GAS–LIQUID CHROMATOGRAPHY OF FREE FATTY ACIDS ON GLASS BEAD COLUMNS*

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SUMMARY

Columns were prepared from soda-lime glass beads, pretreated with alkali metal halides (NaCl and KCl) which have low solubility in the stationary phase (FFAP). The columns have a high efficiency (about 2000 theoretical plates per metre) and give good gas chromatographic separations of complex mixtures of free fatty acids in a short time.

"Salting out" effects for fatty acids have been obtained with microbeads pretreated with NaCl or KCl, and LiI which is soluble in the stationary phase. Analyses of free fatty acids in real samples are shown.

INTRODUCTION

Glass microbeads have low surface areas ($2 \cdot 10^{-2}$ m^2/cm^3 for the 80–100 mesh size) compared to the traditional support Chromosorb G and W. These supports have surface areas of about $30 \cdot 10^{-2}$ m^2/cm^3, practically independent of the mesh size. Hence gas chromatographic (GC) columns packed with microbeads contain lower amounts (10–20 times) of stationary phase than columns packed with Chromosorb G and W, and consequently have capacity ratios, $k' = KV_1/V_{gas}$, similar to those of capillary columns (K is the distribution coefficient, V_1 the volume of the stationary phase and V_{gas} the volume of the column). In fact for capillary columns the ratio V_1/V_{gas} is normally about 10^{-3}, whilst for glass microbeads and Chromosorb G or W columns coated with the minimum amount of stationary phase [$5 \cdot 10^{-2}$% (w/w) for microbeads, packing density, $d = 1.6$ g/cm^3; 1% Chromosorb G, $d = 0.58$ g/cm^3; 2% Chromosorb W, $d = 0.24$ g/cm^3] this ratio is about $2 \cdot 10^{-3}$ and $15 \cdot 10^{-3}$ respectively. This means, in practice, that the retention time of a given solute on a glass microbead column is twice and on a Chromosorb G or W column is fifteen times that obtained on a capillary column of the same length, under the same conditions.

With the low values of V_1/V_{gas} it is possible to analyse, in short times, polar low volatile substances on polar packed columns, symmetrical peaks being obtained, and to analyse, at lower temperature, low volatile and thermally unstable substances.

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In previous papers[1,2] polar and non-polar columns (exhibiting about 1200 theoretical plates per metre) were prepared from glass microbeads pretreated with carbon by pyrolysis of methylene chloride or with sodium dodecylbenzenesulphonate to eliminate the "pudding effect". This type of columns was used for analysis of aromatic polycyclic hydrocarbons[3] and of drugs[4]. Furthermore, columns (exhibiting about 1500 theoretical plates per metre) were prepared from untreated glass microbeads coated with a nematic liquid crystal, N,N'-bis(p-methoxybenzylidene)-α,α'-bi-p-toluidine, and used for the separation of complex mixtures of aromatic polycyclic hydrocarbons[5].

Free fatty acids are difficult compounds to analyze by GC because their peaks show marked tailing. To avoid this difficulty, the acids are usually converted into the methyl esters. However, the esterification step makes the analytical procedure longer and in some cases it may alter the analytical results, giving non-quantitative recoveries of the esters of the lower acids[6]. For these reasons a direct analysis is desirable. Several procedures have been developed to analyze fatty acids in free form[7–14].

This paper reports the GC analysis of free fatty acids using very efficient packed columns of free fatty acid phase (FFAP) on glass microbeads pretreated with alkali metal halides (NaCl or KCl) insoluble in the stationary phase, which eliminate the "pudding effect", and on glass microbeads pretreated with mixtures of NaCl or KCl and LiI which is soluble in the stationary phase and yields a "salting-out" effect for fatty acids.

EXPERIMENTAL

The apparatus used was a Carlo Erba gas chromatograph Model GI (Carlo Erba, Milan, Italy) equipped with a flame ionization detector (FID); this apparatus allows injection directly into the column. Glass columns (1.9 m × 2 mm I.D.) were used.

Materials

The glass microbeads, obtained from Analabs (North Haven, CT, U.S.A.), were sieved and the 80–100 mesh was used. The stationary phase was FFAP (DANI, Monza, Italy), a reaction product from Carbowax 20M and 2-nitroterephthalic acid. FFAP was dissolved in methylene chloride (5 g/l).

The alkali metal halides (E. Merck, Darmstadt, G.F.R.) were dissolved in water (NaCl, KCl; 50 g/l) or methanol (LiI, 50 g/l). The free fatty acid standards (Eastman-Kodak, Rochester, NY, U.S.A.) had concentrations of 1 g/l in chloroform and 0.5 μl were injected. Formic acid (E. Merck) was 98% pure.

Preparation of glass microbead columns

Glass microbeads (10 g) (washed with water plus detergent, water alone, acetone, diethyl ether, dried in an oven at 120°C for 1 h and cooled, at room temperature, in a desiccator) were transferred into a 50-ml beaker and 5 ml of methanol were added. A volume of a solution of one or two halides, sufficient to give the required superficial layer, was then added and the stirred solvent evaporated with hot air. The support was then cooled in a desiccator for about 20 min, in order to avoid adsorption of water from the room. (Traces of water can be responsible for a non-homoge-

neous deposition of stationary phase on the surface of the glass microbeads.) Subsequently, using a similar procedure, the stationary phase FFAP was deposited. Before use, each column, was conditioned for 1 h, at the working temperature, with nitrogen plus formic acid vapour as carrier gas. The column containing LiI was conditioned and used with pure nitrogen (≈ 10 ppm O_2) as carrier gas.

RESULTS AND DISCUSSION

Free fatty acids are not eluted from columns packed with soda-lime glass beads, even when the latter are coated with polar phases. However, symmetrical peaks are obtained by dynamic deactivation of the column, by saturation of the carrier gas, at room temperature, with formic acid vapour.

Formic acid greatly reduces adsorption of free fatty acids on microbeads. Using a column of FFAP on glass microbeads, we have observed that the response factor of palmitic acid relative to a hydrocarbon (phenanthrene) is constant up to the detection limit of the detector employed (FID) (1 ng; sample injected, 0.5 μl of 2 ppm chloroform solution).

Choice of the stationary phase

The choice of stationary phase for the GC analysis of free fatty acids is critical. For several reasons, such as the low volatility of these compounds, solute–solute associations (formation of dimers), solutes–support interactions (chemisorption with tailing of the peaks) and the difficulty in separating saturated and unsaturated acids having the same number of carbon atoms ($C_{16:0}/C_{16:1}$, $C_{18:0}/C_{18:1}/C_{18:2}/C_{18:3}$, $C_{20:0}$), it is necessary to employ polar phases, which should be also thermally stable.

Satisfactory columns for the separation of C_{14}–C_{20} fatty acids in the free form were prepared with DEGS-PS (polydiethylene glycol succinate containing orthophosphoric acid) on 80–100 mesh Supelcoport, an acid-washed and dimethylchlorosilane-treated diatomaceous earth support (Supelco, Bellefonte, PA, U.S.A.)[13]. With these columns, surprisingly high values of the separation factors between saturated and unsaturated acids are obtained. On the other hand, the stationary phase is highly susceptible to moisture or oxygen in the carrier gas and is unstable over long periods above 200°C. Moreover, insufficient efficiency for the separation of complex real mixtures is obtained (about 1100 theoretical plates per metre).

We have selected FFAP as a polar phase, which when deposited on glass microbeads can be used up to 230°C without column deterioration. The FFAP was deposited on microbeads either untreated or pretreated with alkali metal halides. It has been observed that high humidity is critical for column efficiency. For this reason, it is inadvisable to prepare columns in rooms in which the relative humidity exceeds 50–60%. The effect of humidity is greater when the microbeads are pretreated with hygroscopic materials such as sodium dodecylbenzenesulphonate (NaDBS) used previously[2] and alkali metal halides, particularly lithium iodide.

In Table I are reported the values of the minimum height equivalent to a theoretical plate and the corresponding value of the carrier gas velocity obtained for some columns. All the columns had the same percentage of stationary phase, 0.05% (w/w), but they differed in the type and percentage of the pretreating material. The highest efficiency was obtained with microbeads pretreated with alkali metal halides

TABLE I

MINIMUM HEIGHT EQUIVALENT TO A THEORETICAL PLATE, h_{min}, AND CORRESPONDING LINEAR CARRIER GAS VELOCITY, $\bar{u}_{min}$, OBTAINED FOR PALMITIC ACID AT 200°C ON GLASS MICROBEAD (80-100 MESH) COLUMNS PRETREATED WITH DIFFERENT ALKALI METAL HALIDES AND COATED WITH 0.05% (w/w) FFAP

Column	% NaCl	% KCl	% LiI	h_{min} (mm)	$\bar{u}_{min}$ (cm/sec)
1	0.025	—	—	0.46	5.5
2	—	0.025	—	0.44	6.0
3	—	0.050	—	0.48	4.7
4	—	—	0.025	1.35	8.0
5	—	0.025	0.025	0.52	8.9

which were of low solubility in the stationary phase, such as NaCl or KCl (columns 1–3: about 2000 theoretical plates per metre). A very low efficiency, on the other hand, was obtained with microbeads pretreated with alkali metal halides soluble in the stationary phase, such as lithium iodide (column 4). Finally, microbeads pretreated with both types of halides (column 5) had an efficiency comparable to that obtained with microbeads treated with NaCl or KCl alone.

Columns 1 and 2, in Table I, gave good separations, in a short time, of complex mixtures of free fatty acids. Fig. 1 shows the separation of a $C_3–C_{18}$ standard mixture, obtained in about 30 min, while Fig. 2 shows the acids present in the free form in an Italian "pecorino", extracted with chloroform.
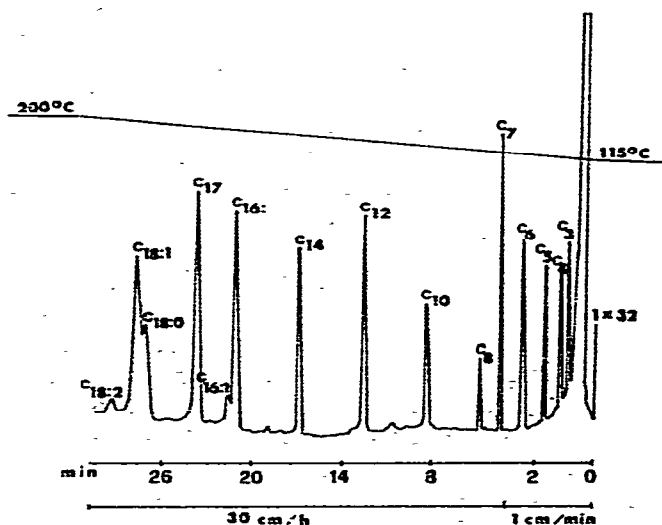

Fig. 1. Gas chromatogram of a $C_3–C_{18}$ standard mixture of free fatty acids. Glass column (190 × 0.2 cm I.D.) loaded with 0.05% (w/w) FFAP on glass microbeads (80-100 mesh) pretreated with 0.05% (w/w) KCl. Column temperature: programmed from 115°C to 200°C at 5°C/min. Injector temperature: 200°C. Carrier gas: nitrogen saturated with formic acid. Flow-rate: 5 ml/min. Detector: FID.
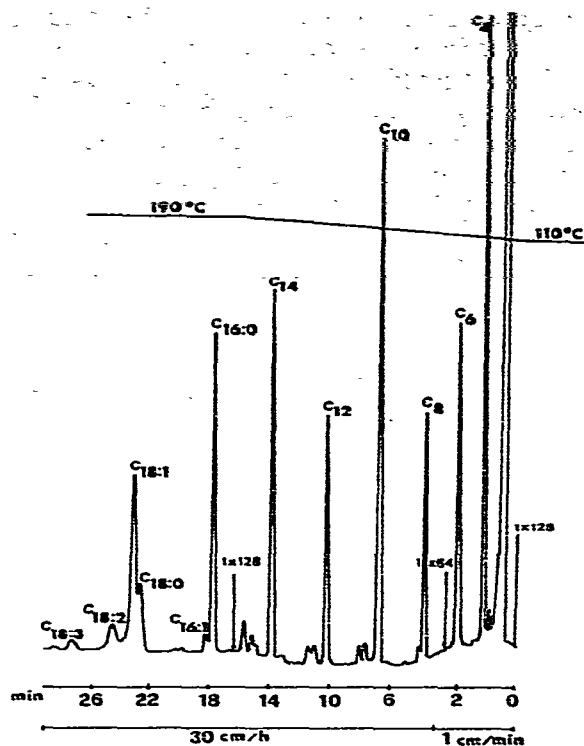
Fig. 2. Gas chromatogram of fatty acids present in free form and extracted with chloroform from an Italian cheese "pecorino". Column temperature: programmed from 110°C to 190°C at 5°C/min, then isothermal. Same column and conditions as in Fig. 1.

Salting-out of free fatty acids on glass beads pretreated with lithium iodide

The addition of alkali metal halides soluble in the stationary phase yield a "salting-out" (acceleration) or "salting-in (retardation) effect for some classes of substances[15-18] because it affects the solubility of these compounds in the stationary phase. This change of solubility is due to interactions which may take place in the stationary phase between molecules of solute and ions of the electrolyte.

Considerable salting-out has been obtained for free fatty acids on microbead columns pretreated with lithium iodide. This salt is soluble in FFAP at 200°C at a ratio of about 1:1.

Table II reports the capacity ratio, k', for palmitic acid at 190°C on microbead columns differently treated and coated with FFAP (0.05%, w/w) and on a conventional column (DEGS-PS on Supelcoport). It is interesting to observe the (about 50%) reduction of k' obtained with column 6 relative to columns 1–3.

The salting-out effect for free fatty acids is of interest, because it greatly reduces the analysis time of these compounds, especially those of higher molecular weight. In addition, different salting-out effects have been observed for fatty acids of different degrees of saturation. These differences are of great importance for pairs which are difficult to separate, such as $C_{16:0}/C_{16:1}$ and $C_{18:0}/C_{18:1}$, present in all oils and fats.

Fig. 3 illustrates the plot of log α (separation factor) *versus* the inverse of the

absolute temperature for the stearic/oleic acid pair, obtained on columns of 0.05% (w/w) FFAP on glass microbeads without pretreatment (curve 1), pretreated with NaCl or KCl (curves 2 and 3 respectively) and pretreated with KCl and different percentages of LiI (curves 4–6). At percentages of LiI greater than 0.04% (w/w), column packing is difficult because of the high hygroscopicity of the salt. Fig. 3 shows the considerable differences in the separation factor for the $C_{18.0}/C_{18.1}$ pair on microbeads untreated and pretreated with NaCl or KCl and pretreated with KCl and LiI.

In Fig. 4 is shown the gas chromatogram of a C_{10}–C_{18} standard mixture of free fatty acids obtained at 210°C on a column of FFAP on microbeads pretreated with

TABLE II

CAPACITY RATIO, k', AT 190°C FOR PALMITIC ACID OBTAINED ON GLASS MICROBEAD (80–100 MESH) COLUMNS PRETREATED WITH ALKALI HALIDES AND COATED WITH 0.05% (w/w) FFAP AND ON SUPELCOPORT (80–100 MESH) COLUMN COATED WITH 5% (w/w) DEGS-PS

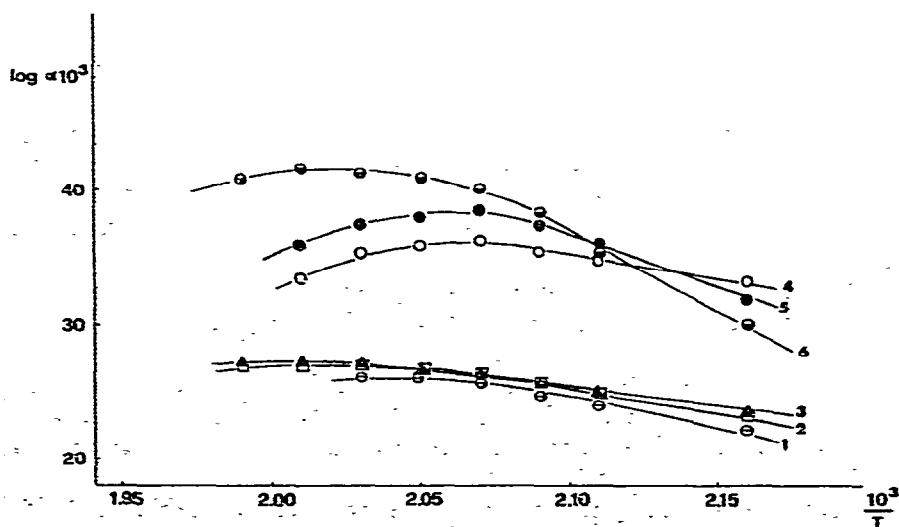| Column	Support	% NaCl	% KCl	% LiI	k'
1	Glass microbeads	—	—	—	20.2
2	Glass microbeads	0.025	—	—	18.5
3	Glass microbeads	—	0.025	—	17.8
4	Glass microbeads	—	0.025	0.012	15.8
5	Glass microbeads	—	0.025	0.025	14.0
6	Glass microbeads	—	0.025	0.040	9.5
7	Supelcoport	—	—	—	40.0

Fig. 3. Plots of log α (separation factor) of stearic/oleic acid pair versus $1/T$ (°K^{-1}) obtained on a column of 0.05% (w/w) FFAP on glass microbeads untreated (curve 1) and pretreated with 0.025% (w/w) NaCl (curve 2), with 0.025% (w/w) KCl (curve 3), with 0.025% (w/w) KCl and 0.012% (w/w) LiI (curve 4), with 0.025% (w/w) KCl and 0.025% (w/w) LiI (curve 5) and with 0.025% (w/w) KCl and 0.04% (w/w) LiI (curve 6).
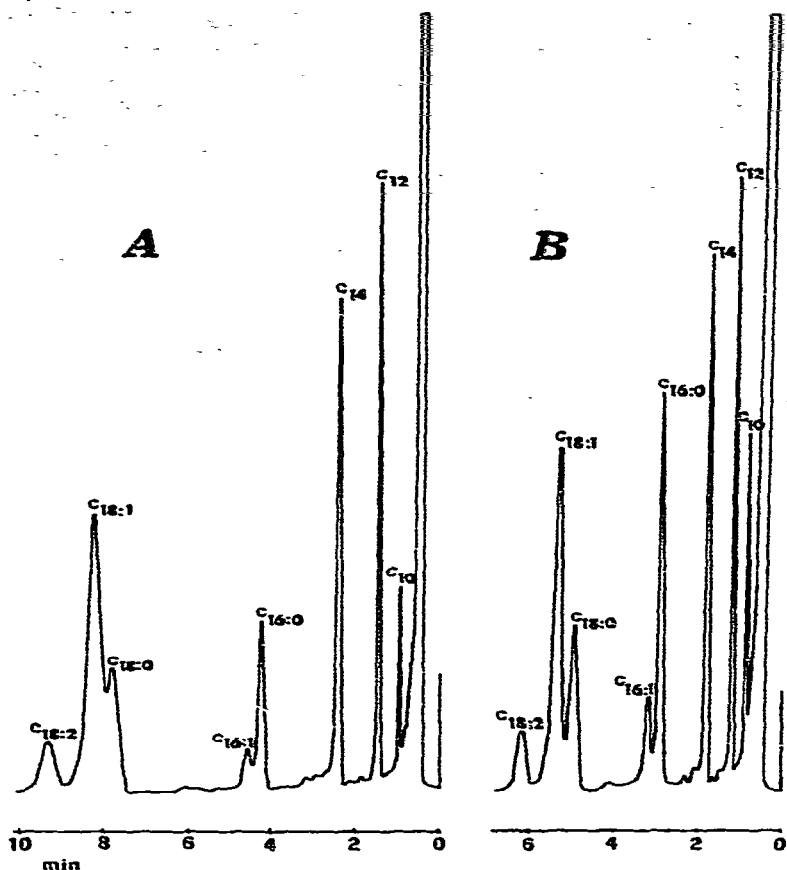
Fig. 4. Gas chromatogram of a C_{10}–C_{18} standard mixture of fatty acids. Glass column loaded with 0.05% (w/w) FFAP on glass microbeads (80–100 mesh) pretreated with 0.025% (w/w) KCl (A) and 0.025% (w/w) KCl and 0.025% (w/w) LiI (B). Column temperature: 210°C. Injector temperature: 220°C. Other conditions as in Fig. 1.

KCl (A) and pretreated with KCl and LiI (B). Chromatogram B shows a reduction of the analysis time and an increase in the separation of the $C_{16:0}/C_{16:1}$ and $C_{18:0}/C_{18:1}$ pairs.

Finally Fig. 5 shows a chromatogram of the acids of a peanut oil after saponification, obtained on a column of microbeads pretreated with 0.025% (w/w) KCl and 0.025% (w/w) LiI.
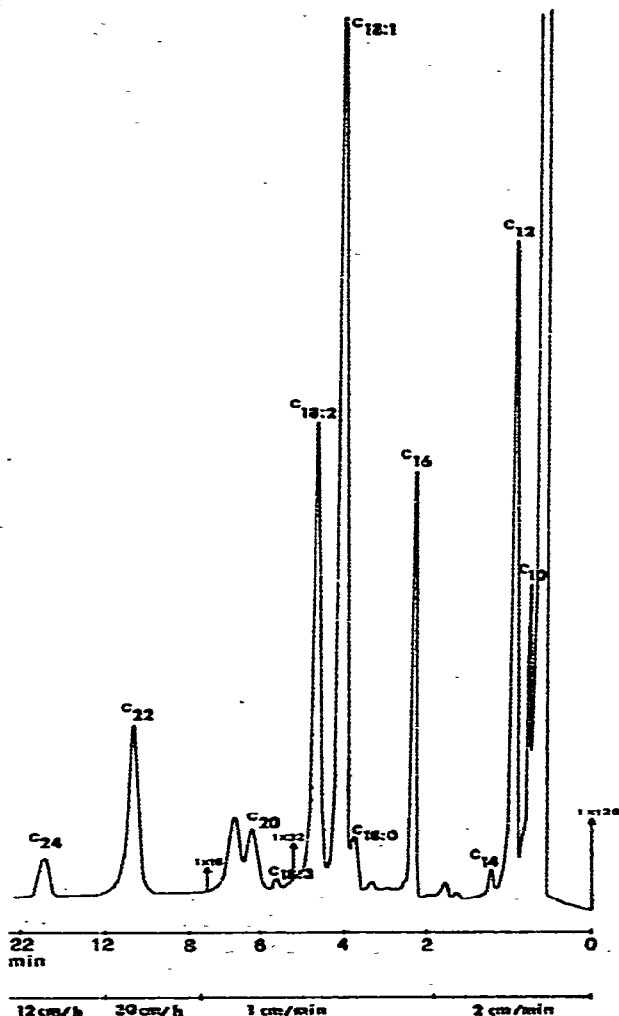
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Fig. 5. Gas chromatogram of fatty acids of a saponified peanut oil. Glass column loaded with 0.05% (w/w) FFAP on glass microbeads (80–100 mesh) pretreated with 0.025% (w/w) KCl and 0.025% (w/w) LiI. Column temperature: 215°C. Injector temperature: 230°C. Other conditions as in Fig. 1.

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