Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1024 (2004) 245–254
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Analysis of drying oils used as binding media for objects of art by capillary electrophoresis with indirect UV and conductivity detection

Izabella Surowiec [a,1], Isabella Kaml [a,b], Ernst Kenndler [a,*]

[a] *Institute for Analytical Chemistry, University of Vienna, Währingerstrasse 38, A 1090 Vienna, Austria*
[b] *Department of Conservation and Restoration, Academy of Fine Arts, Vienna, Austria*

Received 26 August 2003; received in revised form 13 October 2003; accepted 16 October 2003

Abstract

Capillary electrophoresis (CE) was applied to analyse the long-chain fatty acid composition of vegetable oils, and their degradation products formed upon ageing when drying oils are used as binding media. The analytes were detected with contactless conductivity detection (CCD) and indirect UV absorption, both detectors positioned on-line at the separation capillary. The long-chain fatty acids were resolved in a background electrolyte (BGE) consisting of phosphate buffer (pH = 6.86, 15 mM) containing 4 mM sodium dodecylbenzensulfonate, 10 mM Brij® 35, 2% (v/v) 1-octanol and 45% (v/v) acetonitrile. As in this system dicarboxylic analytes, the products of oxidative degradation of unsaturated fatty acids, cannot be determined, a suitable background electrolyte was developed by the aid of computer simulation program PeakMaster. It makes use of a 10 mM salicylic acid, 20 mM histidine buffer, pH 5.85, which combines buffering ability with the optical properties obligatory for indirect UV detection. This buffer avoids system eigenpeaks, which are often impairing the separation efficiency of the system. Separation of the dicarboxylic analytes was further improved by a counter-directed electroosmotic flow (EOF), obtained by dynamically coating the capillary wall with 0.2 mM cetyltrimethylammonium bromide. Long-chain fatty acids and their decomposition products could be determined in recent and aged samples of drying oils, respectively, and in samples taken from two paintings of the 19th century.

Keywords: Art analysis; Oils; Fatty acids; Triglycerides; Dicarboxylic acids

1. Introduction

Drying oils are used since centuries for coatings, especially in Western art they are applied as binding media for pigments in oil paintings. In Italian painting walnut oil has been introduced in the 15th century, but linseed oil became more common from the 16th century on [1]. These oils have in common a high content of unsaturated fatty acids, mainly consisting of 18 carbon atoms. Presence of two or three double bonds in the fatty acid chain seems to be essential for the drying process. This process is a based on a radical-induced abstraction of a hydrogen atom preferably from a CH group in an allyl configuration. Such groups are present between the double bonds of linolic (all-*cis*-9,12-octadecadienoic, 18:2) and linolenic (all-*cis*-9,12,15-octadecatrienoic, 18:3) acid, the main constituents of linseed, walnut and poppyseed oil [2,3] (note that these unsaturated fatty acids have isolated double bonds, not conjugated ones). Other main fatty acids in these oils are the mono-unsaturated oleic (9-*cis*-octadecenoic, 18:1), and the saturated palmitic (hexadecanoic, 16:0) and stearic (octadecanoic, 18:0) acid. In this notation of the acids the first figure indicates the number of carbon atoms, the second the number of double bonds in the chain.

The drying process itself results in a polymerisation upon uptake of oxygen. It has a complex mechanism, which consists besides other reactions by an oxidative degradation of the fatty acid chain, which leads, e.g. to the intermediate formation of an aldehyde at the position of one C–C double bond, followed by autooxydation of the aldehyde to the carboxy-group [2–5]. As a result, in aged samples dicarboxylic acids are formed as decomposition product, mainly pimelic (1,7-heptanedioic, 7di), suberic (1,8-octanedioic, 8di), azelaic (1,9-nonanedioic, 9di) and sebacic (*n*-decanedioic, 10di) acid. Although these compounds

* Corresponding author. Tel.: +43-1427752305; fax: +43-142779523.
E-mail address: ernst.kenndler@univie.ac.at (E. Kenndler).
[1] Permanent address: Laboratory for Flow Analysis and Chromatography, Department of Chemistry, Warsaw University, Poland.

0021-9673/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2003.10.065

are also found in recent oils, their concentration is low here. Higher concentrations can be taken as indicator for ageing.

In objects of art, analysis of the triglycerides used as binding media is normally carried out by gas chromatography [3,6–14]. In most cases it is not the native lipid, which is of interest, it is its fatty acid composition. This is normally determined after hydrolysis of the triglycerides to glycerol and the free fatty acids, the latter usually being converted into their less polar derivatives (e.g. into alkyl- or silylesters). These GC methods are routinely used, they are highly efficient, and deliver additional structure information in combination with mass spectrometry.

Recently, capillary electrophoresis (CE) has been shown to bear an interesting analytic potential for the determination and characterisation of underivatised long-chain fatty acids [15–33]. However, separation of mixtures of saturated and unsaturated long-chain fatty acids by CE is not a trivial task due to several reasons. One reason is the very similar pK_a values and ionic mobility of these analytes, because an additional CH_2 group in the long chain does not affect the dissociation constant, on the one hand, and causes only a marginal decrease in actual mobility. The same argument holds for acids with the same length and various degree of unsaturation, where the effect of the long chain on ionic mobility superimposes possible contributions from double bonds. It must be noted that most of these analytes are poorly absorbing in a UV range above 210 nm, which makes the application of indirect UV detection or conductivity detection advantageous. Taking these restrictions into account, and in order to overcome the low solubility of the analytes in pure aqueous solution, the group of Tavares [33] worked out a complex system for the determination of long-chain *cis* and *trans* fatty acids of interest in nutrition industry. This system implements separation selectivity and allows indirect detection by using a polymer (Brij®), acetonitrile, octanol, and dodecylbenzensulfonate. It was taken in the present paper to examine the content of long-chain fatty acids in drying oils mostly used as binding media. This was the one goal of this paper.

The second goal was to analyse the dicarboxylic acids formed as degradation products during the drying process, mainly 7di, 8di, 9di, 10di. For the analysis of such dicarboxylic acids, background electrolytes (BGEs) for CE with indirect UV detection were described in the literature; they consist of napthalene dicarboxylic acid [34,35], pyridinedicarboxylic acid [36] or phthalic acid [34], respectively. As all these systems did not lead to satisfactory results, we developed an appropriate system for separation and indirect UV and conductivity detection of these analytes. We based the selection of the BGE constituents on computer simulation with PeakMaster program [37].

It should be pointed out that it was not the aim of the present work to deal with a full quantitative procedure for the determination of the analytes, including derivation of the different response factors, etc., our interest was rather directed to the qualitative determination of the respective acid patterns. However, the data allow a semi-quantitative interpretation of the results.

2. Materials and methods

2.1. Chemicals

Stearic acid (95%), suberic acid (98%), pimelic acid (98%), linolenic acid (99%) palmitic acid (90%), linoleic acid (approximately 95%), oleic acid (>99%), Brij® 35, salicylic acid (ACS reagent) and cetyltrimethylammonium bromide were obtained from Sigma–Aldrich (Steinheim, Germany). Dodecylbenzenesulfonic acid sodium salt (“techn.”), L-histidine (“puriss.”), sebacic acid (purity ≥ 95.0%) and azelaic acid (“techn.”; ∼85%) were purchased from Fluka (Buchs, Switzerland). $Na_2HPO_4{\cdot}12H_2O$, $NaH_2PO_4{\cdot}H_2O$ (both analytical grade), 1-octanol (extra pure), methanol (gradient grade) and diethyl ether were obtained from E. Merck (Darmstadt, Germany). Acetonitrile (HPLC reagent) was provided by Mallinckrodt Baker (Deventer, The Netherlands) and 1.0 M sodium hydroxide solution for HPCE was from Hewlett-Packard (Waldbronn, Germany).

Linseed oil and walnut oil samples were from Kremer Pigmente (Aichstetten, Germany). Old dried samples of linseed and walnut oil, and the samples from old paintings were taken from the collection of the Academy of Fine Arts, Department of Conservation and Restoration, Vienna.

Stock solutions of individual acids were prepared at 50 mM concentration by dissolving appropriate amounts of the selected acids in methanol. Bidistilled water was used for preparation of the BGEs.

2.2. Instrumentation

All experiments were carried out with a HP^{3D}CE Instrument (Agilent Technologies, Waldbronn, Germany) with fused-silica capillaries (75 μm i.d. × 375 μm o.d., Composite Metal Services, Hallow, UK), total length 48.5 cm, 33.4 cm length to the contactless conductivity detector (CCD), 40.0 cm to the diode array detector (DAD). The laboratory-made CCD is described in [38]. The separation capillary and the CCD cells were thermostated at 25 °C. A separation voltage of +20 kV and a hydrodynamic injection of 75 mbar.s were used for long-chain fatty acid analysis; it was −10 kV and 15 mbar s for the analysis of dicarboxylic fatty acids. A lab pH mater (WTW pH 538, Weilheim, Germany) was employed to measure the pH of the BGEs.

2.3. Procedure

2.3.1. Background electrolytes

BGEs for the analysis of long-chain fatty acids together with the capillary cleaning procedure were as described by Oliveira et al. [33]. The BGE consisted of phosphate buffer (pH 6.86, 15 mM) containing 4 mM sodium dodecylbenzen-

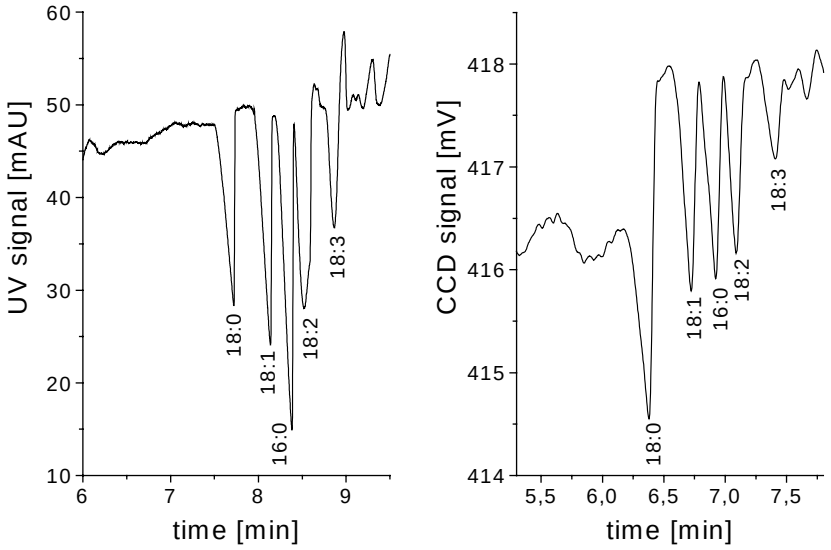

Fig. 1. Separation of long-chain fatty acid standards: (left) indirect detection at 224 nm wavelength; (right) contactless conductivity detection. BGE: phosphate buffer, pH 6.86, 15 mM, 4 mM sodium dodecylbenzensulfonate, 10 mM Brij® 35, 2% (v/v) 1-octanol and 45% (v/v) acetonitrile. Capillary: total length 48.5 cm, 33.4 cm length to the CCD, 40.0 cm to the DAD; 75 μm i.d. Voltage +20 kV, temperature 25 °C, 75 mbar.s injection. Symbols: stearic acid, 18:0; oleic acid, 18:1; palmitic acid, 16:0; linoleic acid, 18:2; linolenic acid, 18:3. Fatty acid concentration 2 mM each.

sulfonate, 10 mM Brij® 35, 2% (v/v) 1-octanol and 45% (v/v) acetonitrile. Stock solutions of electrolyte components were prepared at 100 mM concentration. Phosphate buffer was prepared by weighing equimolar amounts of mono- and di-hydrogenphosphate sodium salts. Before measurements the capillary was conditioned by flushing with 1 M NaOH solution (5 min), ethanol (3 min), 100 mM phosphate buffer pH 2.5 (15 min), bidistilled water (5 min) and electrolyte solution (10 min). In between runs the capillary was flushed with 100 mM phosphate buffer pH 2.5 (2.5 min), bidistilled water (1 min) and electrolyte solution (2.5 min).

The computer program PeakMaster was employed to calculate the composition of the BGE for the analysis of the dicarboxylic acids. The calculated composition of the BGE at the finally measured pH 5.85 (according to a theoretical pH 6.00) consisted of 10 mM salicylic acid and 20 mM L-histidine, with 0.2 mM cetyltrimethylammonium bromide added in order to reverse the electroosmotic flow (EOF).

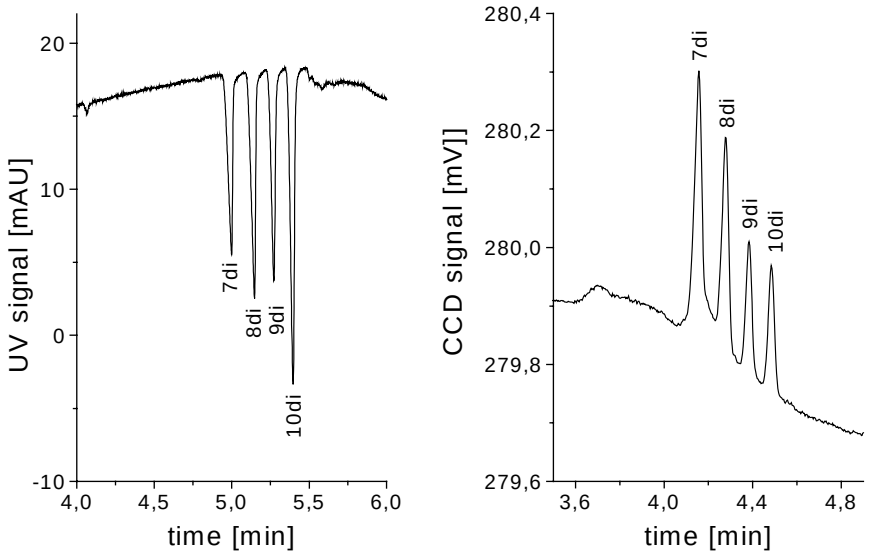

Fig. 2. Separation of dicarboxylic acid standards: (left) indirect detection at 224 nm wavelength; (right) contactless conductivity detection. BGE: 10 mM salicylic acid, 20 mM L-histidine, pH 5.85; 0.2 mM cetyltrimethylammonium bromide added to reverse the electroosmotic flow. Capillary: total length 48.5 cm, 33.4 cm length to the CCD, 40.0 cm to the DAD; 75 μm i.d. Voltage −10 kV, temperature 25 °C, 15 mbar.s injection. Symbols: pimelic acid, 7di; suberic acid, 8di; azelaic acid, 9di; sebacic acid, 10di. Dicarboxylic acid concentration 1 mM each.

Before measurements the capillary was conditioned by rinsing with 1 M NaOH solution (10 min), bidistilled water (10 min) and electrolyte solution (5 min). In between runs the capillary was washed by rinsing with 1 M NaOH solution, bidistilled water and electrolyte solution, 3 min each.

In both systems no EOF marker was needed to record the clear signal from the EOF.

2.3.2. Sample preparation

Weighted amounts of oil sample or sample from old painting (between about 1 and 2 mg) were mixed in an Eppendorf vial with 100 μl of 3 M NaOH, the vial was closed and heated to ∼80 °C for 4 h in an oven. Then the supernatant liquid sample was transferred to another vial, acidified by addition of 200 μl of 3 M HCl, 500 μl of diethyl ether were added and the mixture was vortexed for about 10 s. The ether fraction was transferred to a third vial, the solvent was evaporated to dryness and the residue re-dissolved in 100 μl (oil samples) or 20 μl (old painting samples) of methanol. An aliquot was then injected into the CE apparatus.

3. Results and discussion

3.1. Separation of standards mixtures

3.1.1. Long-chain fatty acids

The same BGE as developed by the group of Tavares [33] for the analysis of the relevant *cis* and *trans* fatty acids in Brazil nuts was used in the present paper to determine the main fatty acids constituting drying oils. Beside the saturated palmitic (16:0) and stearic (18:0) acid analytes of interest are the unsaturated oleic (18:1), linolic (18:2) and linolenic (18:3) acids. The resulting electropherograms of a

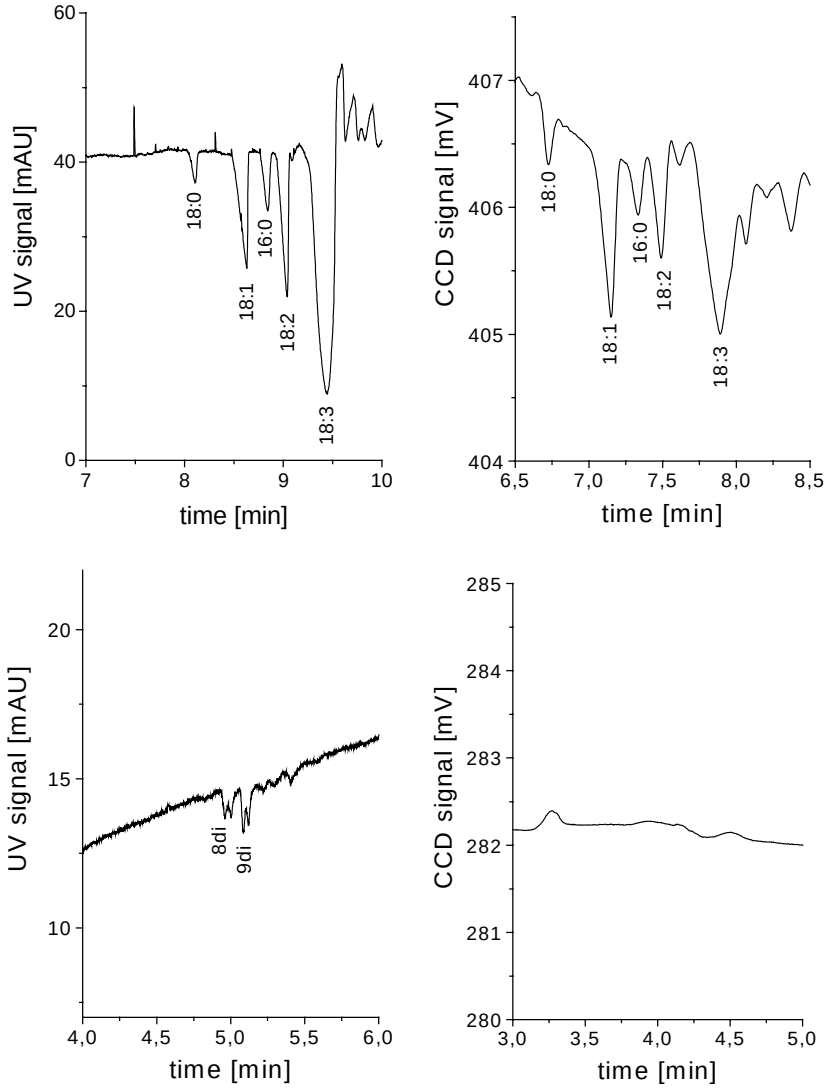

Fig. 3. Electropherograms of the long-chain fatty acids (upper panel) and the dicarboxylic acids (lower panel) obtained for recent linseed oil: (left) indirect detection at 224 nm wavelength; (right) contactless conductivity detection. Experimental conditions and symbols are as given in Figs. 1 and 2. Amount of sample analysed: 2.0 mg.

standard mixture of these acids measured with the UV absorbance and the contactless conductivity detector on-line are presented in Fig. 1. Although there is some fluctuation of the baseline, the analytes can be clearly distinguished. It should be pointed out that such fluctuations are often observed when universal detectors are applied, because slight changes in the BGE composition, which are common in CE, are recorded here. These changes are often not seen in non-UV absorbing BGEs, although present as well. It should also be pointed out that the relatively broad peaks observed are not untypical for systems with indirect UV absorbance detection and are due to the relatively high sample concentrations needed for detection. However, separation selectivity is high enough to overcome the relatively low efficiency.

It was found that the system is not well suited for the determination of the shorter chain dicarboxylic acids, the degradation products of interest, because these analytes exhibit a too large effective mobility in this BGE. As this mobility is close to that of the counter-directed electroosmotic flow under the present conditions, the analytes will not be detected thus within a reasonable time, say within 60 min. Instead of modifying the system capable for long-chain fatty acid analysis it was decided to determine these dicarboxylic acids separately in an appropriate BGE.

3.1.2. Dicarboxylic acids

BGEs for the separation of the dicarboxylic analytes pimelic (7di), suberic (8di), azelaic (9di) and sebacic (10di) acid have already been described in the literature; they

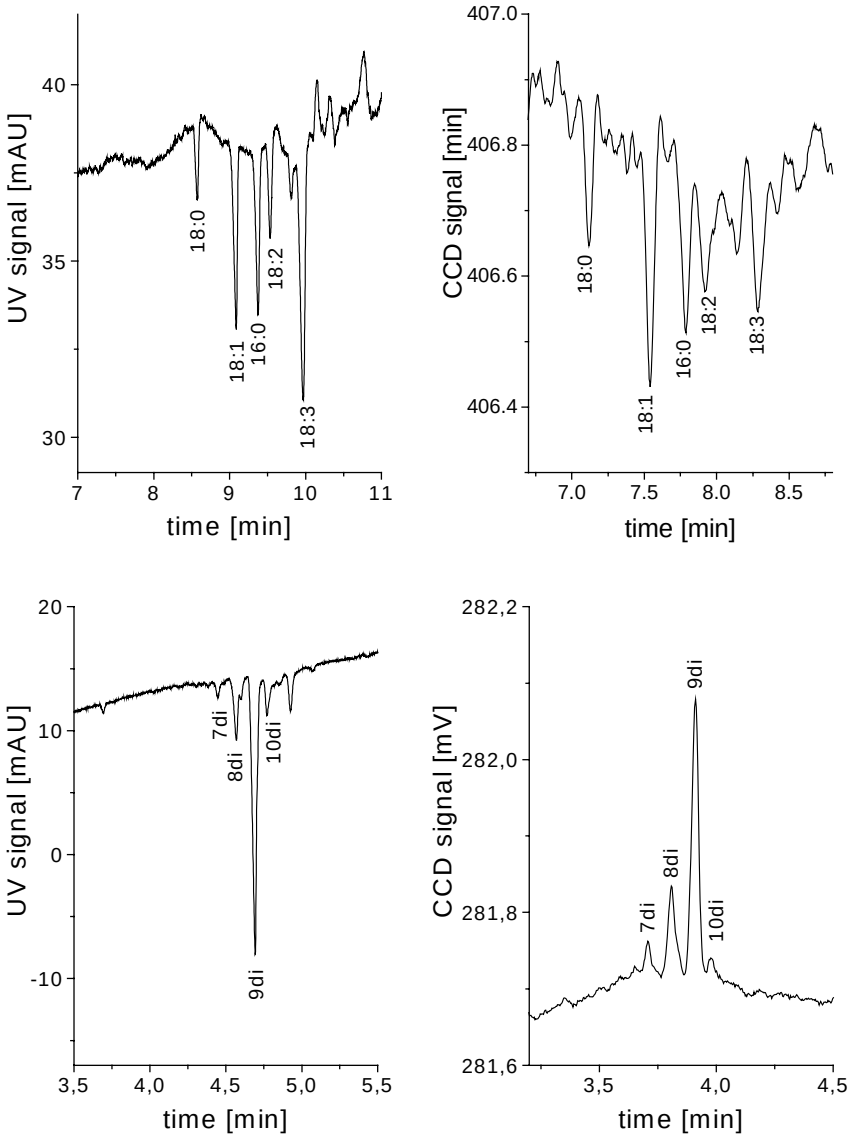

Fig. 4. Electropherograms of the long-chain fatty acids (upper panel) and the dicarboxylic acids (lower panel) from a dried, rubber-like sample of linseed oil: (left) indirect detection at 224 nm wavelength; (right) contactless conductivity detection. Experimental conditions and symbols are as given in Figs. 1 and 2. Amount of sample analysed: 0.8 mg.

contain naphthalene dicarboxylic acid [34,39], pyridinedicarboxylic acid [36] or phthalic acid [34] as constituent, mainly rather as chromophor than as buffering compound. In our work, the system with pyridinedicarboxylic acid caused problems with sudden drop and decrease of the current; moreover, the peaks were strongly triangulating; this extreme peak asymmetry hindered resolution. Similar effects were observed with the other BGEs. The reason for the disturbing asymmetry effects is most probably the presence of the divalent aromatic acids as BGE constituents; BGEs with more than two ions should not be applied when system eigenpeaks are to be avoided [40–45]. This general assumption was supported by simulations with PeakMaster program [37], which indeed simulates the strongly triangulation of the peaks. Therefore the divalent BGE ions were replaced by monovalent salicylate, which was used as chromophor, in conjunction with histidine as counter-ion, which is buffering at the chosen pH. The resulting electropherograms (under the appropriate pH and ionic strengths conditions as calculated by the aid of PeakMaster) for the standard mixture of the four dicarboxylic acids are shown in Fig. 2 (a cationic additive reverses the EOF). Indeed relatively sharp and symmetrical peaks are obtained, and all analytes are baseline resolved.

3.2. Recent drying oils

We show here the results of the analysis of a typical drying oil—recent linseed oil—which possesses a high content of 18:3, namely between 48 and 60%. The electropherograms

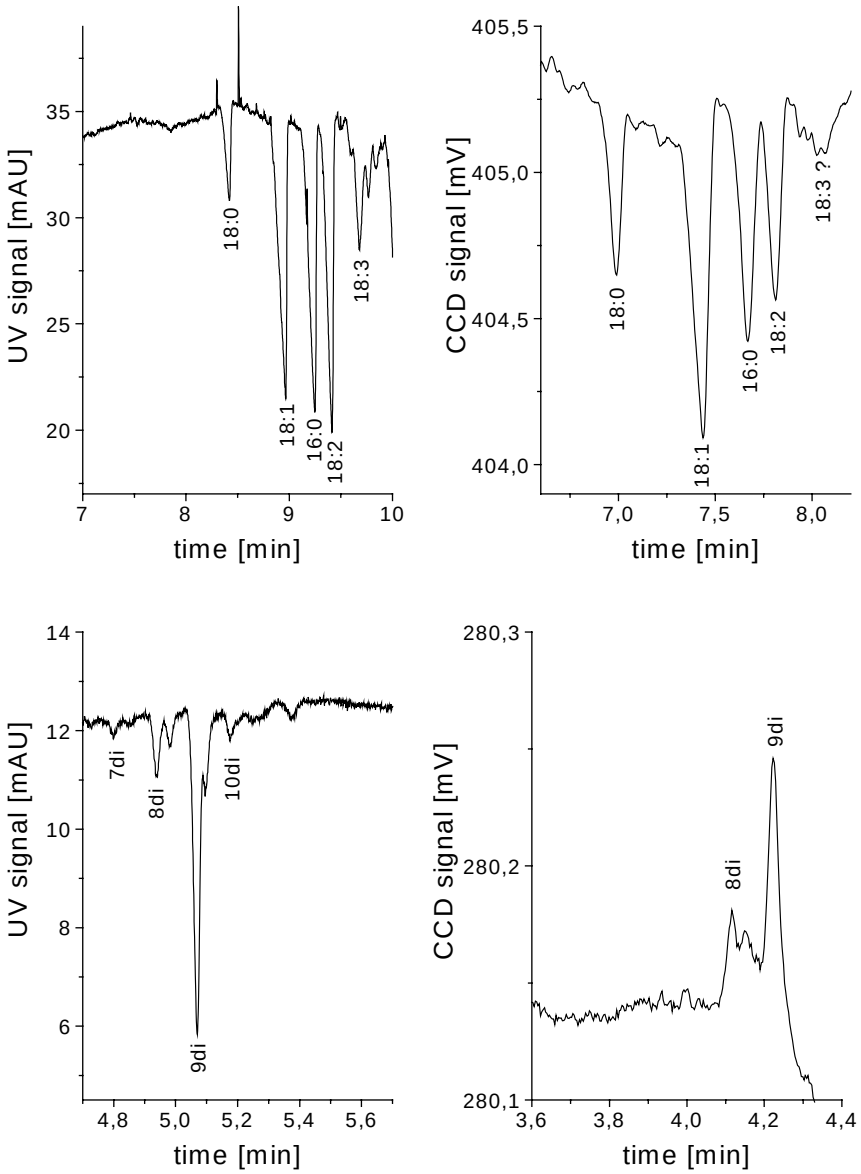

Fig. 5. Electropherograms of the long-chain fatty acids (upper panels) and the dicarboxylic acids (lower panels) from a dried, rubber-like sample of walnut oil: (left) indirect detection at 224 nm wavelength; (right) contactless conductivity detection. Experimental conditions and symbols are as given in Figs. 1 and 2. Amount of sample analysed: 0.5 mg.

of the long-chain fatty acid are shown in Fig. 3. The most prominent peak is indeed that of 18:3, but the other unsaturated acids can be recognised as well. The according electropherogram for the oxidation products, on the other hand, shows the occurrence of an only minute amount of dicarboxylic acids, at a concentration not high enough to be detectable by the CCD. As proved by comparison with the ionic mobilities, the dicarboxylic acids are most probably 8di and 9di.

3.3. Aged drying oils

From aged linseed and walnut oil, respectively, which reached already a soft rubber-like consistency due to polymerisation samples were taken and analysed. It is known that the conversion of the unsaturated fatty acids is a long-term process, strongly determined by the conditions of the surrounding, also depending on the presence of UV light. Thus in aged and dried samples unchanged unsaturated fatty acids can still remain; they are indeed found in both samples shown in Figs. 4 and 5. The lower content of 18:3 in the walnut oil sample compared to the linseed oil is not necessarily the result of the advanced degradation process; it can simply reflect the higher initial 18:3 content of linseed oil.

In contrast to recent oil, dicarboxylic acids are present at much higher concentration, as can be seen from these Figures (lower panels) as well. The peak of 9di as the major degradation product is clearly detected. It is formed from the unsaturated acids, which have a double bond at position 9. This double bond is oxidatively cleaved and the carbon

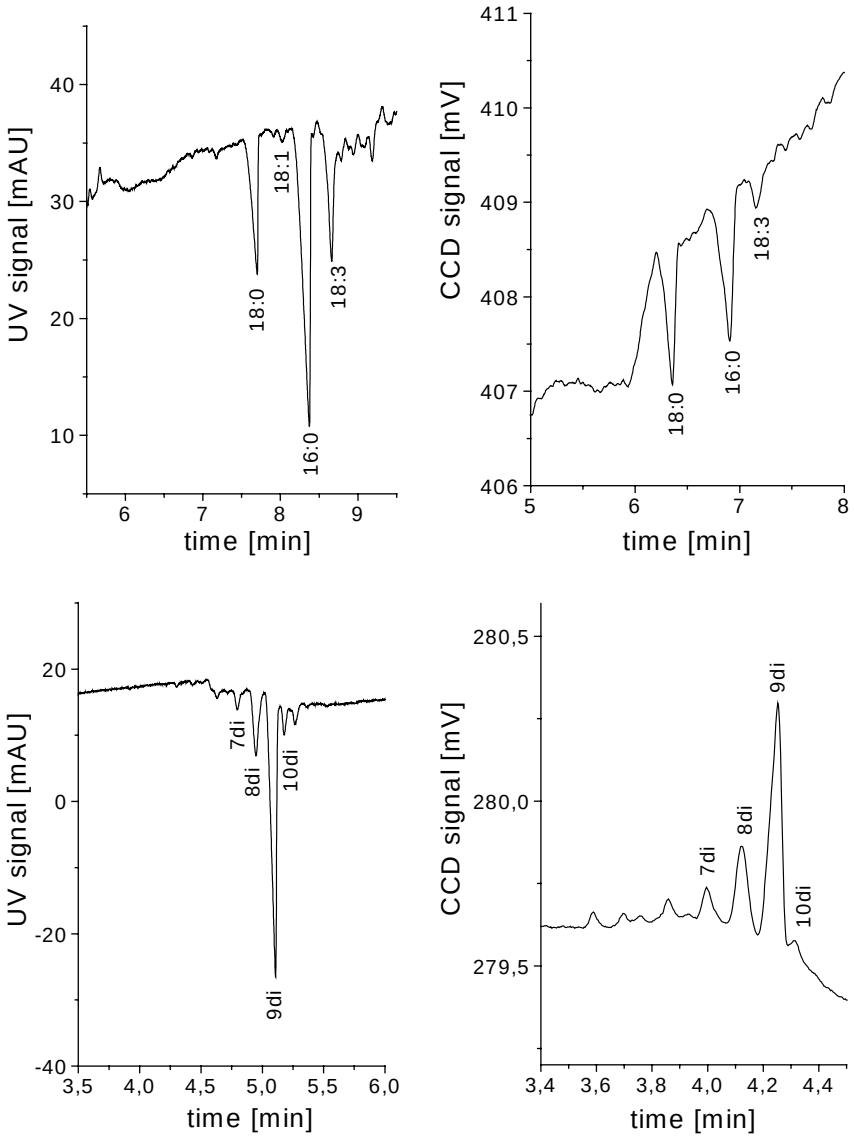

Fig. 6. Electropherograms of the long-chain fatty acids (upper panels) and the dicarboxylic acids (lower panels) from a sample taken from a painting from the 19th century (oil on canvas) “portrait of a woman” (anonymous): (left) indirect detection at 224 nm wavelength; (right) contactless conductivity detection. Experimental conditions and symbols are as given in Figs. 1 and 2. Amount of sample analysed: 1.1 mg (for details see text).

number 9 in the chain is oxidised to the carboxylic function. Thus, together with the initial carboxylic group at position 1 nonanedioic acid is resulting.

The rough comparison between the content of the dicarboxylic acids in the aged sample and that in recent oil (compare Figs. 4 and 5 with Fig. 2) allows the conclusion of an about 50-fold increase upon drying.

3.4. Samples from oil paintings

We have analysed samples taken from two paintings (oil on canvas) from the 19th century, which are part of the historical collection of the Institute for Restoration and Conservation at the Academy of Fine Arts, Vienna. The artists of both paintings are unknown. Sample A was taken from the brown background of the painting "portrait of a woman", which consisted of ground, brown paint layer and varnish. Sample B was from a bloom of the middle of the painting "still life with flowers", consisting of ground, green pigment layer, violet pigment layer and varnish. The different layers could be recognised upon visual inspection under a stereo-microscope. The amount of sample taken from both objects was few milligrams each. It should be mentioned in this context that the major matter of such samples consists of pigments, not of binders.

The samples were hydrolysed as described, extracted in diethylether, the solvent evaporated, the residue dissolved in methanol, and an aliquot injected into the CE instrument. The resulting electropherogram of both samples (Figs. 6 and 7) allow detection of the fatty acids and the dicarboxylic

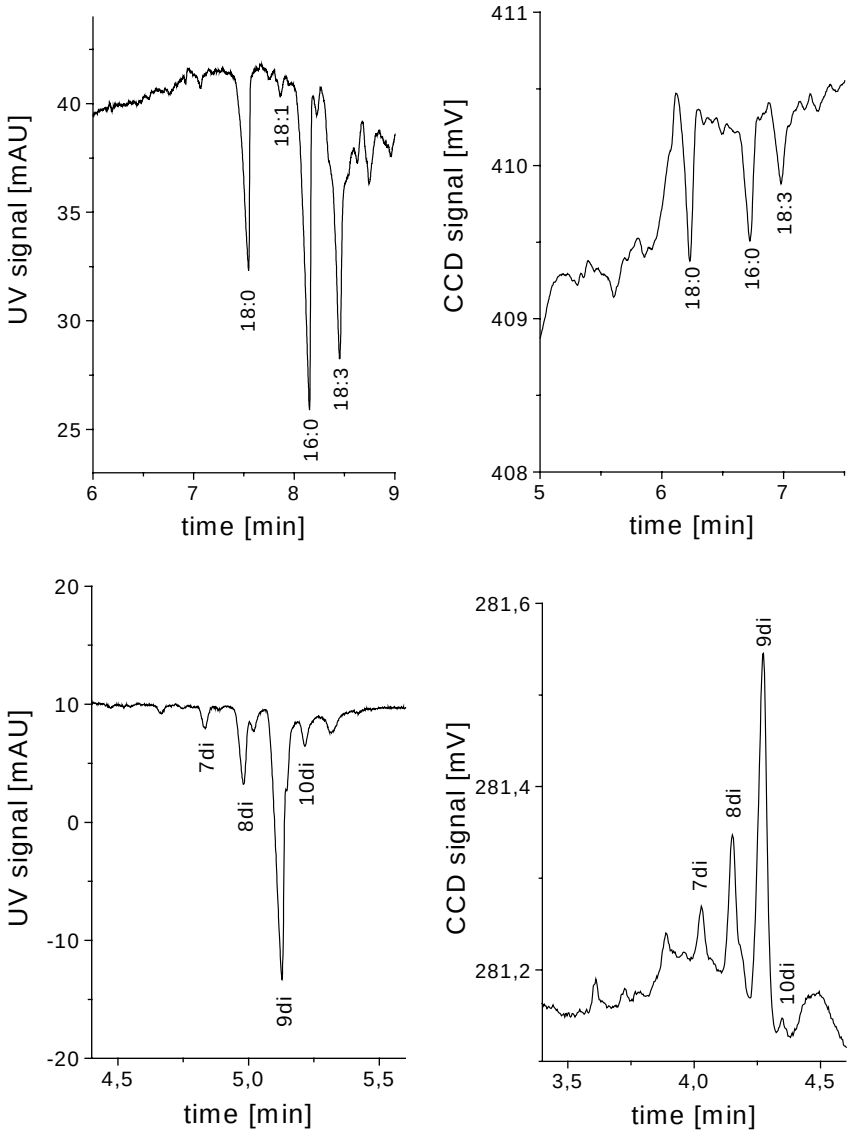

Fig. 7. Electropherograms of the long-chain fatty acids (upper panels) and the dicarboxylic acids (lower panels) from a sample taken from a painting from the 19th century (oil on canvas) "still life with flowers" (anonymous): (left) indirect detection at 224 nm wavelength; (right) contactless conductivity detection. Experimental conditions and symbols are as given in Figs. 1 and 2. Amount of sample analysed: 1.7 mg (for details see text).

degradation products. Interestingly, both samples show the unsaturated C16 and C18 fatty acids as main lipid constituents, but 18:3 can still be recognised. As described above, oxidative reaction during the drying process led to a significant amount of the dicarboxylic acids, mainly 9di, as can be seen from the lower panels of the figures. The results demonstrate the suitability of the method for the analysis of real, aged samples, which contain also other constituents like pigments, varnishes, etc. as well.

4. Conclusions

Capillary electrophoresis with indirect or conductivity detection allows the determination of the long-chain fatty acids of drying oils, and the dicarboxylic acids formed upon oxidative degradation which occurs simultaneously with the polymerisation process, both in their underivatised forms. Sample preparation is limited to the common hydrolysis followed by extraction from acidic solution with diethylether. Determination of the analytes is carried out in two different BGEs. Although the CE methods are simple and give unequivocal results, some critical comments are justified, especially when compared to the well-established GC method of the according acid derivatives (esters or silyl compounds). (i) As often observed for indirect detection systems, the peaks tend to be broad, and separation efficiency is relatively low. Compared to the methodology commonly used for fatty acid analysis—capillary GC—the present CE method has much lower separation efficiency; however, due to the appropriately adjusted separation selectivity it is high enough to resolve the analytes of interest. (ii) For the given experimental conditions, indirect UV detection seems to be superior to conductivity detection, but it must be mentioned that these conditions were selected mainly for indirect UV. It should be mentioned that, in fact, the combination these two detectors does not increase the information attained by only one of them; they are not complementary. (iii) Compared to GC, both detection schemes are inferior concerning sensitivity and detection limit. Detectibility is, however, high enough to determine the analytes even in real samples, which are normally available in the milligram amount, otherwise handling and sample manipulation would be too cumbersome. Considering an end volume of the sample solution being in the few 10 μl range, analyte concentration is high enough to overcome the unfavourable sensitivity and detection limit of the CE method. (iv) Compared to GC (which must be operated in the temperature gradient mode due to the large volatility range of the analytes) CE has the advantage of shorter analysis times. This holds even when the rinsing steps between the CE runs are taken into account (and the according equilibration time of the temperature in GC after the re-set of the column temperature). However, we think that the shorter analysis time of the CE method might be relevant only in few cases in practice, as the number of samples in laboratories dealing with museum objects is relatively low, e.g. compared with industrial routine laboratories.

Acknowledgements

A grant for I.S. from the Austrian Exchange Service (CEEPUS H-76) is acknowledged. The authors thank B. Gas, Charles University Prague, for donation of the CCD, and for the valuable discussion about system eigenpeaks.

References

[1] J.S. Mills, R. White, The Organic Chemistry of Museum Objects, Butterworth, Heinemann, London, 1994.
[2] F. Rasti, G. Scott, Stud. Conserv. 25 (1980) 145.
[3] J.S. Mills, Stud. Conserv. 11 (1966) 92.
[4] H. Wexler, Chem. Rev. 64 (1964) 591.
[5] K.U. Ingold, Accounts Chem. Res. 2 (1969) 1.
[6] D. Scalarone, M. Lazzari, O. Chiantore, J. Anal. Appl. Pyrol. 58–59 (2001) 503.
[7] A. Casoli, P. Cremonesi, G. Palla, M. Vizzari, Ann. Chim. (Rome) 91 (2001) 727.
[8] R. Mateo-Castro, J.V. Gimeno-Adelantado, F. Bosch-Reig, A. Domenech-Carbo, M.J. Casas-Catalan, L. Osete-Cortina, J. De la Cruz-Canizares, M.T. Domenech-Carbo, Fresenius J. Anal. Chem. 369 (2001) 642.
[9] R. Newman, M. Derrick, Mater. Res. Soc. Symp. Proc. 712 (2002) 291.
[10] L.E. Plahter, U. Plahter, Conserv. Restor. Pict. Art (1976) 36.
[11] M.P. Colombini, F. Modugno, R. Fuoco, A. Tognazzi, Microchem. J. 73 (2002) 175.
[12] W. Nowik, Stud. Conserv. 40 (1995) 120.
[13] A.M. Shedrinsky, R.E. Stone, N.S. Baer, J. Anal. Appl. Pyrol. 20 (1991) 229.
[14] M.P. Colombini, F. Modugno, M. Giacomelli, S. Francesconi, J. Chromatogr. A 846 (1999) 113.
[15] G. Gutnikov, W. Beck, H. Engelhardt, J. Microcol. Sep. 6 (1994) 565.
[16] R. Roldan-Assad, P. Gareil, J. Chromatogr. A 708 (1995) 339.
[17] W. Buchberger, K. Winna, Mikrochim. Acta 122 (1996) 45.
[18] J. Collet, P. Gareil, J. Capillary Electrophoresis 3 (1996) 77.
[19] E. Drange, E. Lundanes, J. Chromatogr. A 771 (1997) 301.
[20] R. Neubert, K. Raith, J. Schiewe, Pharmazie 52 (1997) 212.
[21] O. Schmitz, S. Gaeb, J. Chromatogr. A 767 (1997) 249.
[22] K. Heinig, F. Hissner, S. Martin, C. Vogt, Am. Lab. 30 (1998) 24.
[23] B. Vallejo-Cordoba, M.A. Mazorra-Manzano, A.F. Gonzalez-Cordova, J. Capillary Electrophoresis 5 (1998) 111.
[24] T.L. Wang, H.P. Wei, S.F.Y. Li, Electrophoresis 19 (1998) 2187.
[25] L.G. Blomberg, Lipid Technol. 11 (1999) 140.
[26] M.-J. Chen, H.-S. Chen, C.-Y. Lin, H.-T. Chang, J. Chromatogr. A 853 (1999) 171.
[27] F. Haddadian, S.A. Shamsi, I.M. Warner, J. Chromatogr. Sci. 37 (1999) 103.
[28] N. Mofaddel, A. Desbene-Monvernay, Analusis 27 (1999) 120.
[29] D.L. Gallaher Jr., M.E. Johnson, Anal. Chem. 72 (2000) 2080.
[30] M.A.L. de Oliveira, G.A. Micke, R.E. Bruns, M.F.M. Tavares, J. Chromatogr. A 924 (2001) 533.
[31] M. Ohman, H. Wan, M. Hamberg, L.G. Blomberg, J. Sep. Sci. 25 (2002) 499.
[32] M.E. Bohlin, M. Ohman, M. Hamberg, L.G. Blomberg, J. Chromatogr. A 985 (2003) 471.

[33] M.A.L. de Oliveira, V.E.S. Solis, L.A. Gioielli, B. Polakiewicz, M.F.M. Tavares, Electrophoresis 24 (2003) 1641.
[34] D.H. Craston, M. Saeed, J. Chromatogr. A 827 (1998) 1.
[35] E. Dabek-Zlotorzynska, M. Piechowski, M. McGrath, E.P. Lai, J. Chromatogr. A 910 (2001) 331.
[36] H. Adler, H. Siren, M. Kulmala, M.L. Riekkola, J. Chromatogr. A 990 (2003) 133.
[37] http://www.natur.cuni.cz/~gas/.
[38] B. Gas, J. Zuska, P. Coufal, T. van de Goor, Electrophoresis 23 (2002) 3520.
[39] E. Dabek-Zlotorzynska, M. Piechowski, M. McGrath, E.P. Lai, J. Chromatogr. A 910 (2001) 331.
[40] B. Gas, P. Coufal, M. Jaros, J. Muzikar, I. Jelinek, J. Chromatogr. A 905 (2001) 269.
[41] M. Jaros, K. Vcelakova, I. Zuskova, B. Gas, Electrophoresis 23 (2002) 2667.
[42] M. Stedry, M. Jaros, B. Gas, J. Chromatogr. A 960 (2002) 187.
[43] M. Stedry, M. Jaros, K. Vcelakova, B. Gas, Electrophoresis 24 (2003) 536.
[44] J. Bullock, J. Strasters, J. Snider, Anal. Chem. 67 (1995) 3246.
[45] R.L. Williams, B. Childs, E.V. Dose, G. Guiochon, G. Vigh, Anal. Chem. 69 (1997) 1347.