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EVALUATION OF AN IMMOBILIZED CYANOSILICONE, 60-CN, AS A STATIONARY PHASE FOR CAPILLARY GAS CHROMATOGRAPHY

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SUMMARY

The influence of several factors, such as the method used for the synthesis of the stationary phase, the type of fused-silica capillary tubing, surface deactivation, stationary phase immobilization, column rinsing and ageing, on the properties of capillary columns coated with silicones having 60% cyano substitution has been investigated. The column properties studied were efficiency, deactivation, acid base status, polarity and thermal stability. The column properties were found to be generally improved by stationary phase immobilization. However, an increase in the HETP of *n*-alkanes is a disadvantage that may be encountered. Well deactivated, relatively efficient columns were prepared with good reproducibility. The properties were largely retained after column ageing at 220°C.

INTRODUCTION

Columns that give selective separations have been of great importance in packed-column gas chromatography (GC), where they have made difficult separations possible despite very poor plate numbers. Because non-polar capillary columns readily give high separation efficiencies, it has been considered that selectivity should be of minor importance in connection with capillary columns. This conclusion has been further supported by the fact that, owing to technical difficulties with column preparation, the performance of such columns has been only modest. However, the performance characteristics of columns that separate selectively has been much improved during recent years, and the merits of selectivity in capillary GC have thereby become more generally recognized. Selective columns are not only indispensable for critical separations, but they may also give more rapid separations, provided, of course, that suitable selectivities are available.

Cyanosilicones are at present the most polar stationary phases that are suitable for capillary GC. Nowadays, the demands on column reproducibility and stability are stringent, and are difficult to achieve with the cyanosilicones. It is difficult to synthesize cyanosilicones in a reproducible manner; in addition, column preparation may be critical when a high reproducibility of column properties is required. Finally, their thermal stability is considerably lower than that of, *e.g.*, dimethylsilicones.

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In our laboratory, for several years we have been investigating different methods of cyanosilicone synthesis and of preparing efficient, deactivated, thermally stable and reproducible columns coated with these stationary phases[1]. Lee and co-workers have also contributed to the development of these new cyanosilicone phases[2].

In a recent paper[3], the first part of a rigorous evaluation of these columns was presented. In this paper, the evaluation is continued. The consequences of immobilization on the elution of *n*-alkanes and the influence of several factors on column polarity are emphasized.

EXPERIMENTAL

Reagents

Cyclosiloxanes for deactivation were prepared as described earlier[4]. A cyanosilicone gum stationary phase with 60% cyanopropyl substitution (60-CN) was prepared as described previously[1].

Column preparation

Fused-silica tubing of 0.32 mm I.D. (Chrompack, Middelburg, The Netherlands, and Quartz et Silice, Paris, France) and of 0.10 mm I.D. (SGE, Ringwood, Victoria, Australia) was used. Some columns were coated directly after flushing with dry nitrogen at room temperature for at least 3 h, whereas other columns were pretreated according to a procedure reported earlier[1,3], consisting of the following steps: (1) leaching with 20% (v/v) hydrochloric acid at 150°C for 15 h; (2) rinsing with two column volumes each of hydrochloric acid (pH 3) and methanol; (3) dehydration by heating at 260°C for 5 h while flushing with dry nitrogen; and (4) high-temperature silylation with bis(cyanopropyl)cyclosiloxane immediately after dehydration.

For the silylation, a plug of 10–20% of the column length [2% (w/w) of siloxane in dichloromethane] was pushed at a rate of 2 cm/s through the column, which then was connected to a buffer column. After flushing with dry nitrogen for at least 6 h, the column was sealed with a microtorch while being evacuated at both ends. The column was heated at 5°C/min to 395°C and kept at this temperature for 2 h. One column end was opened under dichloromethane, the column then being rinsed with about 4 ml of dichloromethane. Before coating, the column was flushed with dry nitrogen for several hours.

Column coating

The stationary phase was dissolved in dichloromethane (analytical-reagent grade) in appropriate concentrations. All columns were coated by the static method at room temperature. After coating, the columns were flushed with dry nitrogen overnight in order to remove residues of dichloromethane.

Cross-linking

Cross-linking was achieved by the use of azo-*tert.*-butane (ATB) (Ventron, Karlsruhe, F.R.G.). The coated columns were flushed at room temperature for 60 or 30 min with nitrogen, saturated with ATB. The columns were then sealed with a microtorch and cured at 220°C for 1 h[5] after being heated at a rate of 4°C/min. The characteristics of the prepared columns are given in Table I.

TABLE I

CHARACTERISTICS OF CAPILLARY COLUMNS COATED WITH 60-CN STATIONARY PHASE

Column			*Type of fused silica*	d_f *(μm)*	β	*Deactivation*	*ATB (min)*
No.	*Length (m)*	*I.D. (mm)*					
1	20	0.32	Chrompack	0.3	266	+	
2	20	0.32	Chrompack	0.3	266	+	60
3	20	0.32	Chrompack	0.3	266	+	60
4	20	0.32	Q. et S.*	0.3	266	+	30
5	20	0.32	Q. et S.	0.3	266	–	30
6	20	0.32	Q. et S.	0.3	266	–	30
7	18	0.32	Q. et S.	0.09	888	–	30
8	20	0.32	Q. et S.	0.09	888	+	30
9	11	0.10	SGE	0.09	266	+	60
10	19	0.32	Chrompack	0.64	125	+	30
11**	20	0.32	Q. et S.	0.3	266	–	30

* Quartz et Silice.
** 95-CN stationary phase.

Column evaluation

All columns were evaluated on the same instrument, a Hewlett-Packard (HP) 5790A gas chromatograph connected to an HP 3390A integrator. The integrator was adjusted to give retention times in minutes to three decimal places, and the time constant was set to the lowest value (peak width 0.01). The columns were evaluated by use of a polarity test mixture. This was split-injected at 100°C, with the injector temperature at 220°C and the flame ionization detector at 250°C. Hydrogen was used as the mobile phase at a rate of 50 cm/s. Column dead-times were determined by the iterative method of Guardino *et al.*[6] using the retention times of *n*-alkanes in the test mixture.

Each column was tested at least three times. The first test was performed after heating at a rate of 0.5°C/min to 220°C and then isothermally for 11 h. The second test was performed after immobilization with ATB and conditioning by the same method as that used prior to cross-linking. A third test was performed after rinsing with dichloromethane and conditioning as above. A few columns were conditioned at higher temperatures.

Test mixtures

The main test mixture consisted of the C_{16}–C_{20} *n*-alkanes, decanol, aniline, 2-methylnaphthalene, nicotine, 2,6-dimethylphenol (DMP), 2,6-dimethylaniline (DMA), methyl myristate and phenol. The concentrations were *ca.* 100 ng/μl, except for the *n*-alkanes, where the concentrations were 20 ng/μl. Generally, 1–2 μl of the polarity mixture was injected at 100°C with a splitting ratio of 1:100, 1–2 ng of the polar test solutes and 0.2–0.4 ng of the *n*-alkanes thus passing through the column. In case of difficulties in the determination of *n*-alkane retention times, a polarity mixture containing 100 ng/μl of the alkanes was used. For column 9, a splitting ratio of 1:250 was used because of the low sample capacity of this column. For the determination of equivalent chain lengths (ECL)[7], a fatty acid methyl ester (FAME) sam-

ple, rapeseed oil (Cat. No. 4-7019, Supelco, Bellefonte, PA, U.S.A.), was used. Injection of 1 μl at a splitting ratio of 1:20 was made, the column temperature being maintained at 170°C. Further, a complex *cis–trans* mixture (Cat. No. 4-5170, Supelco) was used for testing purpose.

RESULTS AND DISCUSSION

Some aspects of stationary phase synthesis

The columns described in this paper were coated with the same 60-CN stationary phase as the ones used in previous work[3]. In the synthesis of this phase, the equilibration step was kept very short, as pointed out by Ogden and McNair[8]. Equilibration is a process in which polymer bonds are continuously broken and reformed. When the equilibration of a polymer containing differently substituted silicon atoms is allowed to proceed for a longer period of time, a silicone having a relatively random distribution of different substituent groups may result. However, the strength of the oxygen–silicon bond in the backbone depends on the nature of the substituent groups, and a truly random distribution may therefore occur in only a few instances. A prerequisite for good results is, of course, that the relatively volatile cyclic compounds that may be formed on equilibration are effectively hindered from leaving the reaction vessel.

In the synthesis of the 60-CN stationary phase, vinyl groups were included by the addition of methyl(vinyl)cyclopentasiloxane, and the object of a short equilibration time in this instance was to hold the methyl(vinyl) groups together as much as possible, thereby facilitating subsequent cross-linking. After the synthesis, it is generally advantageous to remove the cyclic compounds, because they may otherwise obscure the immobilization. Further, remaining cyclic compounds may cause problems with column bleeding. It was pointed out by Lee *et al.*[9] that removal of these cyclic compounds may lead to alterations in polymer composition, as the distribution of substituents in the cyclic compounds may not be the same as for the entire polymer. In a more recent paper[10], the same group reported that a fraction of the low-boiling dimethyl monomer may be lost in the hydrolysis or polymerization step, leading to an unexpectedly high content of substituents that correspond to higher boiling monomers. However, analysis by ^{1}H NMR spectroscopy indicated that the cyano content of the 60-CN polymer used in this work was 62%. According to the NMR spectrum, the polymer did not contain any carboxamide substitution.

Elution of n-alkanes

Elution of *n*-alkanes from columns coated with this polymer is satisfactory (Figs. 1a and 2a). However, the peak shapes of the *n*-alkanes are not acceptable after conditioning at 250°C (Fig. 1c). Immobilization with ATB leads to severe tailing of hydrocarbons (Fig. 2b). Column rinses with a solvent after immobilization lead to a slight improvement (Fig. 2c), but after further conditioning the hydrocarbon adsorption again becomes severe (Fig. 2d). The *n*-alkane elution problem also leads to an increased HETP (*cf.*, HETP values for C_{17}, column 2, Table II). It seems that the solubility of *n*-alkanes is drastically reduced on ATB-treated columns. An improvement could be achieved by use of a lower cross-linking density, *e.g.*, by using a prepolymer having a lower vinyl content, but that would lead to a relatively high

percentage washout after immobilization. Of the solutes tested, it was only the *n*-alkanes that gave severe problems.

It is now recognized that Gibbs surface adsorption of *n*-alkanes may occur on strongly polar stationary phases[11]. Such an adsorption may lead to concentration-dependent retention times in the range of concentrations that are generally being used (Table III). The retention times will thereby become ill-defined, to say the least. In the Kováts retention index system, *n*-alkanes are used as standards, and on adsorption of these the retention indices may become uncertain. In this work, column polarities have been indicated by Kováts retention indices (Table IV), but as a check on the significance of the results we have also determined polarities in the ECL system

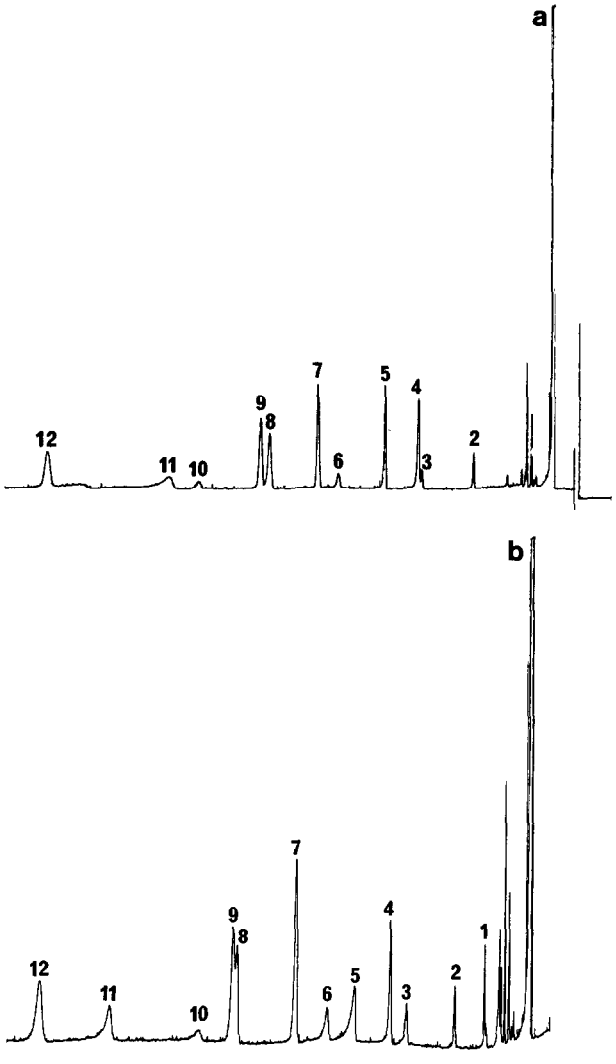

Fig. 1.

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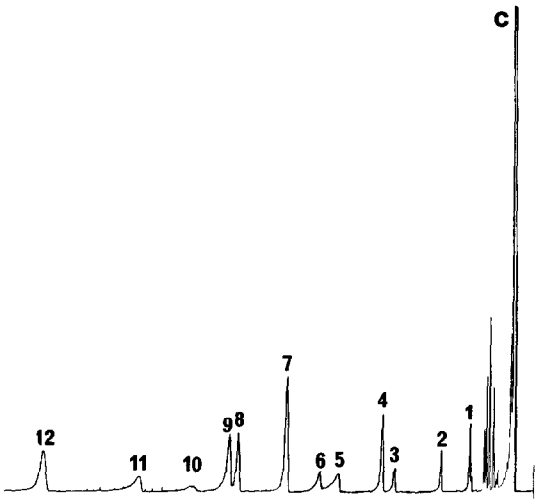

Fig. 1. Gas chromatograms (flame ionization detection) of a polarity mixture on a fused-silica capillary column (20 m × 0.32 mm I.D.) coated with non-immobilized 60-CN stationary phase (column 1, Table I). Conditions, isothermal at 100°C. (a) After conditioning at 220°C for 11 h; (b) after conditioning at 220°C for 44 h; (c) after further conditioning at 250°C for 12 h. Peaks: 1 = hexadecane; 2 = heptadecane; 3 = octadecane; 4 = decanol; 5 = aniline; 6 = nonadecane; 7 = 2-methylnaphthalene; 8 = 2,6-dimethylphenol; 9 = 2,6-dimethylaniline; 10 = eicosane; 11 = phenol; 12 = methyl myristate.

(Table IV). The retention times of the FAME tested here were not affected by the amount of sample in the range 0.8–6.5 ng.

Different types of capillary tubing

It is well known that the properties of fused silica may vary from manufacturer to manufacturer and from batch to batch[12]. Fused silica from three different sources (Chrompack, Quartz et Silice and SGE) was utilized in this work. The SGE silica was used for only one column, which had a smaller I.D. (column 9, Table I). For a film thickness of 0.3 μm, columns made from Quartz et Silice silica were more polar than columns made from Chrompack silica (*cf.*, columns 2 and 4, Table IV). For practical reasons, the use of different types of fused-silica capillary tubing could not be avoided in this work.

Reproducibility of column preparation

Columns 5 and 6 were prepared under identical conditions, although not from the same coating solution. This may explain the slight difference in k' values (Table II). The column polarities were similar at first, but ATB treatment and the subsequent conditioning led to some small differences (Table IV). Chromatograms from the test solutions were also similar (Fig. 3). Moreover, the ratios of the peak areas of DMP and DMA were similar (cf., columns 2 and 4 and columns 5 and 6, Table V).

The polarity of column 1 (Table IV) was lower than expected. The column was prepared exactly as the other columns, except that the coating solution was accidentally pumped out during the static coating, and the column then had to be rinsed and coated again.

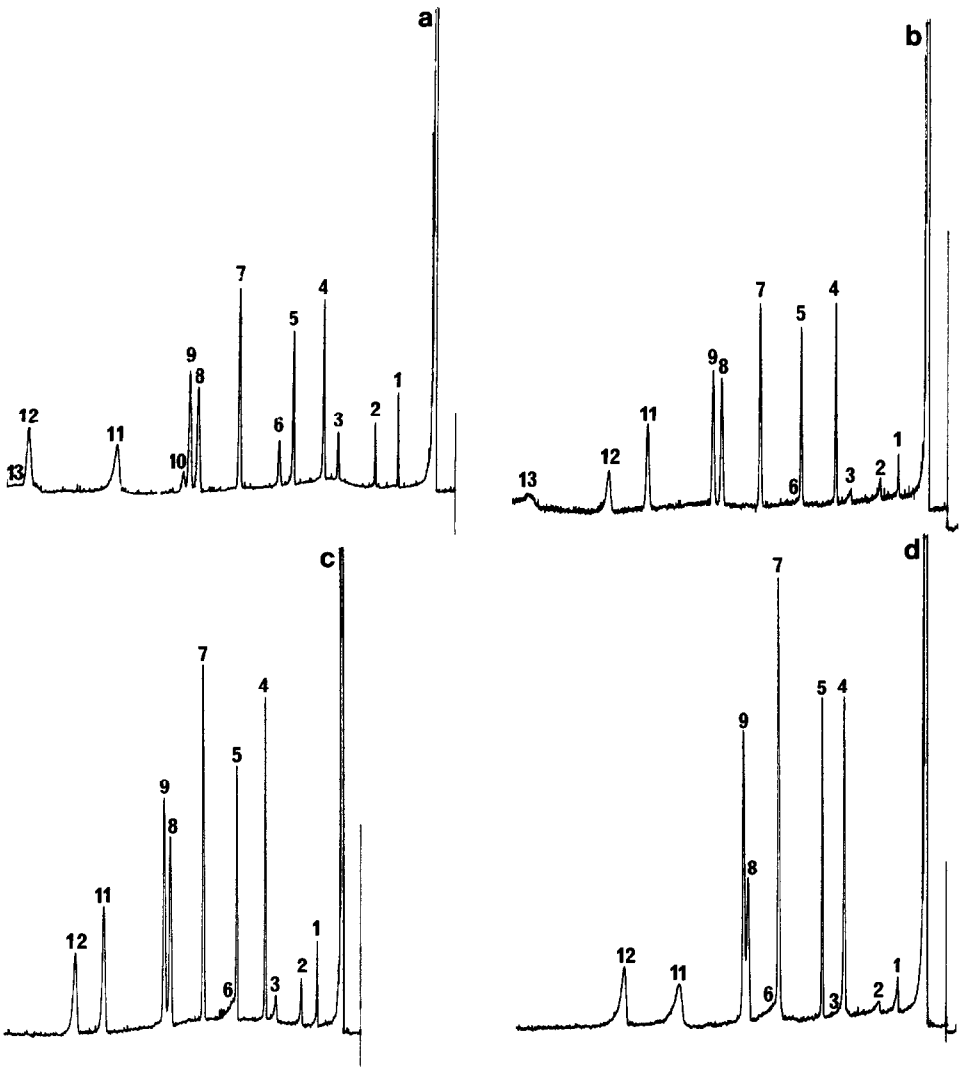

Fig. 2. Gas chromatograms (flame ionization detection) of a polarity mixture on a fused-silica capillary column (20 m × 0.32 mm I.D.) coated with 60-CN stationary phase (column 2, Table I). Conditions, isothermal at 100°C. (a) After conditioning at 220°C for 11 h; (b) after ATB treatment and further conditioning at 220°C for 11 h; (c) after extraction and further conditioning at 220°C for 11 h; (d) after a further conditioning at 240°C for 66 h. Peaks as in Fig. 1; 13 = nicotine.

Column deactivation and stationary phase film thickness

The deactivation method used here involves heat treatment of the inner surface of the capillary with 20% hydrochloric acid, and it is expected that this would lead to columns of higher acidity.

The DMP/DMA peak-area ratios for columns coated with a 0.3-μm stationary phase film thickness were then found to be similar for the deactivated column 2 and

TABLE II

CHROMATOGRAPHIC DATA

*Column No.**	*Column treatment***	*k' (100°C)*			*HETP (100°C)*		
		2-Methylnaphthalene	*Decanol*	*n-Heptadecane*	*2-Methylnaphtalene*	*Decanol*	*n-Heptadecane*
1	Cond. 220°C, 11 h	11.7	6.7	4.0	0.37	0.71	0.71
	Cond. 220°C, 44 h	11.6	7.0	3.8	0.37	0.65	0.95
	Cond. 250°C, 12 h	11.6	6.7	3.4	0.60	0.85	0.86
2	Cond. 220°C, 11 h	10.0	5.7	3.1	0.34	0.44	0.58
	After ATB, cond. 220°C, 11 h	8.6	4.8	2.5	0.34	0.57	1.23
	Rinsed with CH_2Cl_2, cond. 220°C, 11 h	7.2	4.0	2.2	0.39	0.61	2.15
	Further cond. 240°C, 66 h	7.9	4.4	2.4	0.42	0.85	2.30
3	Cond. 220°C, 11 h	10.2	5.8	3.2	0.33	0.58	0.58
	After ATB, cond. 220°C, 11 h	9.1	5.1	2.7	0.41	0.81	0.84
	Rinsed with CH_2Cl_2, cond. 220°C, 11 h.	7.5	4.2	2.3	0.43	0.73	1.80
4	Cond. 220°C, 11 h	9.0	5.1	2.7	0.34	0.55	0.87
	After ATB, cond. 220°C, 11 h	8.0	4.4	2.2	0.42	0.80	2.33
	Rinsed with CH_2Cl_2, cond. 220°C, 11 h	7.1	4.0	2.1	0.42	0.82	5.42
5	Cond. 220°C, 11 h	8.4	4.7	2.4	0.39	0.73	0.67
	After ATB, cond. 220°C, 11 h	7.5	4.1	2.1	0.47	0.90	3.51
	Rinsed with CH_2Cl_2, cond. 220°C, 11 h	7.2	4.0	2.1	0.49	1.19	4.20
6	Cond. 220°C, 11 h	9.4	5.3	2.7	0.42	0.73	0.75
	After ATB, cond. 220°C, 11 h	8.6	4.8	2.4	0.46	0.79	2.38
	Rinsed with CH_2Cl_2, cond. 220°C, 11 h	7.9	4.4	2.2	0.46	0.84	2.59

7	Cond. 220°C, 11 h	2.9	1.7	1.2	0.36	0.69	0.40
	After ATB, cond. 220°C, 11 h	2.6	1.5	1.0	0.44	0.72	0.64
	Rinsed with CH_2Cl_2, cond. 220°C, 11 h	2.4	1.4	1.0	0.47	0.80	0.75
8	Cond. 220°C, 11 h	3.6	2.0	1.2	0.33	0.66	0.42
	After ATB, cond. 220°C, 11 h	3.2	1.8	1.1	0.39	0.60	0.64
	Rinsed with CH_2Cl_2, cond. 220°C, 11 h	3.0	1.7	1.0	0.48	1.72	1.19
9	Cond. 220°C, 11 h	10.7	6.1	3.8	0.16	0.62	0.91
	After ATB, cond. 220°C, 11 h	9.8	5.5	3.2	0.18	0.27	0.73
	Rinsed with CH_2Cl_2, cond.: 220°C, 11 h	7.6	4.4	2.7	0.20	0.37	0.63
10	Cond. 220°C, 11 h	22.4	12.6	6.5	0.39	0.61	0.79
	After ATB, cond. 220°C, 11 h	19.8	11.0	5.4	0.43	0.82	1.72
	Rinsed with CH_2Cl_2, cond. 220°C, 11 h	14.0	7.9	4.0	0.51	0.90	4.82
11	Cond. 220°C, 11 h	12.8	5.9	2.1	0.38	0.49	0.57
	After ATB, cond. 220°C, 11 h	13.3	5.8	2.1	0.34	0.51	0.64

* See Table I

** Cond. = conditioned at the temperature and for the period indicated.

TABLE III

RETENTION OF *n*-ALKANES AS A FUNCTION OF SAMPLE AMOUNT AT 100°C

Data from column 6 (Table I) after ATB treatment and rinsing with dichloromethane. The retention times here represent mean values from two experiments. Typical values of the Kováts retention index from two such experiments are 1867.66 and 1868.18

Amount (ng)	t_r *(min)*				*Kováts retention index*		*HETP*	
	*Oct.**	*Napht.**	C_{18}	C_{19}	*Oct.**	*Naphth.**	C_{18}	C_{19}
4.0	1.63	3.58	2.77	4.06	1638	1868	3.67	5.87
2.0	1.63	3.58	2.79	4.10	1637	1866	6.89	12.79
1.3	1.63	3.58	2.81	4.17	1636	1864	13.00	15.78
1.0	1.63	3.58	2.82	4.19	1635	1861	17.24	

* Oct. = octanol; napht. = naphthalene.

for the non-deactivated column 5 (Table V), but after ATB treatment and rinsing, the DMP/DMA peak-area ratio was higher for the deactivated column. The acidic properties of deactivated columns made from Chrompack fused silica are further demonstrated by the well shaped phenol and alcohol peaks (Fig. 2a and b), compared with the same peaks on a non-deactivated column made from Quartz et Silice fused silica (Fig. 3a and b). As a point of interest, aniline and DMP are also eluted with better peak shapes from the deactivated than from the non-deactivated column. This may be explained as being a result of the presence of both acidic and basic sites on the capillary surface.

For columns coated with a thinner stationary phase film (0.09 μm), the DMP/DMA peak-area ratio was lower for the deactivated than for the non-deactivated column (columns 8 and 7, Table V), thus indicating a lower acidity for the deactivated column. This result is unexpected, as the non-deactivated column was flushed with nitrogen at room temperature prior to the coating step in order to remove possible residues of hydrochloric acid from the orginal tubing, from which the capillary tubing had been drawn. It may be speculated that the rinsing with methanol and water, together with the dehydration step, removes traces of hydrochloric acid more effectively than the nitrogen flushing. An improvement could possibly be achieved by flushing with nitrogen at a higher temperature.

Before ATB curing, the phenol exhibits worse tailing on the deactivated than on the non-deactivated column (Fig. 4a and c). Curing improves the phenol peak shapes in both instances. Further, the aniline peak shape is better on the deactivated column, especially after curing (Fig. 4b). Also, column 9 had a film thickness of 0.09 μm, but here the β-ratio was lower, which led to longer retention times and thereby increased opportunities for adsorption (Fig. 5a and b). The elution of nicotine was not successful with these columns (*cf.*, Fig. 4b). Possibly, the 60-CN stationary phase contains some acidic adsorptive sites.

The first step in the synthesis of the 60-CN stationary phase was cyclization of dichlorosilanes in the presence of zinc oxide. In the synthesis of another phase, 95-CN, the first step was the formation of dimethoxysilanes by reaction with tri-

TABLE IV

COLUMN POLARITIES

*Column No.**	*Column treatment***	*Kováts retention index (100°C)*		*ECL (170°C)*		
		2-Methyl-naphthalene	*n-Decanol*	*18:1*	*18:2*	*18:3*
1	Cond. 220°C, 11 h	1919	1805	18 44	19.15	19.99
	Cond. 220°C, 44 h	1931	1827			
	Cond. 250°C, 12 h	1933	1821	18.45	19.16	19.99
2	Cond. 220°C, 11 h	1948	1829	18.47	19.21	20.06
	After ATB, cond. 220°C, 11 h	1964	1840	18.49	19.29	20.16
	Rinsed with CH_2Cl_2, cond. 220°C, 11 h	1956	1834	18.50	19.25	20 14
	Further cond. 240°C, 66 h	1939	1820	18.47	19.17	20.00
3	Cond 220°C, 11 h	1940	1823	18.47	19 19	20.03
	After ATB, cond. 220°C, 11 h	1955	1835	18.50	19.26	20.13
	Rinsed with CH_2Cl_2, cond 220°C, 11 h	1951	1832	18.48	19.24	20.11
4	Cond. 220°C, 11 h	1960	1839	18.49	19.25	20.11
	After ATB, cond. 220°C, 11 h	1970	1845			
	Rinsed with CH_2Cl_2, cond. 220°C, 11 h	1957	1837	18.51	19.30	20.19
5	Cond. 220°C, 11 h	1965	1843	18.49	19.25	20.12
	After ATB, cond. 220°C, 11 h	1967	1844	18.52	19.32	20.23
	Rinsed with CH_2Cl_2, cond. 220°C, 11 h	1959	1838	18.50	19.28	20.20
6	Cond. 220°C, 11 h	1965	1844	18.50	19.26	20.14
	After ATB, cond. 220°C, 11 h	1972	1848	18.52	19.32	20.23
	Rinsed with CH_2Cl_2, cond. 220°C, 11 h	1964	1842	18.51	19.30	20.21
7	Cond. 220°C, 11 h	1874	1773	18.37	19.02	19.78
	After ATB, cond. 220°C, 11 h	1884	1777	18.39	19.05	19.82
	Rinsed with CH_2Cl_2, cond. 220°C, 11 h	1876	1771	18.36	19.02	19.79
8	Cond. 220°C, 11 h	1935	1817	18.46	19.18	20.02
	After ATB, cond. 220°C, 11 h	1928	1811	18.46	19.19	20.01
	Rinsed with CH_2Cl_2, cond. 220°C, 11 h	1917	1808	18.43	19.14	19.98
9	Cond. 220°C, 11 h	1905	1794			
	After ATB, cond. 220°C, 11 h	1926	1809			
	Rinsed with CH_2Cl_2, cond. 220°C, 11 h	1917	1803	18.45	19.14	20.00
10	Cond. 220°C, 11 h	1956	1837	18.48	19.23	20.08
	After ATB, cond. 220°C, 11 h	1976	1850	18.52	19.31	20.21
	Rinsed with CH_2Cl_2, cond. 220°C, 11 h	1965	1843	18.46	19.24	19.95
11	Cond. 220°C, 11 h	2074	1913	18.67	19.62	20.69
	After ATB, cond. 220°C, 11 h	2081	1909	18.67	19.62	20.69

* See Table I.

** Cond. = conditioned at the temperature and for the period indicated.

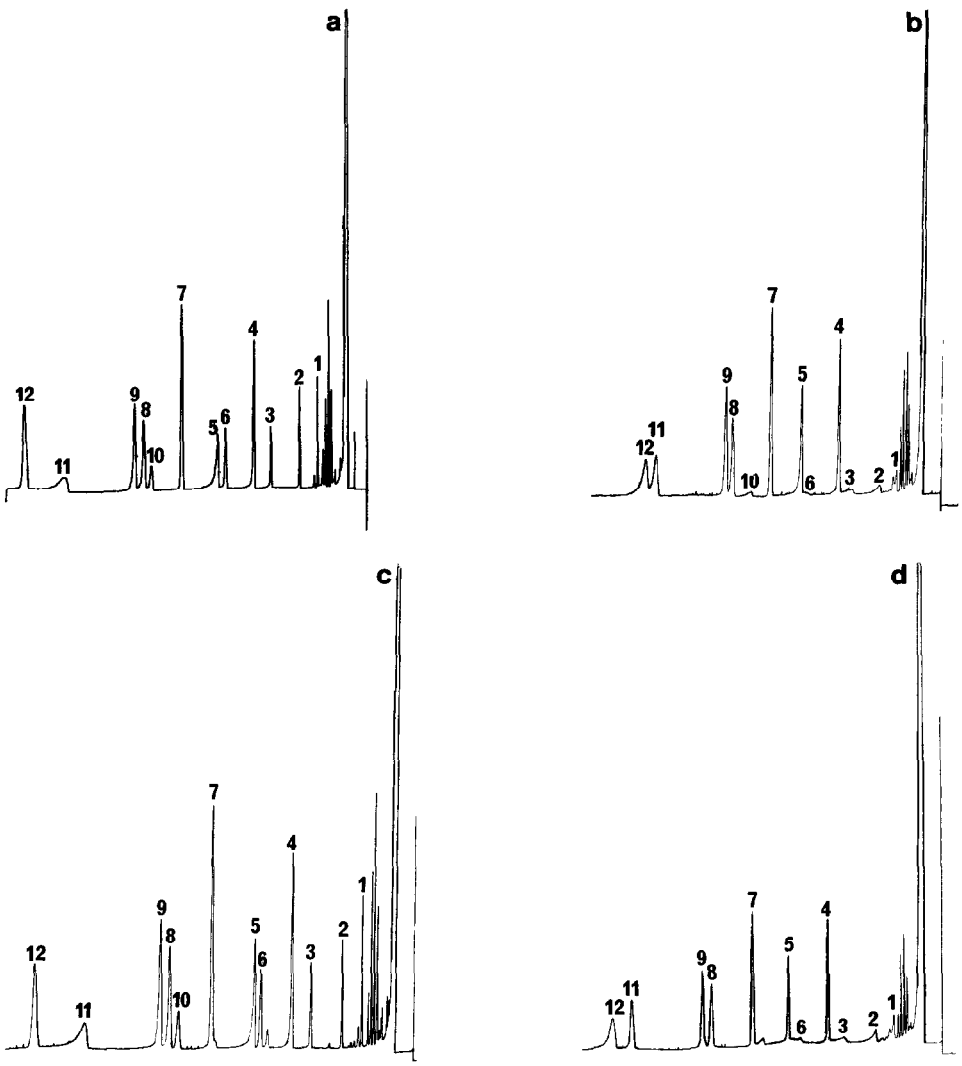

Fig. 3. Gas chromatograms (flame ionization detection) of a polarity mixture on two non-deactivated, fused-silica capillary columns (20 m × 0.32 mm I.D.) coated with 60-CN stationary phase. Conditions, isothermal at 100°C. (a) Column 5, Table I, after conditioning at 220°C for 11 h; (b) the same column after ATB treatment and conditioning at 220°C for 11 h; (c) column 6, Table I, after conditioning at 220°C for 11 h; (d) the same column after ATB treatment and conditioning at 220°C for 11 h. Peaks as in Fig. 1.

methyl orthoformate[1]. The composition of this phase was 94.7% cyanopropyl, 1.8% methyl and 3.5% vinyl. No carboxamide could be detected by ^{1}H NMR spectroscopy. Columns coated with this latter stationary phase gave an acceptable elution of nicotine (Fig. 5c).

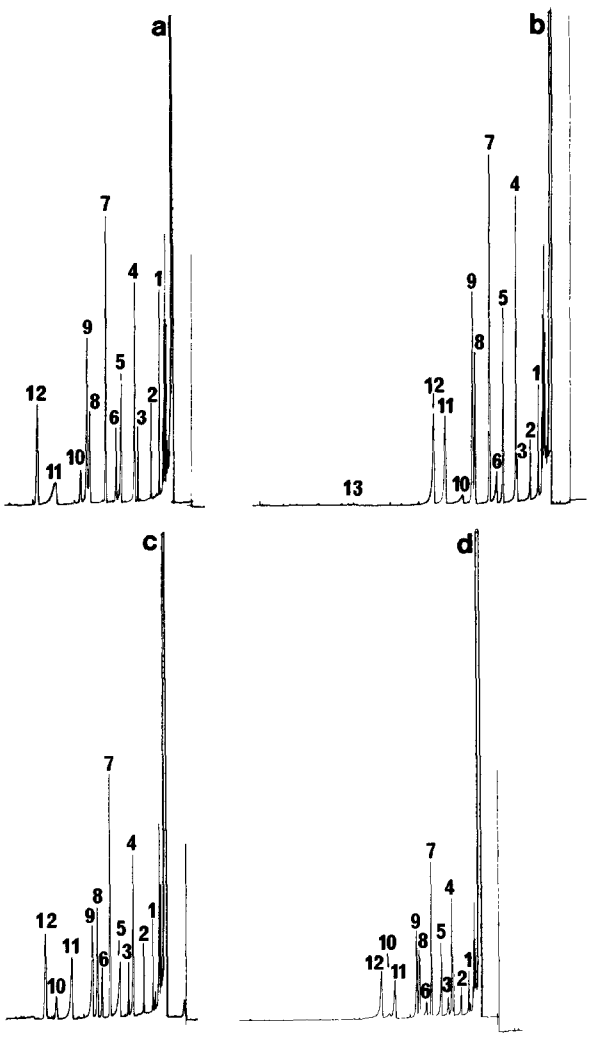

Fig 4. Gas chromatograms (flame ionization detection) of a polarity mixture on (a, b) a deactivated, fused-silica capillary column (20 m × 0.32 mm I D.) coated with a thin film of 60-CN stationary phase (column 8, Table I) and (c, d) on a non-deactivated, fused-silica capillary column (18 m × 0.32 mm I.D.) coated with a thin film of 60-CN stationary phase (column 7, Table I). Conditions, isothermal at 100°C. (a) Column 8 after conditioning at 220°C for 11 h; (b) the same column after ATB treatment and further conditioning at 220°C for 11 h; (c) column 7 after conditioning at 220°C for 11 h; (d) the same column after ATB treatment and further conditioning. Peaks as in Figs. 1 and 2.

Column polarity

It was stated previously[3] that the polarity of 60-CN, when immobilized with dicumyl peroxide, was lower on a deactivated than on a non-deactivated column. In this work, ATB was used for immobilization, and then only small differences in polarity between deactivated and non-deactivated columns were observed when the

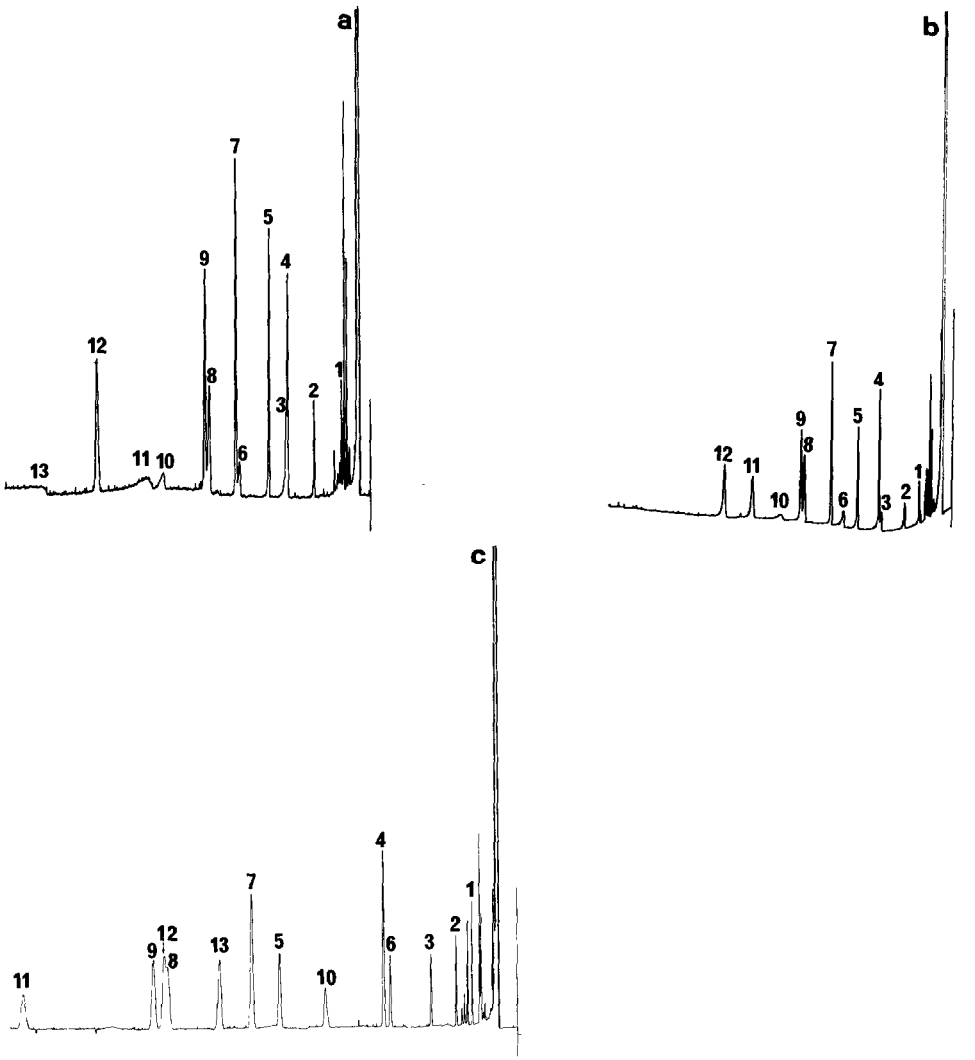

Fig. 5. Gas chromatograms (flame ionization detection) of a polarity mixture on (a, b) a fused-silica capillary column (11 m × 0 10 mm I.D.) deactivated and coated with 60-CN stationary phase (column 9, Table I) and (c) a fused-silica capillary column, non-deactivated and coated with 95-CN stationary phase (column 11, Table I). Conditions, isothermal at 100°C. (a) Column 9 after conditioning at 220°C for 11 h; (b) the same column after ATB treatment and conditioning at 220°C for 11 h; (c) column 11 after conditioning at 220°C for 11 h. Peaks as in Figs. 1 and 2.

film thickness was 0.3 μm (see, *e.g.*, the ECL values and Kováts retention indices for columns 4 and 6, Table IV).

With thin films of stationary phase, the influence of the surface is obviously much greater than when thicker films are applied. The Kováts retention index for 2-methylnaphthalene for columns coated with a film thickness of 0.09 μm was 1935 for a deactivated column, whereas the non-deactivated column showed only 1874 (*cf.*, columns 7 and 8, Table IV, and Fig. 4). Deactivation was performed with a

TABLE V

DIMETHYLPHENOL TO DIMETHYLANILINE PEAK-AREA RATIO

Column No *	*DMP/DMA peak-area ratio*		
	Before ATB	*After ATB*	*After rinsing*
2	0.86	0.67	0.81
4	0.87	0.77	0.74
5	0.81	0.72	0.68
6	0 78	0.76	0.73
7	0.84	0.75	0.76
8	0.64	0.72	0.60
9	0.70	0.77	0.75

* See Table I.

reagent that contains 100% CN substitution, bis(cyanopropyl)cyclotetrasiloxane and this may raise the overall polarity. However, the polarity of the deactivated thin-film column is still lower than that of a correspondingly thicker film column (*cf.*, columns 8 and 4, Table IV). The ECL values of columns 8 and 4 (Table IV) are similar, and it may be speculated that the difference in polarity observed when using the Kováts retention index refers mainly to the elution of hydrocarbons. A more rapid elution of hydrocarbons is thus observed with an increase in polarity. Finally, the non-deactivated thin-film column shows the lowest polarity of all the columns. A possible explanation for this would be that a substantial part of the polar groups in the stationary phase are oriented toward the capillary surface by the action of active surface groups. With thin films of stationary phases, this would lead to reduced polarities.

Stationary phase immobilization

Immobilization with ATB leads to increased Kováts retention indices for 2-methylnaphthalene and decanol (Table IV). Owing to the increased adsorption of *n*-alkanes after immobilization, a test solution containing a higher concentration of *n*-alkanes (100 ng/μl) had to be used for the determination of retention data in this instance. This may result in an increase in the Kováts retention index of *ca.* 4 units for naphthalene (Table III). The ECL values of FAME 18:1, 18:2 and 18:3 are increased by immobilization (Table IV). It may be speculated that the solubility of *n*-alkanes in the stationary phase is decreased on curing and this, in turn, may lead to column overloading and thus decreased *n*-alkane retention times.

Periods of 60 or 30 min for the introduction of ATB vapour into the columns did not affect the chromatographic properties. Moreover, immobilization on non-deactivated columns resulted in tailing of methyl myristate (peak 12 in the polarity mixture; *cf.*, Fig. 4a and b). This effect is obvious in the analysis of rapeseed oil (Fig. 6).

Column polarity is dependent on temperature, and in order to achieve comparable results in temperature-programmed tests, all factors that influence the elution temperatures must be standardized. As an example of a source of errors, it may be mentioned that curing and conditioning led to lower k' values (Table II) and this, in turn led to decreased elution temperatures, all other conditions being constant. In our opinion, small shifts in column properties are detected with the greatest certainty in isothermal tests.

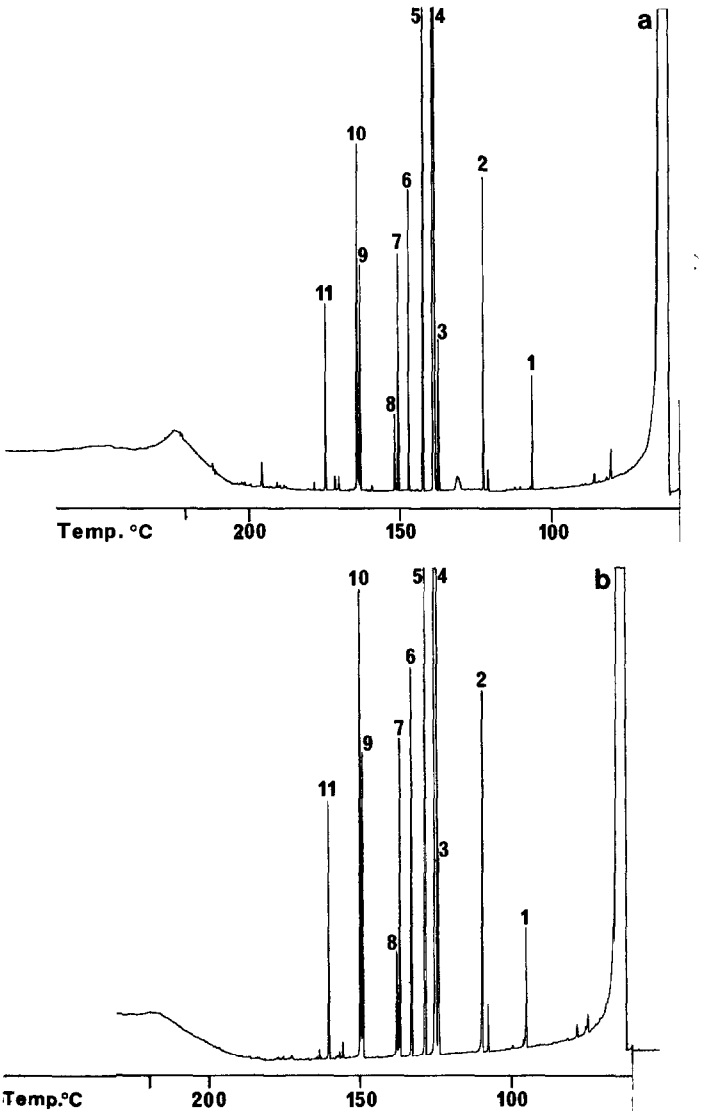

Fig. 6. Gas chromatograms (flame ionization detection) of a fatty acid methyl ester test mixture (rapeseed oil, Supelco) on a fused-silica capillary column (18 m × 0.32 mm I.D.) coated with the stationary phase 60-CN (column 7, Table I). Conditions, injection at 70°C and, after 2 min, programmed at 5°C/min to 220°C. (a) Before immobilization; (b) after immobilization and rinsing. Peaks: 1 = 14:0; 2 = 16:0; 3 = 18:0; 4 = 18:1; 5 = 18:2; 6 = 18:3; 7 = 20:0; 8 = 20:1; 9 = 22:0; 10 = 22:1; 11 = 24:0.

Influence of column rinsing

Rinsing with a solvent is performed in order to remove silicone fragments, consisting mostly of cyclic compounds, that have not been immobilized by the ATB treatment. These may be residues from the synthesis of the stationary phase, but they may also be products of thermal degradation of the stationary phase that has taken

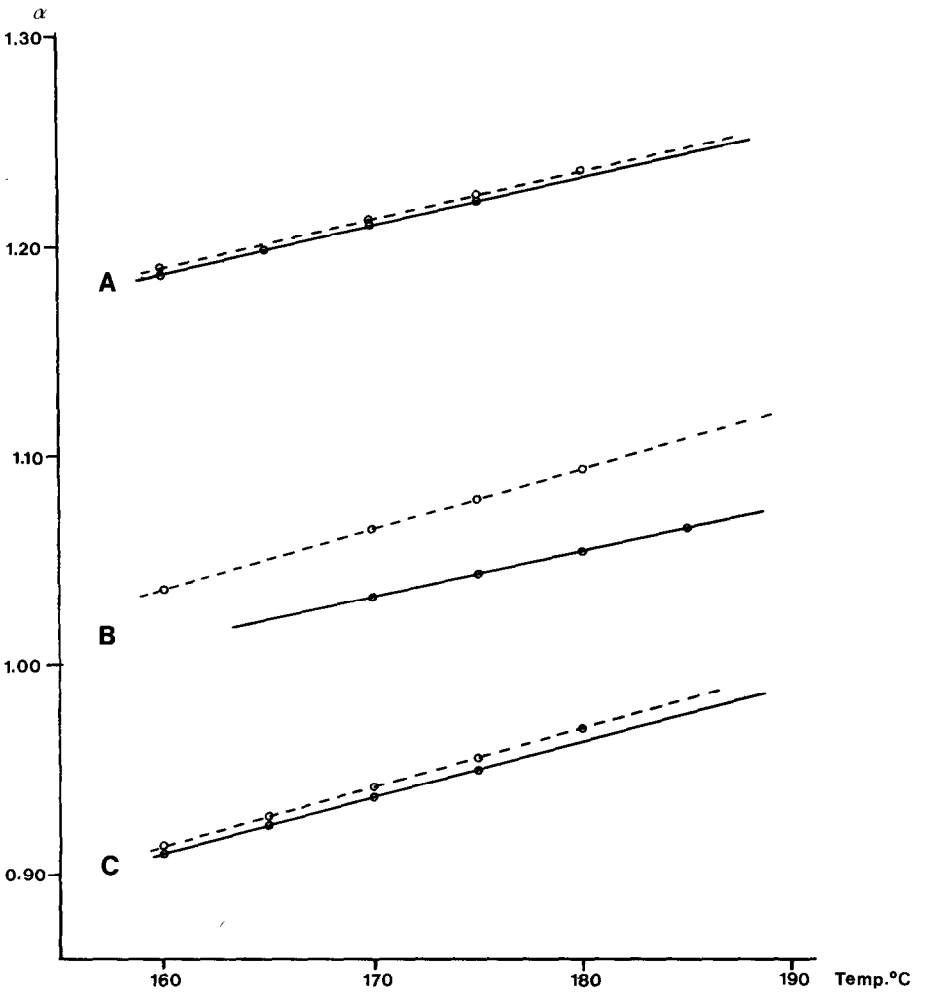

Fig. 7. Relative retention, α, of fatty acid methyl esters $C_{18:3}$ and $C_{20\,0}$ as a function of temperature in isothermal experiments. Solid lines, before curing; dotted lines, after curing and extraction. (A) Column 11; (B) column 5; (C) column 7 (Table I). Correlation coefficients are in the range 1.00–0.997.

place during the conditioning step. Polar or moderately polar substituents are often over-represented in these mixed cyclic compounds compared with the overall polymer composition[13]. This can be explained by the fact that the polymer backbone is generally more easily broken in the vicinity of such substituents. In this work, in all instances rinsing was found to result in decreased polarities, thus reflecting the removal from the column of cyclic compounds having relatively high contents of cyanopropyl substituents (Table IV and Fig. 2c).

Column ageing

Column polarities were further reduced on thermal ageing of the columns (*cf.*, columns 2 and 3, Table IV). This may be interpreted as being a result of the relative

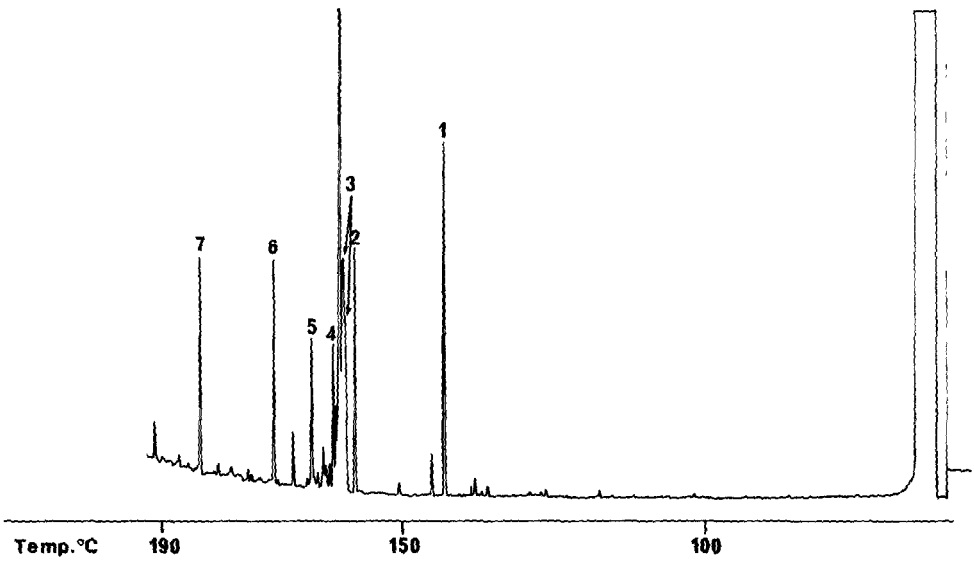

Fig. 8. Gas chromatogram (flame ionization detection) of a fatty acid methyl ester *cis–trans* test mixture (Supelco) on a fused-silica capillary column (20 m × 0.32 mm I.D.) deactivated and coated with 60-CN stationary phase (column 2, Table I). Conditions, injection at 70°C and, after 2 min, programmed at 5°C/min to 200°C. Peaks: 1 = 16:0; 2 = 18:0; 3 = 18:1 *trans*-4 isomers; 4 = 18:1 *cis*-4-isomers; 5 = 18:2; 6 = 18:3 + 20:0; 7 = 22:0.

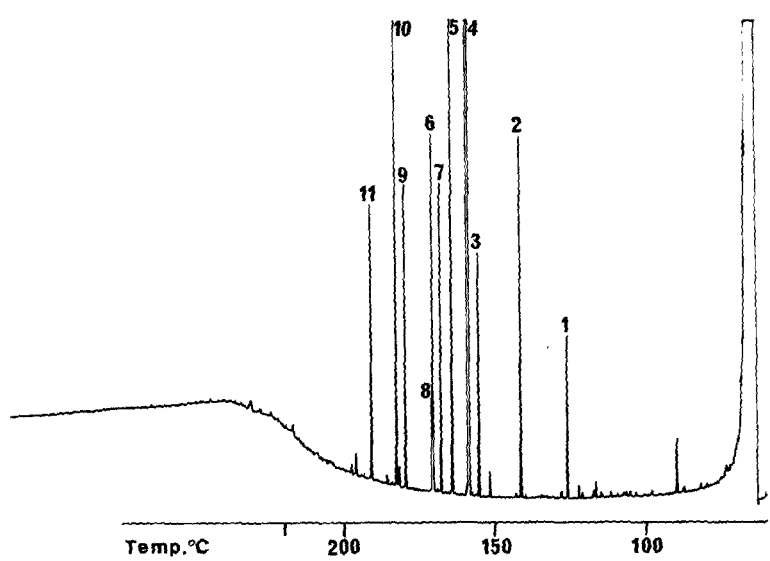

Fig. 9. Gas chromatogram (flame ionization detection) of a fatty acid methyl ester test mixture (rapeseed oil, Supelco) on a fused-silica capillary column (20 m × 0.32 mm I.D.) non-deactivated and coated with 95-CN stationary phase (column 11, Table I). Conditions and peaks as in Fig. 6.

over-representation of cyanopropyl-substituted silicone moieties in the bleed products.

Conditioning at 220°C of columns coated with an immobilized stationary phase led to increased values of HETP for *n*-alkanes, whereas the HETP for 2-methylnaphthalene and decanol remained fairly constant (column 3, Table II, and Fig. 2c). However, conditioning at 240°C for 66 h destroys such a column (Fig. 2d). The HETP of *n*-alkanes in columns coated with a non-immobilized stationary phase was found to be relatively stable on conditioning for 11 h at 220°C (column 1, Table II, and Fig. 1a). Conditioning for 44 h at 220°C led to increased solute adsorptivity (Fig. 1b), and the column properties were further impaired on conditioning for 12 h at 250°C (Fig. 1c).

The stability of column properties depends on the temperatures at which the column is being used. In our experience, columns coated with immobilized 60-CN stationary phase fulfil high demands on stability when used for prolonged periods at temperatures up to 220°C.

Effect of temperature on column polarity

As mentioned above, column polarity depends on temperature. This is demonstrated in Fig. 7, where the relative retention, α, of FAME 18:3 and 20:0 is plotted against the temperature used in isothermal experiments. The polarity, as expressed here, was found to increase with increasing temperature over the range studied. This condition has long been recognized[7,14].

Applications

The columns are suitable for the separation of FAME. The separation of a *cis–trans* mixture is shown in Fig. 8 and of a rapeseed oil sample in Fig. 9.

CONCLUSIONS

Elution of *n*-alkanes may easily lead to overloading of columns coated with 60% cyano-substituted silicones. On stationary phase immobilization, this problem becomes severe. FAME are very satisfactorily eluted from a non-immobilized phase, but they show slight tailing after stationary phase immobilization, especially with non-deactivated columns. Deactivation leads to relatively neutral columns, as reflected by the elution of phenol and aniline. On the basic side, nicotine presents difficulties, which may depend on the adsorptive activity in the stationary phase itself.

Immobilization improves the phenol and aniline peak shapes. Columns were prepared with a high degree of reproducibility with regard to acid–base status, efficiency, k' values and polarities. However, immobilization and subsequent conditioning led to some small differences in the polarity. Typically, the values of the Kováts retention index for *n*-decanol on two different columns were 1838 and 1842, respectively.

The column polarity is influenced by (1) stationary-phase film thickness, thinner films resulting in lower polarities; (2) column deactivation; for $d_f = 0.3$ μm non-deactivated columns were slightly more polar than deactivated columns, and for $d_f = 0.09$ μm the situation was the reverse; (3) immobilization, generally leading to increased polarities; (4) rinsing, which leads to decreased polarities, and (5) conditioning at 220°C, leading to decreased polarities.

The properties of columns coated with non-immobilized stationary phases were not stable on prolonged conditioning at 220°C. Columns coated with immobilized 60-CN stationary phase fulfilled high demands on stability when conditioned for a long period at 220°C. Only the elution of *n*-alkanes deteriorated.

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