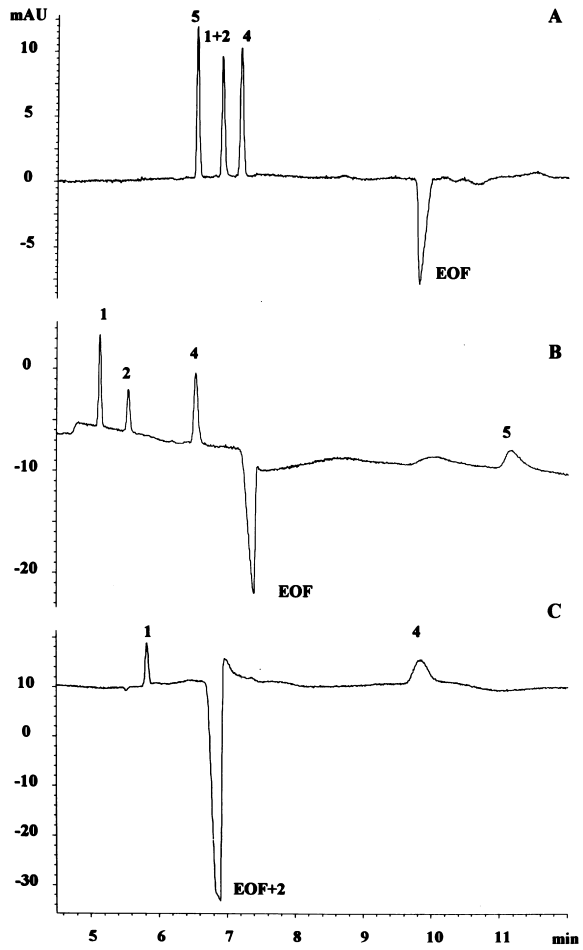

Fig. 4. Electropherogram from the center points. BGE: phosphate buffer, pH 3.00 (I=0.035), with 0.9, 4.5 or 15 m*M* TDC, applied voltage 17.5 kV, temperature 25°C, injected plug length 4.2 mm, stacking five-times lower ionic strength than in the BGE. (A) 0.9 m*M* TDC, (B) 4.5 m*M* TDC, (C) 15 m*M* TDC. Peptide numbers as in Fig. 1.

low ionic strength, since the field strength is lower (stacking effect). This involves a delay of peptides strongly distributed to the micelles, like LEA. For such peptides conventional conditions for stacking may have the opposite effect regarding peak width.

The results from the center points at all three TDC concentrations are compared in Table 8. The peak width of LE [$w_{t(LE)}$] at 0.9 m*M* TDC was broader than the peak width of TGG [$w_{t(TGG)}$], but LE had a lower mobility. When correcting the peak widths for migration time according to Eq. (1), they were

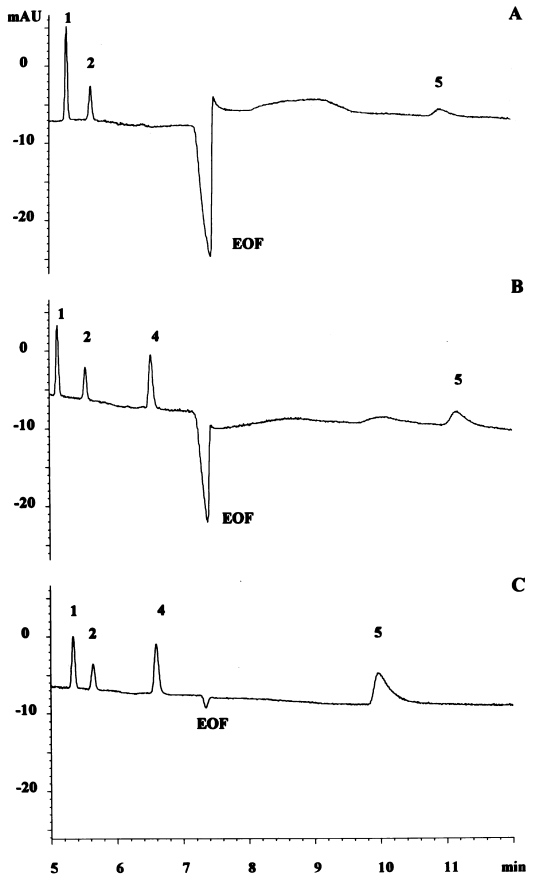

Fig. 5. Influence of stacking on the peak-to-noise level. BGE: phosphate buffer, pH 3.00 (I=0.035) with 4.5 mM TDC; ionic strength of the injected solution (A) 0.0035, (B) 0.019, (C) 0.038.

almost identical. The peak width for TGG was lower at 4.5 mM than at 0.9 mM TDC. However, the spatial peak widths were identical at both TDC concentrations. The peptide had no association to the micelles at 4.5 mM TDC. The spatial peak width for LE increased at 4.5 mM TDC and the peptide had a small degree of association to the micelles (k'= 0.42).

The peptide TGG had some association to the micelles at 15 mM TDC, which is indicated by low k' value, which might be the reason for higher peak width at this TDC concentration. The peak width for LE had increased even more, both for w_t and w_s. At the same time the degree of association to the micelles was much higher (k'=1.3). The peak width for the peptide with low association to the micelles was at least four-times smaller than for the peptide with high association to the micelles, after correction according to Eq. (1) the difference was 2.5-times. The regression coefficients from the PLS regression analysis of $w_{t(TGG)}$ (data not shown) was similar for all parameters at all TDC concentrations. The regression coefficients for $w_{t(LE)}$ were, however, similar to $w_{t(TGG)}$ at 0.9 and 4.5 mM, but at 15 mM TDC stacking is significant, negatively correlated, like the interaction term between plug width and stacking (see Fig. 6). Applied voltage was significant at all TDC concentrations, higher voltage gave narrower peak widths. For the spatial widths (w_s) the applied voltage had a smaller effect. The injected plug length contributed to the peak broadening at all TDC

Table 8
Results from the center points at the different TDC concentrations

	TDC (mM)								
	0.9			4.5			15		
Responses		TGG	LE		TGG	LE		TGG	LE
μ_{EOF} (10^{-4} cm^2/V s)	1.4			2.5			2.6		
μ_{MC} (10^{-4} cm^2/V s)				−0.83			−2.2		
μ_{eff} (10^{-4} cm^2/V s)		1.0	0.72		1.1	0.33		0.43	−0.94
w_s (cm)		0.30	0.33		0.30	0.42		0.32	0.79
w_t (min)		0.058	0.071		0.039	0.071		0.050	0.22
k'		–	–		0	0.34		0.23	1.3

μ_{EOF}=Electroosmotic mobility, μ_{MC}=effective mobility of the micelles, μ_{eff}=effective mobility of the peptides.
w_s=Spatial peak width according to Eq. (1).
$k' = \frac{\mu_{eff,OV} - \mu_{eff,AQ}}{\mu_{MC} - \mu_{eff,OV}}$, $\mu_{eff,OV}$=the overall effective mobility in a micellar BGE, $\mu_{eff,AQ}$=the effective mobility in the aqueous phase containing TDC monomers (0.9 mM).

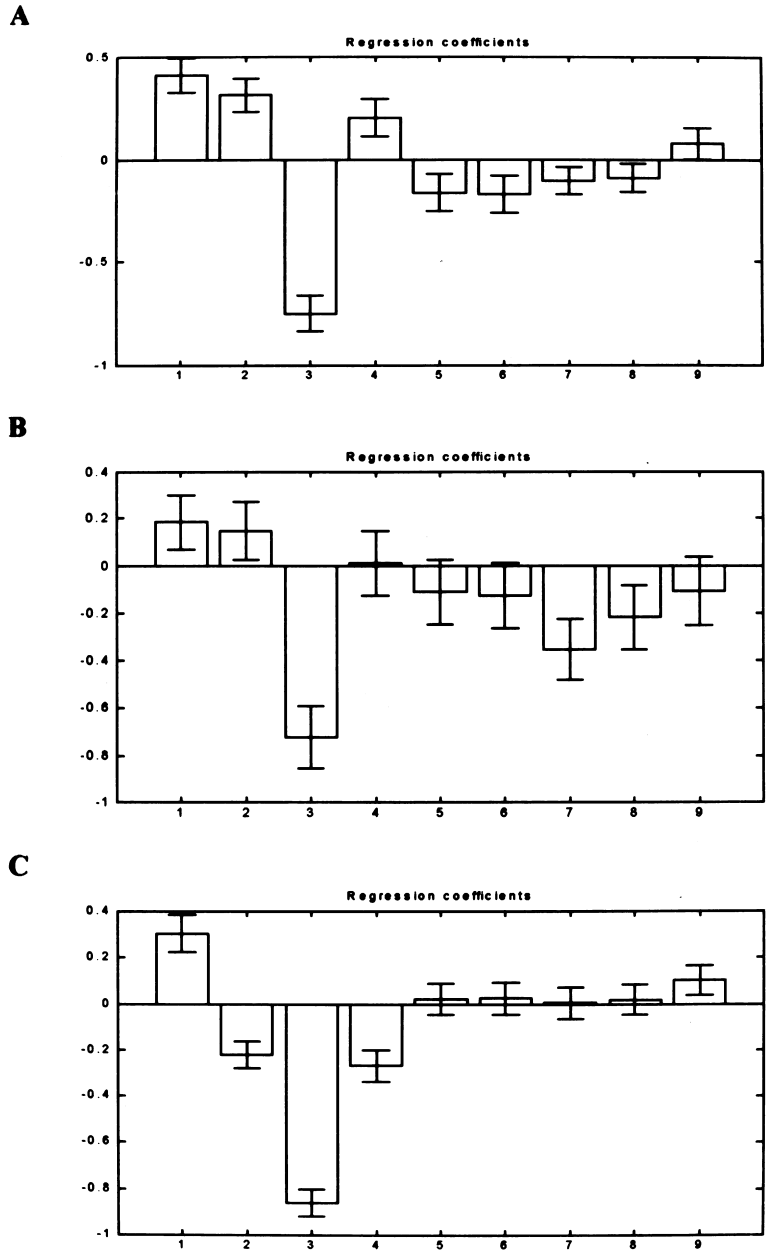

Fig. 6. The regression coefficient for the peak width of LE. (A) 0.9 m*M* TDC, (B) 4.5 m*M* TDC, (C) 15 m*M* TDC. 1=Plug length, 2=stacking, 3=applied voltage, 4=plug length×stacking, 5=plug length×voltage, 6=stacking×voltage, 7=(plug length)^2, 8=stacking^2, 9=voltage^2.

concentrations, injecting smaller plug gave more narrow peak width. Stacking was of no significance at short plug length but was important when large volumes were injected. This effect was seen for both peptides at the lower concentration, illustrated by the response surface for $w_{t(LE)}$ in Fig. 7A. The response surface for $w_{t(LE)}$ at 15 m*M* TDC, however, shows (see Fig. 7B) that when a large volume was injected

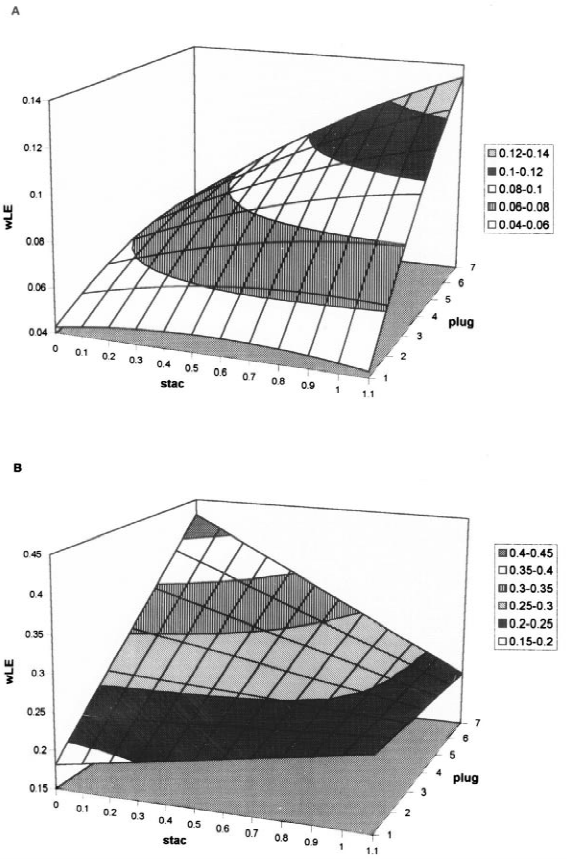

Fig. 7. Response surface plot for the peak width of LE, as a function of stacking and plug length at 17.5 kV. (A) 0.9 m*M* TDC, (B) 15 m*M* TDC.

the peak width was lower when the ionic strength in the injected solution was higher than in the background electrolyte. This can be accounted for by a greater distribution to micelles migrating into the sample solution by a stacking effect on the micelles as discussed before. The largest difference in peak width was seen when the association to the micelles increased by raising the TDC concentration. Therefore the main contribution to the band broadening is assumed to be a slow mass transfer to the micelles. Modelling of $w_{t(LE)}$ over the whole TDC concentration range (0.9–15 m*M*) gave further evidence of this. From the predicted versus measured peak width plot (see Fig. 8) it is clear that more than one phenomenon operated in the system. At low TDC concentrations the migration of the peptides by their own electrophoretic mobilities in the aqueous phase

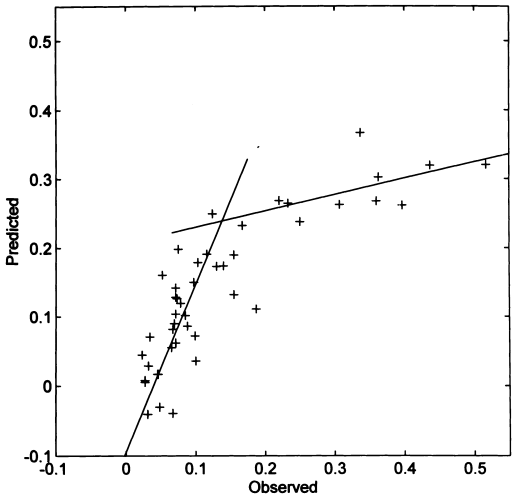

Fig. 8. Predicted versus measured peak width for LE over the whole TDC concentration range (0.9–15 m*M*).

is dominant, giving low peak widths. As the TDC concentration increased so did the association to the micelles and the solubilization to the micelles was now the dominant factor. This resulted in much broader peaks due to slow sorption–desorption kinetics to the TDC micelles. The peptides are positively charged at the pH 3, therefore both hydrophobic and electrostatic interactions are possible with the TDC micelles. Wallingford and Ewing [4] reported increased band broadening due to strong electrostatic interactions. However, our studies with sodium dodecyl micelles did not show increased band broadening for peptides with strong electrostatic interactions to the micelles [11]. The special helical structure of the TDC micelles [12] may therefore play a role in the distribution kinetics.

4. Conclusions

The plot of predicted versus measured peak width for the peptide, LE, and the dependence on the TDC concentration, revealed two different behaviors in the system. At low TDC concentration the peak widths were much smaller than at higher concentrations. Generally, peptides with a low association to the micelles had four-times lower peak widths than peptides with a high degree of association to the

micelles. The difference in peak width was assumed to be due to slow mass transfer to the TDC micelles. Peptides not distributed to the micelles had similar peak widths. This behaviour of the system is probably the reason for the low explained variance and the poor prediction ability in the first CCF design, which spanned over a large range of TDC concentrations. Response surface modelling at each of three different concentrations of TDC (0.9, 4.5 and 15 m*M*, respectively) gave much better models. The results indicated that high voltage and low plug length gave the lowest peak width at all TDC concentrations. The interaction between stacking and injected plug length was significant. Stacking was of no significance at short plug length, but it was important to use lower ionic strength in the injected solution when large volumes were injected in order to reduce band broadening. This effect changed when the peptides were strongly associated with the micelles, then the peak width was reduced by using a higher ionic strength in the sample solution than in the BGE, when large plug lengths where injected. The reason is probably a reversed stacking effect due to a net migration towards the anode with the micelles in the sample solution. The ionic strength of the buffer and temperature had no significant effect on the peak widths in this study.

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