Quantitative Separations of Higher Fatty Acid Methyl Esters by Adsorption Chromatography on Silica Impregnated with Silver Nitrate

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Abstract

A chromatographic adsorbent has been developed for the separation of geometric isomers of fatty acid methyl esters. The adsorbent consists of silicic acid impregnated with silver nitrate.

Quantitative separations of saturated, *cis-*, and *trans*-monoenoic and polyenoic methyl esters in 30 to 100 mg quantities are reported.

Introduction

SINCE THE INVENTION of gas chromatography the quantitative analysis of a mixture of non-isomeric fatty acids has become a routine operation. More recently separations of *cis-* and *trans*-isomers have also been successfully demonstrated on capillary columns (11); however, for large scale separations in mg ranges other techniques merit further development. Separation of isomers has been achieved by fractional crystallization or by treating the isomers with mercuric acetate (9,10). Various metal ions are known to form complexes with unsaturated compounds. As early as 1938, Winstein et al. (14) reported that the reaction between Ag^+ and alkene is reversible and that the equilibrium is reached very rapidly. Gardner et al. (6) found a linear relationship between the log of the equilibrium constant and the heat of hydrogenation of the corresponding alkene, with the result that the stability of the Ag^+-alkene complex increases with the free energy content of the alkene. Ag^+-complexes with *cis*-isomers therefore are more stable than those with *trans*-isomers.

Nichols (12) first predicted the possibility of quantitative separation of methyl oleate and methyl elaidate by countercurrent distribution, using silver nitrate in the solvent mixtures. This was later confirmed by Dutton et al. (5) and De Vries (3). Separations of alkenes and menthenes have been reported using gas-liquid partition chromatography on packed columns of supported solutions of silver nitrate in glycols (7.2). Recently, Duffield et al. (4) described the gas-solid chromatography of lower alkenes using solid silver nitrate on several supports. Silver nitrate in glycol has also been applied to the liquid-liquid partition chromatographic separation of *cis-* and *trans*-5-cyclodecenols (8).

In our laboratories we have developed a novel adsorbent consisting of silica impregnated with silver nitrate for the separation of saturated, monoenoic (*cis-* and *trans-*) and polyenoic methyl esters of fatty acids. A preliminary report on this subject was published earlier (3). The selective properties of the adsorbent and its applications are reported in this paper.

Experimental

Preparation of the adsorbent. One hundred grams of silicic acid (Mallinckrodt, 100 mesh, analytical grade) are suspended in 200 ml aq solution of silver nitrate 50%. The mixture is heated at 100C for 30 min. After cooling it is filtered through a Buchner funnel and the adsorbent dried at 120C for 16 hr. Before use, the adsorbent is ground in a ball mill.

Due to poor reproducibility of the filtration process the adsorbent contained varying amounts of silver nitrate, viz. 0.3–0.4 g per gram of adsorbent. Accordingly, adsorbents obtained from different batches showed slightly different adsorptive properties.

Preparation of the column. Two water-jacketed columns (internal diameters 8 and 14 mm) were used. Ten grams of the adsorbent was mixed with five grams of a filter aid (Celite 535, ex Johns-Manville Corp.) and slurried with 50 ml light petroleum (b.p. 40–60C). The slurry was boiled for 5 min, cooled, and transferred to the column. In the larger column 10 g of adsorbent were used and in the smaller only 2 g. After preparation the columns were protected from light and kept at 15C. During chromatographic runs 3 or 5 ml fractions were collected; after evaporation of the solvent at 60C in a stream of nitrogen their methyl ester content was

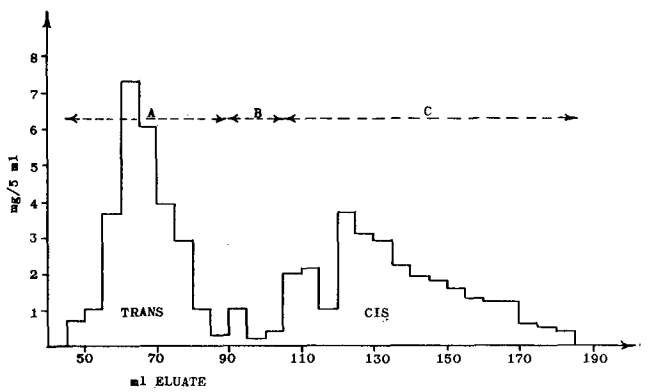

FIG. 1. Chromatography of a mixture of methyl elaidate (25.3 mg) and methyl oleate (25.6 mg). Recovery: A, 26.8 mg (97% *trans*); B, 1.6 mg; C, 27.5 mg (0% *trans*).

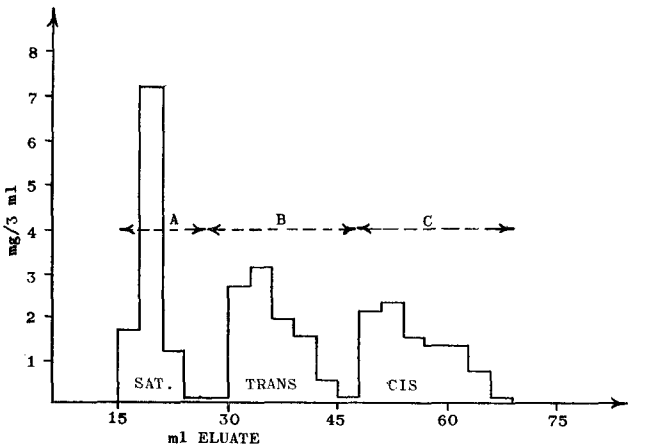

FIG. 2. Chromatography of a mixture of methyl stearate, elaidate and oleate (10 mg each). Recovery: A, 10.2 mg; B, 9.9 mg (96% *trans*); C, 9.3 mg (0% *trans*).

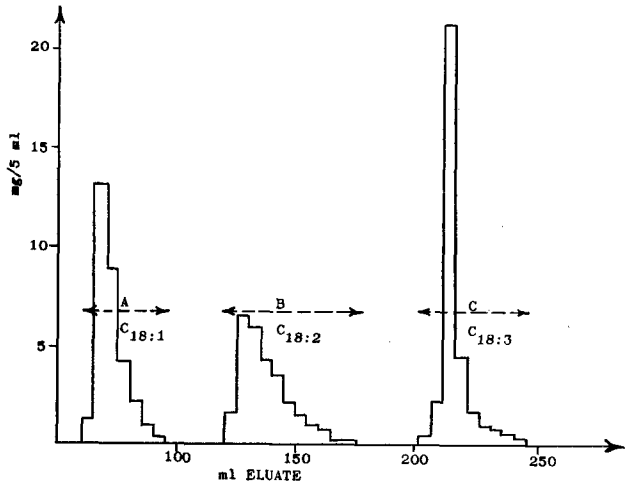

FIG. 3. Chromatography of a mixture of methyl esters of oleic, linoleic, and linolenic acids.

determined. Fractions belonging to the same chromatographic peak were combined and coded A, B, etc. and analyzed by gas chromatography or infrared spectroscopy.

Materials

The methyl esters of stearic, oleic, elaidic, oleic, linoleic, and linolenic acids were employed in the investigation. These esters had 98–100% purity, and were prepared in this laboratory by conventional methods.

A 10 ml sample of methyl oleate was isomerized at 180C with 1 g of nickel on guhr catalyst to which 1.8% of flowers of sulphur (1,13) had been added. The methyl ester analysis of the mixture gave the following result: stearate, 2.5%; oleate (with isomers) 93.0%; linoleate (with isomers) 2.0%; conjugated linoleate 1.0%; linolenate trace; and unidentified 1.0%. Infrared analysis indicated 66.5% *trans* double bonds. By means of osmium tetroxide oxidation the double bonds were located at 4 and 15 and intermediate positions in the C_{18} chain.

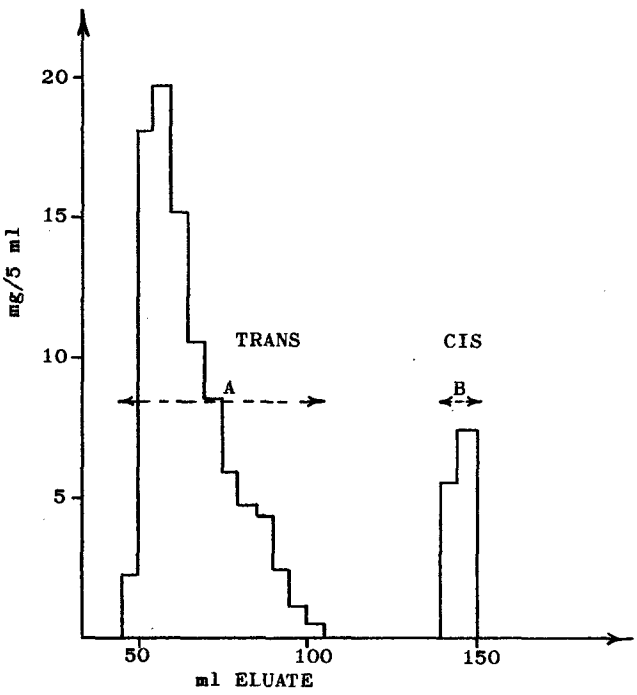

FIG. 4. Chromatography of a mixture of methyl elaidate (102 mg) and methyl oleate (12.9 mg). Recovery: A, 93.5 mg (95% *trans*); B, 12.8 mg (0% *trans*).

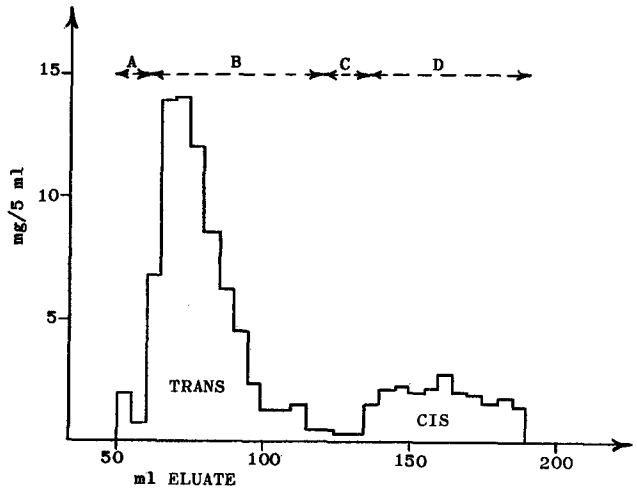

FIG. 5. Chromatography of isomerized methyl oleate.

The gas-chromatographic analysis was carried out on a Pye apparatus equipped with argon ionization detector. The column (120 cm) was packed with polyethylene glycol adipate on Celite, 20% w/w. The determination of *trans* double bonds was carried out on a Unicam SP-200 spectrophotometer using methyl elaidate as standard.

Results and Discussion

The results obtained with mixtures of methyl esters are shown in Figures 1 to 5. The experimental details are summarized in Table I. The recoveries range from 93 to 108%. The analysis of fractions obtained with a mixture of oleate, linoleate, and linolenate (Fig. 3) is shown in Table II. A small quantity of unknown material (6.4% in fraction C) was found, presumably originating from autoxidation of linoleate and linolenate. The results obtained in other experiments gave no evidence of oxidation of unsaturated compounds by silver ions during the chromatographic procedure.

Data obtained with isomerized methyl oleate (Fig. 5) are shown in Table III. Despite a satisfactory separation of *cis*- and *trans*-isomers the author believes that the peaks were less pronounced than those obtained with a mixture of *cis*-9 and *trans*-9 isomers. This is presumably due to the presence of other positional isomers in the mixture, the silver complexes of which are likely to have slightly different stabilities. In a forthcoming paper separations of closely related triglycerides will be reported, using this type of adsorbent.

TABLE I

Experimental Conditions During Chromatography of Methyl Esters

Experiment and figure number	Column [a]	Ester mixture	Solvent sequence [c]
1	a [a]	Elaidate 25.3 mg and oleate 25.6 mg	Benzene in L.P.[b] (28%) (28:72)
2	b [a]	Stearate, elaidate, and oleate 10 mg each	Benzene in L.P. 10 ml 0%; 20 ml each of 10%, 15%, 20%, and 30%.
3	a	Oleate, linoleate, and linolenate 30 mg each	Ethyl ether in L.P. 30 ml 2%; 60 ml 3%; 60 ml 5%; 30 ml 6%; and 60 ml 100%
4	a	Elaidate 102 mg, and oleate 12.9 mg	115 ml benzene in L.P. 1:3; 50 ml benzene
5	a	Isomerized oleate 105 mg	Benzene in L.P. 100 ml 25%; 30 ml 28%; 30 ml 35%; 40 ml 100%

[a] Column a: 14 mm ID; adsorbent 10 g and fraction volume 5 ml. Column b: 8 mm ID; adsorbent 2 g and fraction volume 3 ml.
[b] L.P. = light petroleum 40–60C, optically pure. Benzene, analytical grade, ethyl ether, non-purified.
[c] Elution rate, 0.5 ml/min in all experiments.

TABLE II

Methyl Ester Composition (%) of Fractions Obtained in Experiment 3.

Methyl esters	Fraction A (31.2)[a]	B (27.7)	C (32.1)
Oleate	99.7	0.7	0.4
Linoleate	0.0	98.5	1.0
Linolenate	0.0	0.0	92.0
Unknown	0.3	0.8	6.4

[a] Figures in parentheses indicate the weight of the fraction in mg.

TABLE III

Analysis of Fractions Obtained in Experiment 5

Methyl ester % GLC	Fraction A[a] (2.6)[b]	B (72.7)	C (1.0)[c]	D (21.0)
Monoene	.....	100[c]	.....	93
Diene	.....	0	.....	6
Triene	.....	0	.....	1

[a] Fraction A was crystalline and is presumed to be methyl stearate.
[b] Figures in parentheses indicate the weight of fractions in mg.
[c] I.R. analysis indicated 90% *trans*-double bonds in fraction B, 35% in fraction C, and 0% in fraction D. Fraction C also contained a considerable amount of conjugated diene.

Conclusions

Column chromatography using silica impregnated with silver nitrate can be successfully applied for separations of i) *cis*- and *trans*-isomers of fatty acid methyl esters and ii) fatty acid esters according to degree of unsaturation.

In contrast to separations by means of Hg(II) acetate no chemical operations preceding and following the above-mentioned chromatographic procedure have to be carried out.

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Suspensions of High-Melting Triglycerides[1,2]

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Abstract

A method for preparing stable oil/water suspensions of cottonseed stearine, tristearin, tripalmitin, trimyristin, methyl stearate, and palmitic acid in concentrations up to 10% with minimum concentrations of stabilizing agents is described. Using 2.5% of polyethylene glycol 400 monostearate (based on weight of hard fat), 0.1% of Pluronic F 68, and 0.2–0.25% of Carbopol 934 (the concentrations of these two agents are based on the weight of the aqueous phase), suspensions of the hard fats were prepared by simple stirring, were stable for at least one month at room temperature, and could be sterilized. The size of the dispersed fat particles was 20–40 μ. Apparent viscosities of cottonseed stearine suspensions at 2, 5, and 10% concentrations were 3.59, 5.95, and 6.62 poises at 25C, respectively. Suspensions as described should have utility in those areas of investigation in which solid fatty materials in the form of stable dispersions are desirable.

Introduction

MANY INVESTIGATIONS have been conducted in the general field of specificity and rates of enzymatic hydrolysis of liquid fatty materials. Among the investigations which may be cited are those on the specificity of pancreatic lipase (9,10,11) and on the enzymatic hydrolysis of vegetable and animal fats (8,5,3). For such investigations the liquid fatty materials are dispersed in aqueous media and stabilized by suitable emulsifying agents, or by gels (6), and enzymatic activities are then determined at the usually-employed temperature of 37C, although 45C has been used in order to have a liquid system (7). Higher temperatures for obtaining liquid systems would be expected to destroy enzymatic activity.

Relatively few such enzymatic investigations have been applied to normally solid fatty materials because of the difficulty in maintaining stable dispersions of hard fats. Some portion of an emulsifier molecule may be soluble in a high-melting triglyceride above its melting point and an excellent dispersion may form, but upon cooling and solidification of the fat, solubility of the emulsifier apparently decreases and the emulsifier no longer is effective in maintaining stability of the system, and phase separation results. The few reported systems of dispersed hard fat contain relatively low concentrations of the fatty material; Weber and King emulsified the higher fatty acid monoglycerides with sodium cholate, with 4×10^{-6} moles of substrate in 50 cc (14); tristearin was emulsified by Balls and Matlock, who used a mixture of bile and glycerol and obtained a final concentration of 0.1% (1). Dissolving a high-melting fat in a carrier such as a liquid vegetable oil is of limited value because the solubility is less than 1% at room temperature (13), and the oil carrier would thus be in such large concentration as to interfere with the determination of hydrolysis of the dissolved fat.

The purpose of the present investigation was to develop a system in which high-melting fats could be dispersed and stabilized in concentrations up to 10% with a minimum amount of added stabilizing agents, and which systems could be sterilized if need be. Such systems should have utility in those areas of investi-

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