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Note

The qualitative separation of fatty acids by isotachophoresis

Fatty acids can be determined in various ways, but many methods require complicated or tedious pre-treatments. In general, electrophoretic methods do not require pre-treatments. One of the electrophoretic methods is isotachophoresis, an analytical method for the qualitative and quantitative analysis of ionic species[1–4].

The separation of anionic species has been discussed earlier[2], but many of the fatty acids have about the same effective mobility and some of them are not sufficiently soluble in water. Because methanol[5] can be used as a solvent in isotachophoresis and because it is a better solvent for fatty acids, the separation of fatty acids has been studied with methanol as a solvent.

Apparatus and chemicals

An apparatus similar to that described earlier[5] was used. Most chemicals were obtained from Merck; not all of them were of p.a. quality, but no adverse effects were obtained when small amounts of the ionic species were used.

Before use, the methanol (technical grade, 96 % by weight) was prepared by running it through a column filled with a mixed-bed ion exchanger so as to remove ionic impurities.

TABLE I

RELATIVE STEP-HEIGHTS (mm) FOR THE FATTY ACIDS IN SYSTEMS A, B AND C.

The step-heights are expressed relative to the step-heights of the leading electrolyte, which are 138, 87 and 86 mm, respectively, for systems A, B and C.

Fatty acid	*System A*	*System B*	*System C*
	Leading electrolyte		
	0.02 N Tris, 0.01 N HCl	*0.0085 N Tris, 0.01 N HCl*	*0.01 N Tris, 0.018 N HCl*
Formic acid	37	21	18
Acetic acid	88	64	78
Butyric acid	128	91	110
Isovaleric acid	137	—	122
Caproic acid	148	104	132
Caprylic acid	168	114	144
Pelargonic acid	180	124	148
Capric acid	190	126	156
Lauric acid	204	136	172
Myristic acid	220	146	184
Palmitic acid	240	156	204
Stearic acid	252	166	222
Terminator electrolyte			
Litocholic acid	—	216	256
Cacodylic acid	400	—	—

Experimental

The effective mobility depends on the extent of dissociation, and the choice of the pH of the leading electrolyte therefore plays an important role in the determination of the effective mobility. Maximum differences in effective mobility are necessary if good separations are to be achieved. From electrometric pK measurements on some fatty acids and some possible buffer substances, we chose Tris (tris-

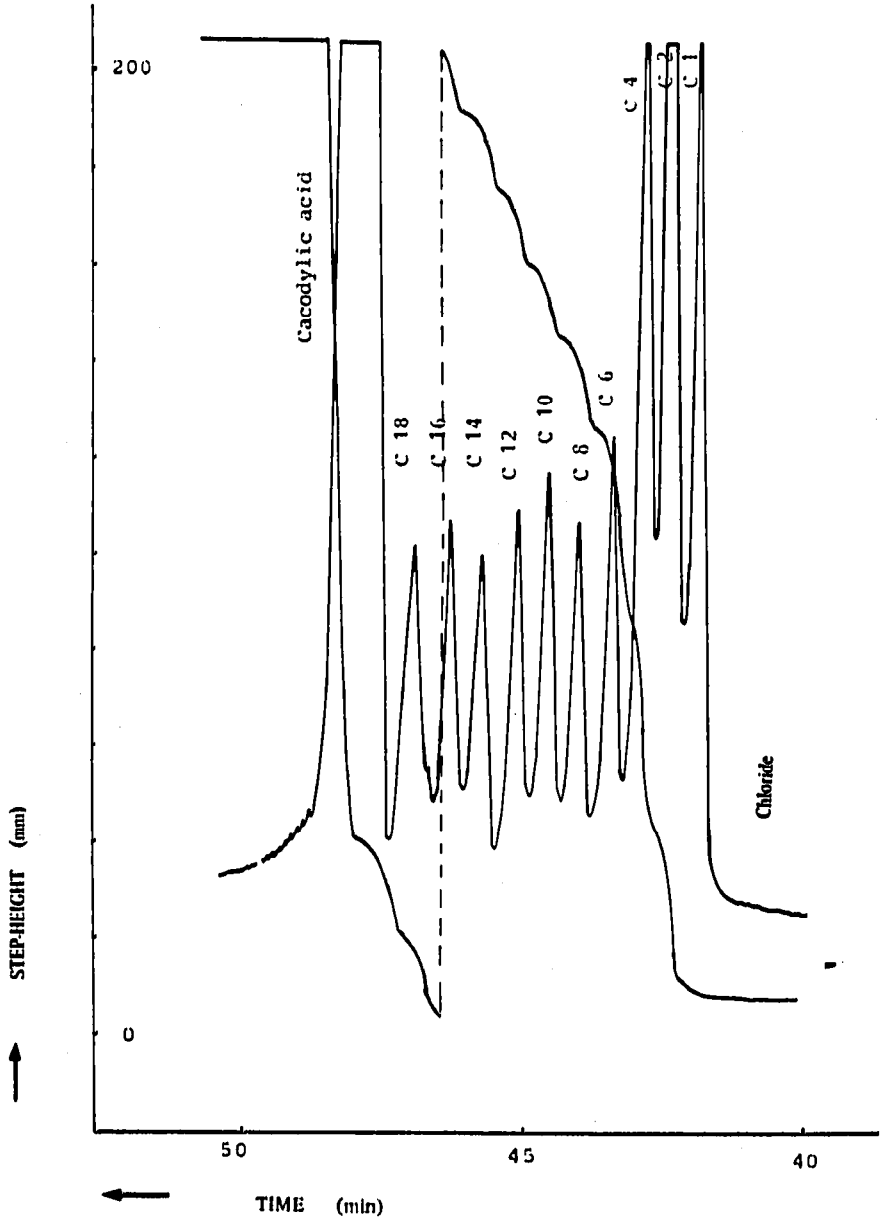

Fig. 1. Separation of a mixture of saturated fatty acids. The terminator solution was cacodylic acid.

hydroxymethylaminomethane) as a buffering counter-ion for the separation of the fatty acids.

Experiments were carried out on three systems and the step-heights measured for some fatty acids are listed in Table I. The electric current was maintained at 70 μA in all the experiments.

The differences between the step-heights in system A are greater than in systems B and C because the effective mobility of the buffer ionic species decreases at higher pH (less HCl in the electrolyte solution). In all systems, however, complete separations could easily be achieved.

In Fig. 1 the separation of some saturated fatty acids is shown for system A. Cacodylic acid was used as a terminator. The cacodylic acid contained some impurities, but after using it as a terminator in a few experiments most of the impurities migrated out of the solution and the isotachopherograms did not show any impurity.

Discussion

By isotachophoresis, the saturated fatty acids can easily be separated with methanol as a solvent and no pre-treatments are required. By this method the minimum detectable amount of the sample is about 10^{-8} g and the reproducibility of the analyses is about 2 % (ref. 3).

Unsaturated fatty acids also can be separated. In general, their effective mobilities do not differ sufficiently from the saturated fatty acids to enable the components of a complex mixture to be analysed simultaneously. A possible method for the analysis of unsaturated fatty acids may involve chemical conversion. The oxidation of the double bond, for example, generally gives a mixture of mono- and dicarboxylic acids, which can be analysed by isotachophoresis. For pure unsaturated fatty acids, this method can be used for the determination of the positions of double bonds, and this is being studied at present[6].

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