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Erim, F.B.; Xu, X.; Kraak, J.C.

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Application of micellar electrokinetic chromatography and indirect UV detection for the analysis of fatty acids

F.B. Erim*, X. Xu, J.C. Kraak

Laboratory for Analytical Chemistry, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, Netherlands

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Abstract

A micellar electrokinetic chromatographic system using indirect UV detection was developed for the analysis of saturated fatty acids. Sodium dodecyl benzenesulfonate (SDBS) was used as micellar surfactant as well as chromophore for the indirect detection. The effects of the addition of acetonitrile and Brij on the migration and solubility of the fatty acids was investigated. With a mixture of 10 mM SDBS + 50% acetonitrile + 30 mM Brij, a complete separation of the C_8 – C_{20} fatty acids was obtained. The applicability of the developed system for the analysis of fatty acids in butter is demonstrated.

1. Introduction

Fatty acids are defined as all saturated and unsaturated aliphatic carboxylic acids with a carbon chain length in the range C_6 – C_{24} . In nature many fatty acids are present, but only the C_8 – C_{22} carboxylic acids are of commercial importance [1]. These relevant compounds can be either isolated from animals and vegetables or synthesized. The determination of the individual fatty acids provides important information about the composition of natural fats and oils produced from animals and vegetables [2]. This necessitates the use of advanced and fast analysis techniques. Gas and liquid chromatography have found to be very suited for the analysis of fatty acids [3–5]. Both techniques are widely applied but there is still a need to speed up the analysis because of the innumerable applications in life.

An attractive alternative separation technique might be capillary electrophoresis [6] and in particular micellar electrokinetic chromatography (MEKC) as introduced by Terabe et al. [7]. The last technique combines electrophoresis and chromatography and can be used to separate neutral solutes as well as ionic compounds [8,9]. An additional advantage of micellar systems is the fact that compounds which are insoluble or slightly soluble in aqueous phases can be solubilized. The solubility of fatty acids in aqueous solutions decreases rapidly with increasing alkyl chain and therefore MEKC seems to be an attractive separation technique for these compounds. However, the absence of a chromophoric or fluorophoric group in the fatty acids excludes direct UV or fluorometric detection and therefore indirect detection has to be used [10].

In this paper we report the results of an investigation to develop a MEKC system for the

* Corresponding author.

separation of 13 fatty acids using indirect UV detection. The applicability of the developed MEKC system will be demonstrated with analysis of real samples.

2. Experimental

2.1. Apparatus

A commercial capillary zone electrophoresis (CZE) injection system (Prince; Lauer Labs., Emmen, Netherlands) in combination with on-column UV detection (Linear 200; Linear Inst., Fremont, CA, USA) was used. The wavelength was set at 198 nm. Unless otherwise stated, the analysis voltage was 20 kV. Sample injection was carried out with pressure (40 mbar, 0.06 min) at the anodic side. A 60 cm $\times$ 50 μ m I.D. fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) was used. The distance to the detection window was 45 cm. The measurements were performed at room temperature ($23 \pm 2^\circ\text{C}$).

2.2. Chemicals

Sodium dodecyl benzenesulfonate (SDBS) was purchased from Fluka (Bornem, Belgium). Brij 35 was obtained from Merck (Darmstadt, Germany) and acetonitrile from Janssen Chimica (Beerse, Belgium). The fatty acids with alkyl chains of C_8 – C_{20} were obtained from different sources: C_8 , C_9 , C_{10} , C_{12} , C_{14} , C_{16} and C_{18} from Merck; C_{11} , C_{17} and C_{20} from Sigma (Bornem, Belgium) and C_{13} , C_{15} and C_{19} from Fluka.

2.3. Procedures

During the night the capillaries were stored in 0.1 M NaOH.

Prior to use, the capillary was flushed for 10 min with water and the actual buffer solution successively. Before each measurement, the capillary was flushed for 2 min with the actual buffer solution.

The actual buffer solutions were prepared by mixing stock solutions of Tris buffer (adjusted with HCl to pH 8.5), surfactants and acetonitrile

and adjusting the final volume with water. Doubly distilled water was used for all solutions. All buffer solutions were filtered prior to use through a 0.1- μ m Millipore (Bedford, MA, USA) filter (type Millex-VV).

Stock solutions of fatty acids were prepared in methanol or acetonitrile. Sample solutions were prepared by mixing measured quantities of stock solutions of fatty acids, buffer, surfactants and organic solvents and adjusting the final volume with water. In this way the composition of the sample can be kept similar to that of the buffer solution. The electroosmotic flow was determined from the residence time of the solvent peak or with methanol. In some cases sudan was used to determine the residence time of an analyte totally incorporated into the micelle.

3. Results

The electrophoretic mobility of the fatty acids decreases with increasing alkyl chains. The C_8 – C_{11} fatty acids can be separated by zone electrophoresis but not the C_{11} – C_{20} fatty acids because the difference in electrophoretic mobility becomes too small. Moreover, the solubility of the fatty acids decreases dramatically with increasing alkyl chain which causes serious detection problems. By adding micelles to the back ground electrolyte, fatty acids will be differentially solubilized in the micelle and this opens the possibility to separate the fatty acids according to their hydrophobicities e.g. length of the alkyl chain. In order to determine the optimum conditions for the separation of the fatty acids the effects of two surfactants, SDBS and Brij were studied including the effect of the addition of acetonitrile. The selection of SDBS was based on the result reported by Sandra et al. [9] who separated some smaller alkylcarboxylic acids using the same surfactant as chromophore for indirect UV detection.

3.1. Migration behaviour of fatty acids with SDBS

The effect of the SDBS concentration on the migration behaviour of fatty acids was studied

with C_{11} – C_{13} , using 5 mM Tris pH 8.5 as buffer. The critical micellar concentration (CMC) of SDBS has been reported to be around 2 mM [11]. In the range of 0.5–2 mM SDBS the solutes gave a negative peak all having about the same migration time. This indicates that no micelles are formed in this concentration range as expected on basis of the CMC. When increasing the SDBS concentration to 4 mM, the elution order changed into $C_{11} = C_{12} < C_{13}$. When further increasing the concentration to 10 mM SDBS the elution order became $C_{11} < C_{12} < C_{13}$ and the three solutes can be completely separated. This elution order is the reverse as expected in zone electrophoresis and demonstrates the micellar effect of SDBS. It can be noted that the baseline stability becomes significantly worse with increasing SDBS and resulted in disturbed and small peaks in particular for the C_{13} acid. Also it was not possible to dissolve the longer fatty acids even in 20 mM SDBS. In order to enlarge the solubility of the larger fatty acids the SDBS was mixed with sodium dodecyl sulfate (SDS) or the non-ionic surfactant Brij and with alcohols or acetonitrile. Mixing of SDBS with SDS caused a tremendous increase of the background noise signal. The addition of alcohols had very little effect on the solubility but the migration times increased due to a decrease of the electroosmotic flow. With the addition of Brij and acetonitrile the solubilities of the fatty acids increased significantly. Therefore the effect of the addition of Brij and acetonitrile on the migration behaviour of the fatty acids was studied more extensively. This will be discussed in the next sections.

3.2. Migration behaviour of fatty acids in SDBS–acetonitrile mixtures

The effect of the addition of acetonitrile to the SDBS system on the migration behaviour was studied with the C_{11} , C_{12} and C_{13} fatty acids as test solutes. Fig. 1 shows the effect of the addition of acetonitrile on the mobility of the fatty acids using 10 mM SDBS. As can be seen the electroosmotic flow decreases and the migration times of the fatty acids increases with increasing acetonitrile percentage. For up to

Fig. 1. Effect of the addition of acetonitrile on the electrophoretic mobility of C_{11} – C_{13} fatty acids (FA, solid lines) and electroosmotic flow (EO, dashed line) in a 10 mM SDBS micellar solution.

20% acetonitrile the elution order is the same but the baseline stability appeared to be worse. Surprisingly at 40% acetonitrile the elution order reverses and the baseline stability improved considerably as is illustrated in Fig. 2. The improvement of the baseline stability at larger organic solvent concentrations was found earlier by Gorse et al. [12]. In 40% acetonitrile the larger fatty acids dissolve considerably better and under these conditions C_8 – C_{17} fatty acids (0.1–0.25 mM of each) could be completely separated except the C_{16} and C_{17} acids which coelute. By increasing the acetonitrile content to 50%, a separation of all the C_8 – C_{19} fatty acids was obtained. However, the solubility of the largest fatty acids was still not sufficient to detect the solutes adequately. Moreover, sometimes bloc-

Fig. 2. Background signal obtained with (a) 10 mM SDBS and (b) 10 mM SDBS + 40% (v/v) acetonitrile.

kage of the capillary occurred arising from precipitates. In order to improve the solubility of the mixture, the acetonitrile content was increased up to 75%. However, this caused a dramatic increase of the background noise.

The effect of the SDBS concentration in 40% acetonitrile on the mobility of the fatty acids is shown in Fig. 3. As can be seen the mobility is hardly influenced by the SDBS concentration.

3.3. Migration behaviour of fatty acids with SDBS–Brij mixtures

The addition of the non-ionic surfactant Brij to an ionic surfactant in MEKC has found to bring about changes in the migration window and selectivity [13]. It is assumed that the changes are the result of the formation of mixed micelles. The effect of the addition of Brij to the SDBS system on the migration of C_{11} – C_{15} fatty acids was studied by varying the Brij concentration in the range 0.5–10 mM, keeping the SDBS concentration constant (10 mM). The CMC of Brij has been reported to be about 0.1 mM and thus at all used concentrations Brij micelles will be formed. The migration behaviour of the fatty acids as function of the Brij concentration is shown in Fig. 4. With 0.5 mM Brij C_{11} – C_{13} fatty acids migrate in the order $C_{11} < C_{12} < C_{13}$ but C_{14} and C_{15} could not be detected. With 1 mM Brij, C_{14} and C_{15} appeared in the electropherogram but as very small and

Fig. 3. Effect of the SDBS concentration on the electrophoretic mobility of some fatty acids (FA, solid lines) and electroosmotic flow (EO, dashed line) in the presence of 40% (v/v) acetonitrile.

Fig. 4. Effect of the addition of Brij on the electrophoretic mobility of fatty acids (FA, solid lines) and electroosmotic flow (EO, dashed line) in a 10 mM SDBS micellar solution.

distorted peaks. When further increasing the Brij concentration to 2–4 mM, the electroosmotic flow decreases gradually. The migration time of C_{11} stays almost constant but that of the larger fatty acids decreases and they migrate close together. Surprisingly with 8 mM Brij a complete reversal of the elution order of the fatty acids occurs: the C_{12} – C_{15} fatty acids elute close together in front of the smaller ones! With 10 mM Brij the C_8 – C_{13} fatty acids could be separated as shown in Fig. 5. Increasing the Brij concentration > 10 mM resulted in more resolution between the solutes but in particular the peaks of the smaller fatty acids become distorted. The solubility of the larger acids increase considerably but no improvement of the resolution between the C_{13} – C_{15} acids occurred. It can be noted that the baseline stability improved

Fig. 5. Electropherogram of fatty acids using 10 mM SDBS + 10 mM Brij as background electrolyte.

with increasing Brij concentration. Further in all electropherograms a system peak was observed which always migrated at the same position of the C_{15} acid. Injection of sudan resulted in a peak with the same migration time as the system peak.

3.4. Migration behaviour of fatty acids with SDBS–Brij–acetonitrile mixtures

With SDBS–acetonitrile all the fatty acids could be separated but the solubility of the C_{18} – C_{20} acids is too small to detect them well. With SDBS–Brij mixtures the solubility of all acids is good but only the C_8 – C_{13} could be separated while the larger fatty acids migrated together. On basis of these findings it was decided to combine the favourable selectivity of acetonitrile with the good solvation ability of Brij. For that purpose the migration of the fatty acids with various mixtures of SDBS–acetonitrile–Brij was investigated. Fig. 6 shows the effect of the Brij concentration on the migration of the fatty acids with 50% acetonitrile and 10 mM SDBS. From this figure it can be seen that by addition of 20 mM of Brij the speciation of all the fatty acids improves significantly and a complete separation of the C_8 – C_{20} fatty acids can be obtained. Increasing the Brij concentration to 30 and 50 mM improves the resolution somewhat but at the cost of the separation time due to a gradual decrease of the electroosmotic flow. In particular

Fig. 6. Effect of the addition of Brij on the electrophoretic mobility of C_8 – C_{20} fatty acids (FA, solid lines) and electroosmotic flow (EO, dashed line) using 10 mM SDBS + 50% (v/v) acetonitrile.

with 50 mM Brij the long migration times causes broader peaks of the C_8 and C_9 fatty acids. A concentration of 30 mM of Brij appears to be a good compromise between solubility, migration time, resolution and peak shapes. An electropherogram of all C_8 – C_{20} fatty acids under these conditions is shown in Fig. 7.

In order to determine the effect of the acetonitrile content and SDBS concentration with 30 mM Brij, some addition experiments were performed. Fig. 8 shows the effect of acetonitrile with 10 mM SDBS and 30 mM Brij. It shows that acetonitrile affects considerably the electrophoretic mobilities and a complete separation of all fatty acids can only be realized at a high percentage of acetonitrile.

Fig. 9 shows the effect of the SDBS concentration on the migration using 30 mM Brij and 50% acetonitrile. As can be seen, the effect on the electrophoretic mobilities is very small. However, from the point of view of detection sensitivity the use of 10 mM SDBS appeared to be favourable. Under these conditions the concentration detection limit of the individual fatty acids was determined to be about 10^{-5} M.

The applicability of the developed separation system is illustrated in Fig. 10 showing the electropherograms of an extract of a dairy-fresh butter (Fig. 10a) and of a butter rich in unsaturated fatty acids (Becel) (Fig. 10b). Prior to analyses the butter was first saponified under alkaline conditions in ethanol. Then the ethanol was removed by evaporation and the residue dissolved in the buffer electrolyte and injected.

Although no attempts were undertaken to optimize the sample pretreatment of the butter, the electropherograms clearly show the usefulness of the developed CE technique for the determination of the fatty acids content in real samples. A complication occurring with the simple pretreatment is the presence of the unsaturated fatty acids in the extract. These compounds show a quite similar migration behaviour and therefore interfere in the electropherogram (the peaks indicated with asterisks). Attempts are now undertaken to shift the migration of the unsaturated fatty acids selectively towards free spaces in the electropherogram. It can be noted

Fig. 7. Electropherogram of the C_8 – C_{20} fatty acids using 10 mM SDBS + 50% acetonitrile + 30 mM Brij as background electrolyte.

that for the unsaturated fatty acids it is not necessary to apply indirect detection since these compounds show native UV absorption.

4. Discussion

The electrophoretic mobility, μ_{FA} , of the C_1 – C_{10} acids are known from literature [14]. The μ values appear to decrease gradually from -56.6

for the C_1 acid to -22.1 for the C_{10} acid. The decrease of μ with increasing length of the alkyl chain in the acid have been also found with C_1 – C_{12} alkyl sulfonates [14]. It is therefore likely to suppose that the same decrease in μ continues with the C_8 – C_{20} fatty acids. This means that in the CZE mode with electroosmotic flow the apparent mobility of the larger fatty acids, μ_{FA}^* , will be larger than the smaller ones, resulting in an expected elution order of $C_{20} < C_{19} \dots < C_8$.

Fig. 8. Effect of the percentage acetonitrile on the electrophoretic mobility of fatty acids (FA, solid lines) and electroosmotic flow (EO, dashed line) using 10 mM SDBS + 30 mM Brij.

Fig. 9. Effect of the SDBS concentration on the electrophoretic mobility of fatty acids (FA, solid lines) and electroosmotic flow (EO, dashed line) using 50% (v/v) acetonitrile + 30 mM Brij as background electrolyte.

Fig. 10. Electropherograms of (a) an extract of a dairy-fresh butter and (b) a butter rich in unsaturated fatty acids (Becel).

In reality this order can only be verified with smaller fatty acids because the solubility of the larger fatty acids in the applied aqueous solutions in CZE is too small.

4.1. Effect of SDBS

When adding the negatively charged surfactant

SDBS to the buffer, micelles are formed and migrate with a net velocity which is smaller than that of the electroosmotic flow. The fatty acids will be distributed between the micelle and the surrounding electrolyte. Since the interior of the micelle is hydrophobic, the partition of the fatty acids towards the micellar phase will increase with increasing length of the alkyl chain. As a

result the larger fatty acids will spent more time in the micellar phase than the smaller ones and therefore will migrate slower. In other words an elution order of $C_8 < C_9 \dots C_{20}$ can be expected and this is indeed found in this study as can be seen in Fig. 2a.

4.2. Effect of organic solvent

The effect of addition of organic solvents, like methanol and acetonitrile, to micellar systems has been studied before [12]. These studies showed that the addition of organic solvent (up to 20%) changes the partition of solutes (e.g. the capacity factors) between the micelles and surrounding liquid. It was suggested that the changes can partly be attributed to a reduction of the size of the micelles and of the surface charge density at the micelle-surrounding liquid interface with increasing solvent content. The addition of an organic solvent usually reduces the electroosmotic flow and thereby extends the separation window. The change was found to be significantly larger with methanol than with acetonitrile. In our study the behaviour of acetonitrile on the migration agrees, up to 20% of acetonitrile, with the findings as mentioned in Ref. [12]. The elution order of $C_8 < C_9 \dots < C_{20}$ indicates that still micelles are present (see Fig. 1). However, at larger percentages of acetonitrile the elution order reverses completely into $C_{20} < C_{19} \dots < C_8$, the expected order under CZE conditions. This indicates that either the micelles are largely disintegrated or the fatty acids migrate after the micelles (e.g. migrate close to t_0). Whether still micelles are present at larger acetonitrile content could not yet be answered by us because the neutral solute sudan, usually used to measure migration time of the micelle (t_{mc}), did not give any detector response.

4.3. Effect of Brij

The addition of a neutral surfactant like Brij to the solution with the charged SDBS micelles results in the formation of mixed micelles. The charge density of the mixed micelles will be smaller than without Brij. As a consequence of

this, the electrophoretic mobility of the mixed micelle decreases which results in a decrease of the t_{mc} (e.g. the separation window becomes smaller) [13]. Ultimately at very large Brij concentrations, the electrophoretic mobility of the mixed micelle will approach that of the electroosmotic mobility (t_0). The migration behaviour of the fatty acids using Brij is in agreement with these expectations as can be seen in Fig. 4. At small Brij concentration the electrophoretic mobility of the mixed micelle is larger than that of the fatty acids and the elution order is $C_8 < C_9 \dots < C_{15}$. At large Brij concentration the electrophoretic mobility of the mixed micelle is smaller than that of the fatty acids and an elution order of $C_{15} < C_{14} \dots < C_8$ is found, the expected CZE order. This indicates that at larger Brij concentrations most probably the SDBS micelles are largely or completely disintegrated as found with larger acetonitrile contents. Although the occurrence of mixed micelles is likely, a definite answer can only given when the t_{mc} under the various conditions can be determined. In that respect it can be noted, that in all recorded electropherograms with SDBS–Brij systems, always a system peak appears just after the C_{15} peak. The migration time of sudan could be determined with larger Brij concentrations and its time coincides with that of the system peak. Therefore the system peak might be a marker for the mixed micelle migration time.

4.4. Effect of Brij + acetonitrile

The observed reversal of the migration order of the fatty acids when adding acetonitrile or Brij to SDBS cannot easily interpreted by the simple MEKC separation mechanism. It becomes even more complicated when both additives are used together. With both additives it seems that the SDBS micelle is largely disintegrated and that the SDBS molecules only act as a chromophore for the indirect detection. This finding is supported by the fact that with larger acetonitrile and Brij concentrations, the SDBS concentration has a minor effect on the migration behaviour of the fatty acids. From some preliminary experiments on the application of the developed sepa-

ration system to unsaturated fatty acids, which can also be detected directly with UV, it was found that the migration behaviour of the unsaturated fatty acids is not altered in the absence of SDBS.

5. Conclusions

MEKC combined with indirect UV detection using SDBS as chromophore can be used for the analysis of fatty acids. To create sufficient solubility and selectivity, the addition of 40–50% acetonitrile and 30 mM Brij to the background electrolyte is necessary. The system is in principle suited for the analysis of the unsaturated fatty acids using direct UV detection.

Whether the developed system for the separation of the fatty acids can be considered as a true MEKC system cannot yet be established.

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