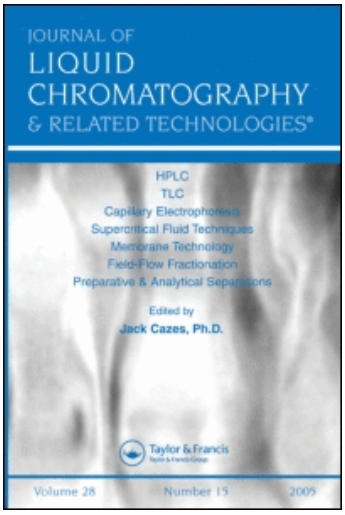

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Considerations on the Enantiomeric Separation by MEKC of N-Bz-Amino Acids with N-Dodecoxycarbonylvaline as Chiral Selector

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CONSIDERATIONS ON THE ENANTIOMERIC SEPARATION BY MEKC OF N-Bz-AMINO ACIDS WITH N-DODECOXYCARBONYLVALINE AS CHIRAL SELECTOR

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ABSTRACT

N-dodecoxycarbonyl-(S)-valine was evaluated for the separation of some benzoylated amino acids (alanine, aminobutyric acid and leucine). Successful enantiomeric separation required blocking of the carboxylic function by the formation of the methyl esters in order to avoid repulsion of the charged carboxylate group by the hydrophobic interior of the chiral micelles. A model of chiral recognition is presented. The highest enantioselectivity was obtained for the 3,5-dinitrobenzoyl derivatives ($\alpha > 1.07$). Based on the data, the generally accepted MEKC-resolution equation was experimentally verified and confirmed.

INTRODUCTION

Capillary electrophoresis (CE) is nowadays widely used for the separation of optically active substances. Direct enantioseparation is usually performed by adding chiral selectors to the separation buffer. Zare and coworkers (1,2) were the first to report on electrokinetic enantioseparations applying a ligand-exchange mechanism with Cu^{2+}-L-histidine as chiral selector. Since that initial work, all chiral recognition principles developed for HPLC, have been applied in CE : formation of inclusion complexes utilizing cyclodextrins or crown ethers, chiral micellar

solubilization and chiral polymer-based recognition i.e. with bovine serum albumine (3). Several reviews on this topic have been published (4-6). In MEKC, two approaches to carry out enantiomeric separations have been developed : the use of chiral surfactants or the addition of chiral additives to achiral surfactants. The chiral surfactants can be of natural origin like bile salts, digitonin etc., or synthetic optically active amino acid derivatives. The first reports in MEKC of separations of racemic compounds date from 1989. Yamaguchi et al. (7,8) used a chiral surfactant L-amino acid derivative e.g. sodium N-dodecanoyl-L-valinate to separate N-acylated amino acid isopropyl esters, while Terabe et al. (9,10) utilized bile salts to separate racemic dansylated amino acids. Scientists in Waters, synthezised the chiral surfactants S- and R-N-dodecoxycarbonylvaline, which were successfully applied for the separation of enantiomers of basic drugs (11). The presented work describes the evaluation of the S chiral surfactant for the enantioselective separation of several derivatives of alanine, amino butyric acid and leucine. An interaction model is proposed. Based on the data obtained with the amino acid derivatives the MEKC-resolution equation, developed by Terabe et al. (12), was verified.

MATERIALS AND METHODS

The analyses were performed on a Quanta 4000 (Waters, Milford, MA, USA). The fused silica capillary (Polymicro Technology, Phoenix, AZ, USA) was 75 μm I.D., 375 μm O.D., 59 cm L. with the detection window at 52 cm. The buffer solutions were prepared with deionised water (Milli-Q, Millipore, Bedford, MA, USA) and consisted of 50 mM N-dodecoxycarbonyl-(S)-valine and 25 mM phosphate/25 mM borate, adjusted to pH 7.8. Experiments were also performed with 100, 150 and 200 mM chiral surfactant. The test samples were composed of the N-benzoyl- (Bz), N-p-nitrobenzoyl- (pNBz), N-m-nitrobenzoyl- (mNBz) or N-3,5-dinitrobenzoyl (DNB) derivatives of alanine (ALA), aminobutyric acid (ABA) and, leucine (LEU) and their corresponding methyl esters. Samples were injected hydrodynamically with an elevation of 10 cm during 5 s. Analyses were performed at room temperature with an applied voltage of 15 kV. Detection was at 214 nm. Formamide and dodecanophenone were used as t_0 and t_{MC} markers, respectivily. All reported results are average values of three measurements. Between each run, the column was rinsed with the separation buffer for 3 min. A PC workstation with maxima

software (version 3.31, Millipore Corp., Bedford, MA, USA) was used for instrument control and data handling.

RESULTS AND DISCUSSION

The enantiomers of the amino acid N-derivatives with free carboxylate group could not be resolved with N-dodecoxycarbonyl-(S)-valine as chiral selector in the concentration range 50 to 200 mM. This is in accordance with ref. 7, in which N-dodecanoyl-L-valinate was used as chiral surfactant. Enantioselective complexation would require that the charged carboxylate group is oriented towards the hydrophobic interior of the micelle (Figure 1A) which is apparently an unfavorable process.

At a relatively low surfactant concentration of 50 mM, the elution order for similarly substituted species was LEU < ABA < ALA, which is corresponding with a pure electrophoretic mouvement of the anions. At higher surfactant concentrations, hydrophobic interaction with the micelles occured, as reflected by the gradually increased retention of leucine, but there was no indication of chiral recognition.

Unlike common polar modifiers, like methanol, acetonitrile and isopropanol, which are aqueous phase modifiers, higher alcohols like butanol are incorporated in the micellar phase and modify the micellar properties (13,14). Because this principle was successfully applied in the MEKC analysis of anionic bitter compounds with SDS (15,16), it was speculated that the addition of butanol to the

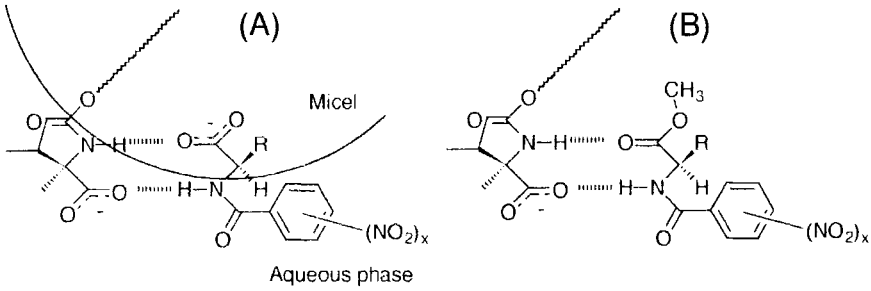

Figure 1. Mechanism of chiral recognition
A. Free carboxylate function; B. Methyl ester.

buffer could facilitate interactions between anionic solutes and micelles, thus creating enantioselectivity. Although some influence on the solute/micelle interaction was noted, no enantioselectivity was observed.

Two other modifications that could increase interactions between similarly charged solutes and micelles are the addition of tetraalkyl ammonium salts (17) or bivalent metal ions (18); and although there is no evidence that they are broadly applicable, they were tried out.

TBAs (tetrabutylammonium salts) have been proposed to improve resolution, but in at least one study dealing with other types of compounds, it was found that TBA had a negative effect on the resolution (19). In this study, the application of TBA modifiers led to buffer systems which gave highly irreproducible and erratic results, serious baseline drift problems and background absorption problems in the case of bromide salts. In those cases where the chlorides were not available, the hydroxydes were applied, followed by pH control with hydrochloric acid. Moreover, addition of 50 mM of these modifiers to 50 mM of surfactant resulted in a strong increase of the current (130 μA and above, at 15 kV). Given the poor quality of the results, and the complete absence of any enantioselectivity, no further attempts were made to systematically treat the data.

Bivalent metal ions can play a role in the diastereomeric complex formation and thus enantiomer recognition (1,2). Cu^{2+} has been used in combination with a chiral surfactant for the separation of dansylated amino acids (20). The results of our own attempts with N-dodecoxycarbonyl-(S)-valine/M^{2+} (M = Cu, Zn) were all negative.. It must be remarked that this surfactant is not likely to form complexes with the metal cations as illustrated in Figure 2.

The N-benzoylated amino acid methyl esters, on the other hand, could be resolved with N-dodecoxycarbonyl-(S)-valine as chiral selector. Figure 3 shows the separation for the N-3,5-dinitrobenzoyl methyl ester derivatives. The data for the other N-derivatives are summarized in Table 1. At 50 mM surfactant, the elution order of similarly substituted species followed the expectation from the hydrophobicity of their side chains (ALA < ABA < LEU).

By analogy to models that have been presented for the chiral recognition in comparable HPLC systems (21), the retention mechanism in Figure 1B is proposed.

With the S-type surfactant, the D-enantiomer elutes before the L-enantiomer in all cases. As L-amino acids have the S-configuration, this means that, according

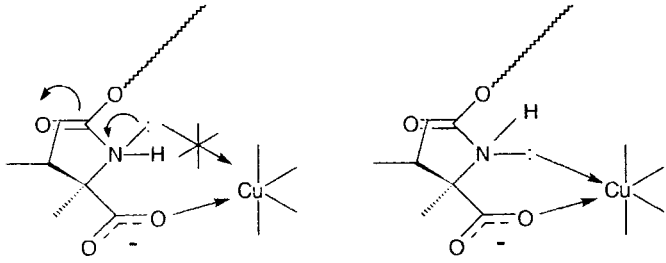

Figure 2. Actual (left) and preferred (right) structure for surfactant/M^{2+} interaction.

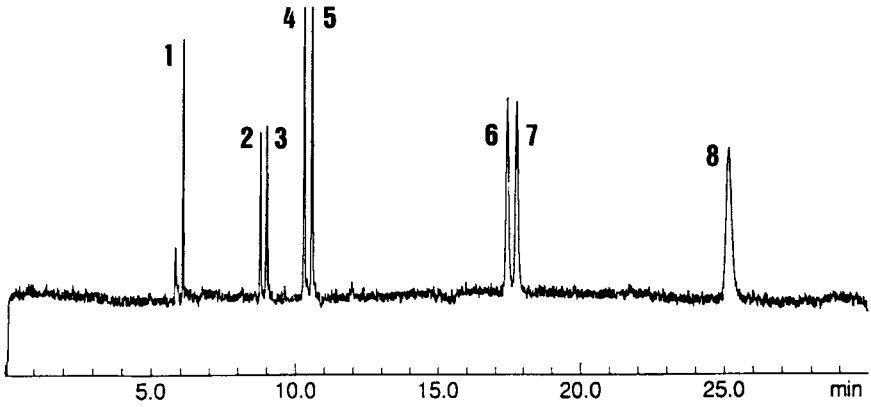

Figure 3. Electropherogram of the N-3,5-dinitrobenzoyl/methyl ester derivatives.
Peaks: 1 = formamide (t_0), 2 = D-ALA, 3 = L-ALA, 4 = D-ABA,
5 = L-ABA, 6 = D-LEU, 7 = L-LEU, 8 = dodecanophenone(t_{MC}).
Buffer : 50 mM N-dodecoxycarbonyl-(S)-valine, 25 mM borax/50 mM phoshate, pH 7.8. Column : 75μm I.D., 52/59 cm L.
Applied voltage : 15 kV. Current : 81 μA.
Temperature : ambient. Detection : 214 nm.

Table 1. CE data for the benzoylated amino acid methyl esters.

Bz	t_R(min)	N	R_s	k'	α
t_0	6.06	166839		0	
D-ALA	9.86	193985		1.06	
L-ALA	9.86	193985	0	1.06	1.000
D-ABA	10.2	199440		1.19	
L-ABA	10.2	199440	0	1.19	1.000
D-LEU	13.6	187275		2.86	
L-LEU	13.85	294737	2.21	3.02	1.056
t_{MC}	24.09	101927		inf	

pNBz	t_R(min)	N	R_s	k'	α
t_0	6.16	165490		0	
D-ALA	8.44	231650		0.55	
L-ALA	8.53	235760	1.2	0.57	1.036
D-ABA	9.73	236093		0.92	
L-ABA	9.85	223762	1.52	0.96	1.043
D-LEU	16.24	183701		4.34	
L-LEU	16.47	166294	1.41	4.54	1.046
t_{MC}	26.13	64482		inf	

mNBz	t_R(min)	N	R_s	k'	α
t_0	5.94	163230		0	
D-ALA	8.00	252647		0.53	
L-ALA	8.12	251875	1.82	0.57	1.075
D-ABA	9.29	268078		0.94	
L-ABA	9.45	273299	2.27	1	1.064
D-LEU	15.48	246229		4.83	
L-LEU	15.77	208056	2.22	5.17	1.070
tMC	23.21	105212		inf	

DNBz	t_R(min)	N	R_s	k'	α
t_0	6.05	151000		0	
D-ALA	8.74	248728		0.68	
L-ALA	8.95	249056	3.05	0.75	1.103
D-ABA	10.25	225613		1.17	
L-ABA	10.52	219476	3.12	1.27	1.085
D-LEU	17.37	158440		6.03	
L-LEU	17.71	150212	1.9	6.49	1.076
t_{MC}	25.19	72261		inf	

to the proposed model, the homochiral associations are more stable. This is in accordance with the results of NMR-studies of diastereomeric dimers of N-acetylvaline t-butyl esters (21). Similar elution orders have also been found with the related surfactant (N-dodecanoyl-L-valinate) and comparable amino acid derivatives (2,3).

The data obtained with the amino acid esters (Table 1) have been used to verify experimentally the MEKC-resolution equation (12). In MEKC, the contribution of the capacity factor and the elution range t_0/t_{MC} to resolution can be expressed as:

$$f_k = \frac{\tilde{k}'}{1 + \tilde{k}'} \cdot \frac{1 - \frac{t_0}{t_{MC}}}{1 + \frac{t_0}{t_{MC}} \cdot \tilde{k}'} \qquad \text{(Eq.1)}$$

For a pair of peaks, the corresponding value is given by:

$$f_k = \frac{\tilde{k}_2'}{1 + \tilde{k}_2'} \cdot \frac{1 - \frac{t_0}{t_{MC}}}{1 + \frac{t_0}{t_{MC}} \cdot \tilde{k}_1'} \qquad \text{(Eq.2)}$$

The same term can also be evaluated indirectly:

$$f_k = \frac{R_s}{\frac{\sqrt{N_{av}}}{4} \cdot \frac{\alpha - 1}{\alpha}} \qquad \text{(Eq.3)}$$

The good agreement between the values obtained with Eq.3 and the curve obtained with Eq.1 is surprising (Figure 4). To the best of our knowledge, this is the first experimental and quantitative confirmation of the general MEKC-resolution equation. Although this result is purely academic, the situation as depicted in Figure 4 and representing real-life values, permits a short discussion on the limitations of capacity factor optimization in MEKC.

Ignoring very small and very large values, In the region around the optimal capacity factor, the gain in resolution contribution that can be expected will rarely exceed a factor 3; on the higher side it will even be less. This means that optimizing the separation in a situation where two peaks are just visibly starting to split (R_s = 0.5) to a full-baseline separation (R_s = 1.5) will be difficult to attain by just varying the surfactant concentration, unless the capacity factors are extremely small or

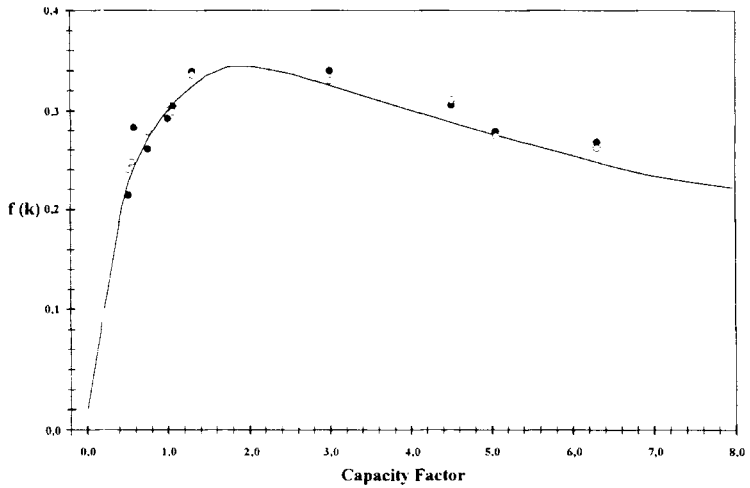

Figure 4. Experimental confirmation of the MEKC resolution equation.
Curve : theoretical values according to Eq. 1 (for $t_0 / t_{MC} = 0.246$, this is the average value for all pooled experiments, which ranged from 0.235 to 0.256).
Open Symbols : f_k calculated according to Eq. 2 (these values were obtained by combining capacity factors and their corresponding t_0 / t_{MC} and reflect the variability that can be expected around the curve which was calculated with an average t_0 / t_{MC}).
Solid Symbols : observed values for f_k, according to Eq. 3.

large. Even in the latter case, it is doubtful whether it will be physically possible to adjust the phase ratio so that the capacity factor changes by more than one order of magnitude. On the lower side there is the limit posed by the CMC value, at the higher side the increase in concentration is limited by the increased current output.

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