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ANALYSIS OF SATURATED NORMAL FATTY ACIDS IN HYDROCARBON MATRICES BY CAPILLARY ISOTACHOPHORESIS*

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SUMMARY

Separation conditions suitable for the analysis of C_1–C_{18} saturated normal fatty acids by capillary isotachophoresis were investigated. Operational systems using water–methanol solvent mixtures were suitable only for the separation of C_1–C_{10} acids, whereas a complete resolution of the studied constituents is possible in methanol. Analyses of the acids present in reaction mixtures after the oxidation of alkanoic and/or alkenoic substrates (the substrate or *n*-butyric acid served as the reaction environment) were carried out without any sample pre-treatment using the operational system proposed in experiments with model mixtures. The time for the analysis ranged from 12 to 25 min, depending on the complexity of the reaction mixture and on the configuration of the separation unit employed.

INTRODUCTION

Fatty acids need to be analysed in a wide variety of matrices and of the analytical methods used for this purpose, gas–liquid chromatography (GLC) by far prevails. The acids are usually analysed by this method as suitable derivatives (*e.g.*, ref. 1), in spite of the fact that under certain conditions GLC analysis of the free acids can be successful (*e.g.*, refs. 2–4). When these constituents need to be determined reliably in a complex hydrocarbonaceous matrix, the use of one of the element-se-

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lective detectors and, obviously, derivatization of the acids using an agent providing a response to such a detector are advantageous (ref. 1, p. 180). However, the simultaneous determination of formic acid can cause some problems[1–4].

High-performance liquid chromatography (HPLC) is the method of choice for the analysis of this group of compounds (*e.g.*, refs. 5–10). Direct analysis of the free fatty acids present in complex samples by this method is, however, of limited analytical utility owing to the lack of sufficiently selective and/or sensitive detectors for this group of separands. To improve the detectability of fatty acids in HPLC, UV light-absorbing or fluorescent derivatives are prepared before the separation or in a post-column reactor[1,5–10]. In this manner, femtomole levels of some saturated fatty acids could be detected using fluorimetric detection, as shown by Tsuchiya *et al.*[7].

High-performance thin-layer chromatography (HPTLC) employed in a reversed-phase mode is also suitable for the separation and analysis of derivatized saturated fatty acids, as recently demonstrated by Gattavecchia *et al.*[11].

Our main task was to develop a procedure applicable to the determination of C_1–C_{18} saturated normal fatty acids in reaction mixtures after oxidation of *n*-alkanes or *n*-alkenes. In some instances the acids need to be determined in very complex non-ionic hydrocarbonaceous mixtures. Therefore, we decided to investigate the potential of capillary isotachophoresis (ITP) for this purpose. The use of ITP seemed promising because of its analytical specificity for the ionogenic compounds. Moreover, the results achieved in the separation of saturated normal fatty acids by Beckers and co-workers[12,13] were encouraging. In general, however, the application of ITP to the analysis of fatty acids is rare. Of other relevant studies, the separation of linoleic acid and its hydroperoxides in ethanol[14] and the determination of lower fatty acids in silage extracts[15] are worth mentioning.

As there have been no reports of the complete ITP separation of C_1–C_{18} saturated normal fatty acids, nor could this possibility be deduced from published work, we carried out an investigation with model mixtures to characterize this performance parameter of ITP. The separations of fatty acids of interest were studied in operational systems using water and methanol as solvents. The optimal analytical conditions found in the separations of the model mixtures were applied to the analyses of saturated normal fatty acids formed by oxidation of *n*-alkanes and *n*-alkenes.

EXPERIMENTAL

Instruments

ITP instruments similar to that described by Everaerts *et al.*[13], with components that come into direct contact with the solutions made of either polytetrafluorethylene (PTFE) or an acrylic, were used in this work. They were employed in a single column configuration or, in experiments using methanolic operational systems, also in a volume-coupling arrangement[16]. The separations were carried out in a 0.3 mm I.D. capillary tube made of fluorinated ethylene–propylene copolymer (FEP) provided with a conductivity sensor[17]. A capillary tube of 0.50 mm I.D. made of the same material was used for the first separation stage when the volume-coupling arrangement was employed. The driving current was delivered by the power supply designed by Havaši[18].

Chemicals

Methanol was obtained from Lachema (Brno, Czechoslovakia) and was doubly distilled when used for the preparation of leading and terminating electrolytes. Water was triply distilled when used for the same purpose.

Histidine was obtained from Reanal (Budapest, Hungary), Tris from Loba-Chemie (Vienna, Austria), morpholinoethanesulphonic acid (MES) from Sigma (St. Louis, MO, U.S.A.), poly(vinyl alcohol) (PVA) from Fluka (Buchs, Switzerland), methylhydroxyethylcellulose 30 000 (MHEC) from Serva (Heidelberg, F.R.G.) and hydroxyethylcellulose (HEC) from Polysciences (Warington, PA, U.S.A.). Chemicals used as ionic constituents in the leading and terminating electrolyte solutions were purified by repeated precipitation (dissolution in water and precipitation on addition of deionized ethanol). Additives to the leading electrolyte (PVA, HEC, MHEC) were purified on a mixed-bed ion exchanger.

The fatty acids obtained from Lachema, Loba-Chemie and Fluka were used without further purification. Only stearic acid (used in methanolic systems as terminating electrolytes) was purified by crystallization.

RESULTS AND DISCUSSION

Separations of model mixtures of fatty acids

The separations of saturated normal fatty acids were studied in mixtures of water with methanol and in methanolic operational systems containing small amounts of water. The former solvent combination was chosen for the investigation of the separability of these acids in solutions that are compatible with instruments made of an acrylic (the methanol content can be up to 40%, v/v).

The operational systems for water–methanol solvent mixtures were derived from those suitable for anionic separations in water[13]. In practice, the desired amount of methanol (containing 1% of water as determined by Fischer titration) was added to a stock aqueous solution of the required electrolyte composition and pH. The addition of methanol caused small deviations of pH from those of the stock solutions[13,19,20]. However, as the procedure for the preparation of the electrolyte solutions performed in this way was reproducible, reproducible results of ITP analyses were also obvious. As the separation unit was aligned vertically, a higher content of methanol had to be used in the terminating electrolyte solutions than in the samples and/or in the leading electrolytes to prevent either loss of the analytes (occurring with a lower density of the sample solutions in comparison with the terminating electrolyte) or a decrease in the load capacity (for a sample solution denser than the leading electrolyte).

Of the saturated normal fatty acids studied, C_1–C_{10} derivatives were well resolved in water–methanol operational systems (the pH of the aqueous stock solutions of the leading electrolytes varied in the range 3–9). The isotachopherogram given in Fig. 1 shows a typical ITP separation of these compounds. As could be expected[21–23], a regular decrease in the effective mobilities of the separands with increasing number of carbon atoms is clear from this isotachopherogram. Lauric acid and homologues having a higher number of carbon atoms, however, did not migrate within the predicted mobility range under identical separation conditions, thus not following this rule. As no solubility problems were observed for these constituents (injected in the

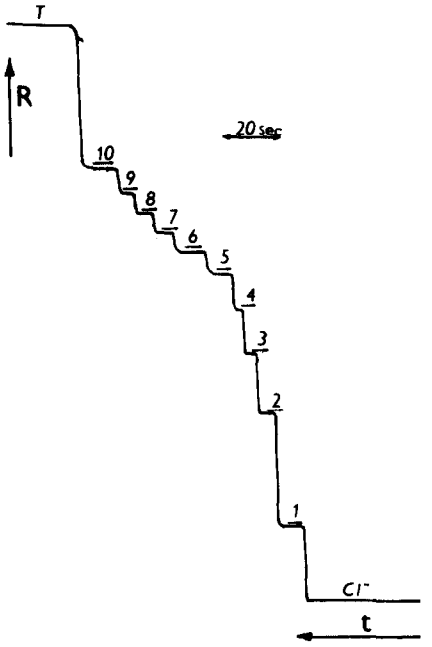

Fig. 1. Analysis of a model mixture of C_1–C_{10} normal fatty acids in operational system No. 1 (Table 1). Driving current, 45 μA. 1 = Formic; 2 = acetic; 3 = *n*-propionic; 4 = *n*-butyric; 5 = *n*-valeric; 6 = *n*-caproic; 7 = *n*-enantic; 8 = *n*-caprylic; 9 = *n*-pelargonic; 10 = *n*-caprinic acid. Cl^-, T = leading and terminating anions, respectively. *R* = Increasing resistance; *t* = increasing time.

form of their salts with solvent-compatible organic cations), their unexpected behaviour can probably be explained through the formation of less mobile micelles[19,24].

With respect to the problems associated with the separation of C_{12}–C_{18} acids in water–methanol operational systems, we decided to use methanol as the solvent. This solvent was effective for the ITP separation of some C_1–C_{18} saturated normal fatty acids in the work of Beckers and co-workers[12,13]. Preliminary results obtained in our laboratory confirmed the suitability of methanol for the complete ITP separation of these compounds[19]. However, when no additives were used in the leading electrolytes, higher fatty acids were not always resolved. This irreproducibility was due to electroosmosis, which was not negligible in methanolic solutions when a lower concentration of the leading constituent was employed in order to minimize the temperature in the capilary tube (low boiling point of methanol).

Of the materials tested that could suppress electroosmotic convection during the separation, the best results were achieved with PVA[19]. An isotachopherogram of the separation of a model mixture of fatty acids in methanol with PVA added to the leading electrolyte is given in Fig. 2a. The zone boundaries of less mobile constituents as revealed by the conductivity detector are not as sharp as would be expected when the electroosmotic flow is suppressed or reversed[25]. Moreover, PVA is not an optimal choice for methanol as it is only sparingly soluble in this solvent.

To improve the zone sharpness, especially for less mobile separands, we investigated the use of non-ionic additives that could have a high memory effect on the

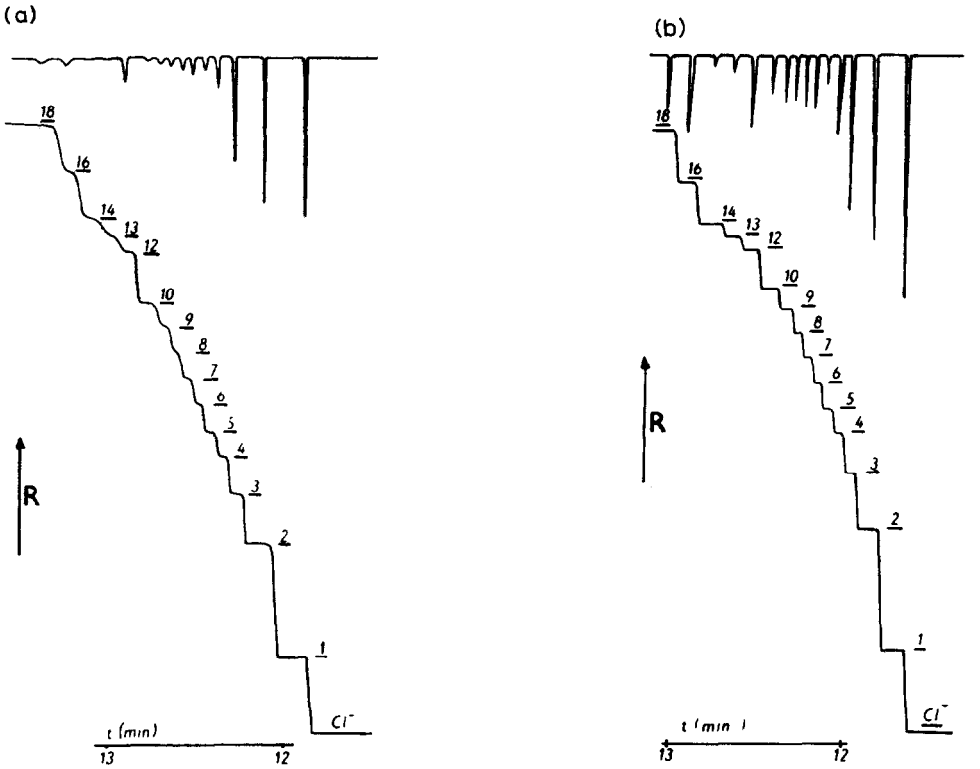

Fig. 2. (a) Isotachopherogram of the separation of a model mixture of saturated normal fatty acids using operational system No. 2 (Table 1). PVA was added to the leading electrolyte. 12 = *n*-Lauric; 13 = *n*-tridecanoic; 14 = *n*-myristic; 16 = *n*-palmitic; 18 = stearic acid (terminating anion). (b) Isotachopherogram of the separation of a model mixture of saturated normal fatty acids in operational system No. 2. The inner walls of the separation compartment were covered with MHRC. Other symbols as in Fig. 1. Driving current, 10 μA.

capillary wall[13,25]. These were applied in aqueous solutions before the analysis. The unretained part of the solute was removed with water to prevent its precipitation on refilling the tube with methanolic solution. In these experiments no other additives were used in the leading electrolytes. The high-molecular-weight MHEC (see Experimental) was very effective when applied in this manner. An isotachopherogram illustrating the separation of a model mixture of saturated normal fatty acids when the walls of the separation compartment were coated with this cellulose derivative is given in Fig. 2b. MHEC exhibited a long memory effect so that its renewal was not necessary in a withinday series of analyses. Obviously, the material of which the capillary tube is made can play an important role in this respect[13,25]. Our results also indicate that high-molecular-weight non-ionic derivatives of cellulose soluble in methanol[26] should be considered as additives to the leading electrolyte for methanolic operational systems.

Analysis of fatty acids present in reaction mixtures

In this part of the work only operational system No. 2 (Table I) was used for the analyses of two types of reaction mixtures. The first type included samples in which the substrate itself (*n*-alkanes or *n*-alkenes) served as a reaction environment and the analysis was carried out in a single column unit. The second type of reaction mixture was taken from the oxidation reactions carried out in *n*-butyric acid, which enhanced the desired conversion rate. A large excess of *n*-butyric acid (in comparison with the acids produced) required the use of a separation unit with a higher load capacity. Of the possible alternatives[27], a volume-coupling arrangement[16] was preferred as the separation unit suitable for analysis in non-aqueous media needed to be only slightly modified and the analysis time remained reasonably short.

TABLE I

OPERATIONAL SYSTEMS USED IN THE SEPARATION OF FATTY ACIDS

Parameter	*Leading electrolyte**		*Terminating electrolyte**	
	1	*2*	*1*	*2*
Solvent	H_2O–CH_3OH	CH_3OH–H_2O	H_2O–CH_3OH	CH_3OH–H_2O
Proportions (v/v)	90:10	95:5	80:20	99:1
Anion	Cl^-	Cl^-	Mes^-	$Stear^-$
Concentration (*mM*)	10	2	5	1
Counter ion	His^+	$Tris^+$	$Tris^+$	$Tris^+$
pH	6.0	8.1**	6.5	8.7**
Additive to the leading electrolyte	HEC	PVA (MHEC)***	–	–
Concentration (%, w/v)	0.2	0.02	–	–

* His = histidine; Stear = stearic acid.
** Measured as recommended in ref. 13.
*** For the application of HMEC, see the text.

Samples containing a large excess of hydrocarbons were, typically, sparingly soluble in methanol. Therefore, they were injected as dilute solutions in this solvent (20 μl volumes with the aid of a sampling valve) with the pH adjusted to that of the leading electrolyte. The isotachopherogram shown in Fig. 3 was obtained from the separation of saturated normal fatty acids formed by the oxidation of *n*-alkanes (C_{14}–C_{18}). When we consider the complexity of the constituents that can be present in the sample mixture at concentrations much higher than the acids to be determined, it is clear that the analytical selectivity of ITP towards ionic constituents provides excellent possibilities for performing this type of analysis. Another advantage of ITP in the analysis of reaction mixtures of this type is the rapid sample preparation (dissolution and pH adjustment). The time required for the complete analysis (*ca.* 20 min) was acceptable for reaction monitoring.

When the oxidation of hydrocarbons was carried out in *n*-butyric acid, the time required for the analysis increased slightly (*ca.* 25 min using the volume-coupling instrument). The procedure for sample preparation was the same as used for the previous sample type. An isotachopherogram of the separation of caproic and formic

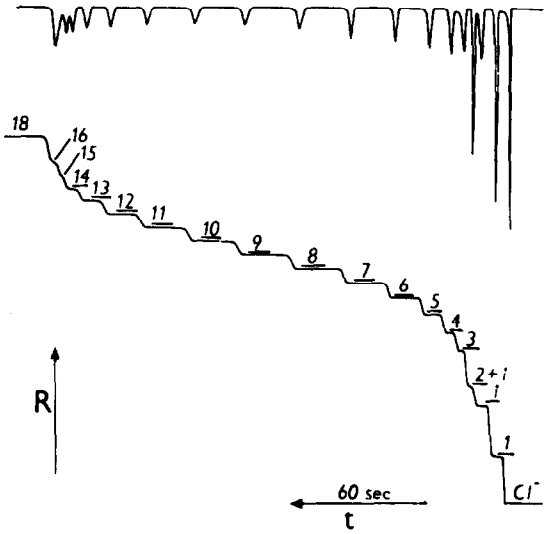

Fig. 3. Analysis of fatty acids present in the reaction mixture after oxidation of *n*-alkanes (C_{14}–C_{18}) by air oxidation followed by a catalytic conversion. 11 = *n*-Undecanoic; 13 = *n*-tridecanoic; 15 = *n*-pentadecanoic acid; i = unidentified impurities. Other symbols as in Figs. 1 and 2. Driving current, 10 μA.

acids formed by ozonization and oxidation of *n*-heptene in *n*-butyric acid is given in Fig. 4. This and another example, shown in Fig. 5, were taken from a series of experiments devoted to the optimization of the conditions of the oxidation process.

The constituents present in both sample types were identified through their step heights obtained from isotachopherograms for model mixtures and from those

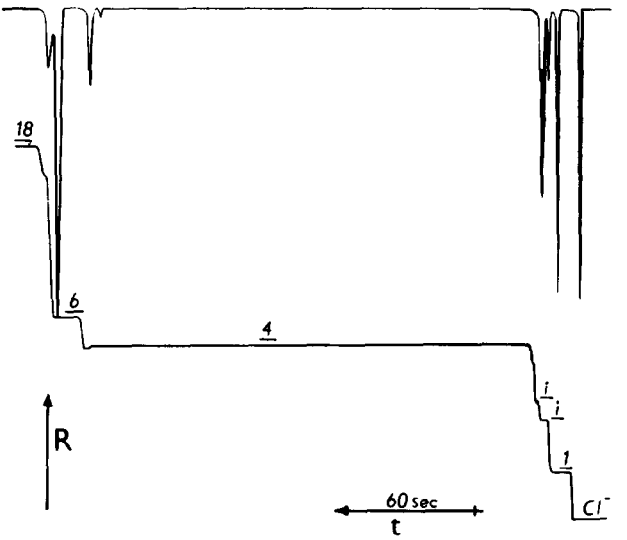

Fig. 4. Analysis of acids formed by the ozonication and air oxidation of *n*-heptene in *n*-butyric acid. Symbols as in Fig. 3. Driving current, 25 μA (17 min), 10 μA (6 min). The isotachopherogram was recorded at a driving current of 10 μA.

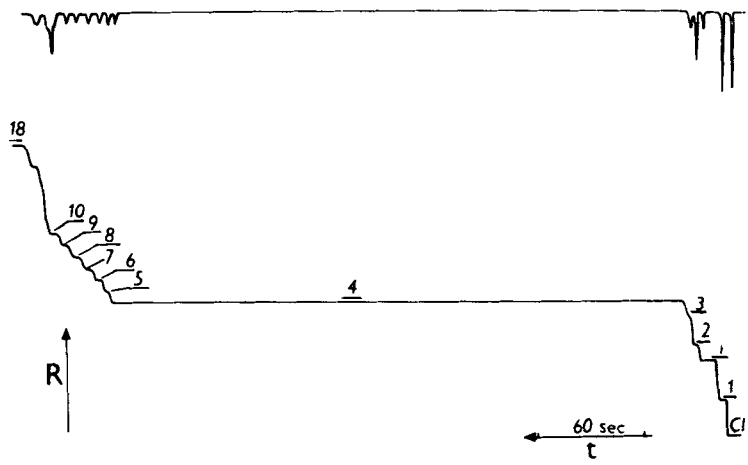

Fig. 5. Analysis of acids present in the reaction mixture after oxidation of C_{12} *n*-alkenes in *n*-butyric acid. Symbols as in Fig. 3. Driving current as in Fig. 4.

for the reaction mixtures. The results coincided well (2% relative standard deviation and better) for the available acids. A plot of the dependence of the relative step heights on molecular weight[22] was used for the prediction of the step heights of *n*-undecanoic and *n*-pentadecanoic acids. This dependence is in some respects equivalent to the relationship between mobility and the reciprocal of the square-root of the molecular weight[23]. The values of the step heights predicted in this way agreed within 1% with those obtained in the analysis of reaction mixtures where the presence of *n*-undecanoic and *n*-pentadecanoic acids was obvious.

Further identification of the acids by other methods was omitted as hardly any other anionic constituents could be present in the samples considering the nature of the substrates (*n*-alkanes or *n*-alkenes) and the relatively mild oxidation conditions used (ozonization and catalytic air oxidation). However, preparative ITP (*e.g.*, refs. 28–31) combined with GLC or mass spectrometry[31,32] offers promising possibilities for increasing the certainty of identification of acids present in samples of this nature.

The relative standard deviations of the determinations of the acids varied in the range 1–5%, using a ruler for the zone length measurements. The reproducibilities for zones shorter than *ca.* 2 sec were not evaluated, as without the use of an adequate zone length measuring technique (*e.g.*, as developed by Reijinga *et al.*[33] or Stover *et al.*[34]) unreasonable results could be achieved. When more precise analyses are required with current instrumentation a higher sample load is necessary, which leads to longer analysis times. Nevertheless, this problem can be solved by using a column-switching configuration in the separtation unit[35]. An investigation along these lines is in progress.

CONCLUSIONS

This work has shown some possibilities for the application of ITP in the analysis of saturated normal fatty acids when mixtures of water with methanol or methanol

containing a small amount of water are used as solvents. The former solvent mixture is suitable only for separations of C_1–C_{10} homologues, whereas methanol enables the complete resolution of at least C_1–C_{18} derivatives to be achieved.

The main problem in analyses using methanolic operational systems was the lack of suitable anticonvective additives. We solved it by coating the separation compartment with high-molecular-weight methylhydroxyethylcellulose.

The analysis of saturated normal fatty acids formed by the oxidation of *n*-alkanes or *n*-alkenes can be carried out without any labour- and time-consuming sample clean-up and/or derivatization. Consequently, the course of the reaction optimized for the formation of saturated normal fatty acids can be followed in *ca.* 20 min, which canot easily be achieved by other analytical methods.

With respect to the high selectivity of ITP towards ionic constituents, its use for the determination of fatty acids also in other non-ionic or simple ionic matrices (*e.g.*, mineral oils) seems very promising. Obviously, the separation conditions found in this work may not be directly applicable to analyses of complex ionic mixtures.

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