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CHARACTERIZATION OF REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC STATIONARY PHASES BY MEANS OF PYROLYSIS-GAS CHROMATOGRAPHY

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SUMMARY

Pyrolysis-gas chromatography has been shown to be a valuable tool for the characterization and evaluation of chemically bonded phases for reversed-phase high-performance liquid chromatography. The chain length of the hydrocarbon group bonded to the silica surface could be determined and the functionality of the silanizing agent was established for different C₁₈ materials. The technique could also be used to investigate whether the materials had been post-silanized with reagents such as hexamethyldisilazane for the capping of residual silanol groups.

INTRODUCTION

The development of chemically bonded stationary phases (CBSP) on micro-porous silica supports for use in high-performance liquid chromatography (HPLC) is one of the major factors that has increased the importance of this technique. Hydrocarbons bonded to the silica support, *i.e.*, phases for reversed-phase chromatography, enjoy great popularity and several reviews on commercial stationary phases have been published^{1,2}.

Because of the increasing number of manufacturers, the type of reversed-phase material for a particular separation is often chosen by chance. However, as frequently pointed out, the packing materials can differ considerably according to their packing characteristics³, their performance^{2,4} and especially their separation properties⁴⁻⁷. Variations from batch to batch for phases from the same manufacturer have also been noted³. Sometimes these differences can be explained in terms of pore size, pore distribution, particle size, particle distribution, surface area, hydrocarbon chain length, hydrocarbon loading, etc., data which are often given by the supplier. However, often the observed differences are not easily explained in these terms⁸.

It has been suggested that the number of unreacted silanol groups on the silica surface can play an important role in reversed-phase chromatography⁹, especially when chromatographing basic substances such as amines^{4,10}.

It appears that more information about the surface properties such as mode

of modification (monolayer, polymer layer), functionality of silanizing reagent, chain length of reagent, degree of silanization and degree of "capping" is necessary before a complete understanding of the chromatographic behaviour is possible and a comparison between different reversed-phase stationary phases can be made⁹. Various chromatographic tests have been employed to evaluate the influence of silanol groups^{8,9} and column performance for comparative purposes¹¹. Screen tests have been used for the evaluation of the separation ability of different bonded phases¹². Methods employed for studying bonded phases, in addition to "standard" elemental analysis, have included IR¹³⁻¹⁶ and photoacoustic spectroscopy¹⁷, UV absorption¹⁸, thermal degradation and mass spectrometry (MS)^{15,19}, alkaline hydrolysis followed by silylation and gas chromatography²⁰ and titration^{21,22}. Unger²³ described various methods for the characterization of HPLC materials.

Pyrolysis-gas chromatography (Py-GC) has found widespread use for the study of polymers²⁴. As the reversed-phase silica support can be regarded as a siloxane polymer modified with hydrocarbons of varying chain length, Py-GC should be a useful technique for the study of these materials. A study of silica gels esterified with saturated alcohols by means of Py-GC-MS has been reported²⁵.

In this investigation we used Py-GC for the characterization of reversed-phase silica gels with different chain lengths and structures of the hydrocarbon moiety. We also studied the influence of "capping" on the pyrolysis and chromatographic results.

EXPERIMENTAL

Apparatus

Pyrolyser. The pyrolyser used was a CDS 150 Pyroprobe (Chemical Data Systems, Oxford, PA, U.S.A.). It consisted of a probe of a platinum-coil type, a quartz tube and a sample holder (see Fig. 1). The sample holder was made by folding a stainless-steel mesh disc for HPLC columns. The probe was inserted into an interface, mounted on the injection port of the gas chromatograph. The interface temperature was set at 275°C. The temperature inside the sample holder was measured by means of an iron-constantan thermocouple.

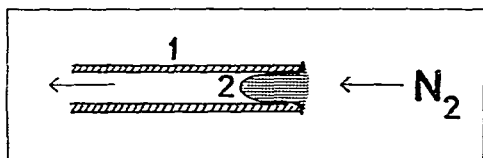


Fig. 1. Diagram showing sample application part placed inside the Pt-coil of the probe. 1 = Quartz tube; 2 = sample holder.

For identification of the pyrolysis products the pyrolyser was connected to the GC part of a Finnigan 4021 mass spectrometer. The Py-GC-MS measurements were carried out using the same conditions as in the Py-GC investigation except for a change from nitrogen to helium as the carrier gas.

Gas chromatography. A Varian 3700 gas chromatograph with a 1.9 mm I.D. stainless-steel column was used. The carrier gas was nitrogen at a flow-rate of 30 ml/

min, and the hydrogen and air flow-rates were 30 and 240 ml/min, respectively. A flame-ionization detector was used at 300°C. The injector temperature was 240°C.

Two GC systems were used: (A) a 1-m Chromosorb 102 column with temperature programming from 50 to 225°C at 20°C/min; and (B) a 2-m column packed with 3% OV-17 on Chromosorb W HP (80–100 mesh) with temperature programming from 70 to 275°C at 20°C/min.

Liquid chromatography. The apparatus, column tubing, fittings and procedure have been described elsewhere²⁶.

Chemicals and packing materials

Octadecyldimethylchlorosilane and octadecylmethyldichlorosilane were purchased from Magnus Scientific (Sandbach, Great Britain). Octadecyltrichlorosilane, vinylmethyldichlorosilane and trimethylchlorosilane were purchased from E. Merck (Darmstadt, G.F.R.). All solvents (E. Merck) were of analytical-reagent grade and all chemicals were used without further purification.

The packing materials studied were LiChrosorb RP-18, RP-8 and RP-2 (E. Merck), μ Bondapak C₁₈ (Waters Assoc., Milford, MA, U.S.A.), Nucleosil C₁₈ (Machery, Nagel & Co, Düren, G.F.R.), Partisil ODS (Whatman, Clifton, NJ, U.S.A.), Spherisorb ODS (Phase Separations Ltd., Queensferry, Clwyd, Great Britain), ODS-Hypersil (Shandon Southern Products, Runcorn, Great Britain) and Zorbax ODS (DuPont, Wilmington, DE, U.S.A.). The silica gels used to prepare the home-made bonded phases were LiChrosorb Si-100 or Si-60 (E. Merck).

Preparation and capping of CBSP

The bonding procedure used for the preparation of home-made CBSP was basically that used by Hemetsberger *et al.*²⁷ but with variation of the reaction time and the type of original silica gel according to Table I.

TABLE I

MATERIALS AND CONDITIONS USED FOR THE PREPARATION OF HOME-MADE CBSP

<i>Type of modifier</i>	<i>Designation of product</i>	<i>Type of silica</i>	<i>Particle diameter (μm)</i>	<i>Reaction time (h)</i>
Trimethylchlorosilane	TMCS-silica	Si-100	5	24
Vinylmethyldichlorosilane	VMDCS	Si-60	10	2
Octadecyltrichlorosilane	OTCS-silica	Si-60	5	1.5
Octadecylmethyldichlorosilane	OMDCS-silica	Si-100	5	24
Octadecyldimethylchlorosilane	ODMCS-silica	Si-100	5	24

The capping of CBSP was carried out in the same way as the preparation of CBSP itself, except that the CBSP was dried at 100°C and 10 mmHg for 1 h and the reaction time was 5 h.

Pyrolysis procedure

The sample (about 60 μ g) was placed in the sample holder of the quartz tube, assembled with the probe and put into the interface. The system was conditioned for

about 10 min, during which time any traces of solvents in the sample were evaporated. When a steady baseline had been achieved, the pyrolyser was set at 800°C, whereupon the sample was pyrolysed in 5 sec. Between each run the quartz tube with the sample holder was flushed with a jet of air in order to remove any traces of previous sample.

RESULTS AND DISCUSSION

Gas chromatography

For the purpose of determining the length and type of the hydrocarbon moiety attached to the silica gel, two different GC systems had to be used. Chemically bonded stationary phases with a chain length up to eight carbon atoms were suitably analysed with GC system A. Using this system a good resolution within a reasonable time was obtained. In order to reduce the time of analysis for CBSPs with longer chain lengths, GC system B was employed. This system gave a poor resolution of hydrocarbons below C₈ but was satisfactory for establishing the identity of longer hydrocarbon groups attached to the silica gel.

Pyrolysis conditions

In order to obtain simple pyrograms, *i.e.*, gas chromatograms obtained from pyrolysis products, it would be desirable to be able to choose a pyrolysis temperature at which mainly the C–Si bond was broken while the hydrocarbon chain was left intact (for representative bond energies, see Table II²⁸). As the energy of the C–Si bond is dependent on the nature of other groups attached to the silicon atom (see Fig. 2), the splitting pattern will vary with the structure around the silicon atom^{16,28,29}. This is in agreement with our experience. Thus, it appears that an exchange of Si–O bonds for Si–CH₃ weakens the Si–C bond of, *e.g.*, the octadecyl groups, resulting in an increase in the proportion between the C₁₈ and C₁₇ peaks on pyrolysis. Although for some compounds it is possible to choose a low pyrolysis temperature at which the C–Si bond is split in preference to the C–C bonds, it appears that for practical work a higher temperature must be used in order to increase the yield of pyrolysis products. At this temperature both C–C and C–Si bonds are split, as shown by the pyrogram in Fig. 3.

TABLE II
BOND ENERGIES

Type of bond	Bond energy (kcal/mole)
Si–C	76
Si–O	108
C–C	82.6
C–H	98.7

The choice of pyrolysis conditions must be a compromise because of the conflicting demands of a high yield of products and simplicity of the pyrogram. It was found that the optimal pyrolysis conditions for the purpose of this investigation were a set pyrolysis temperature and pyrolysis time of 800°C and 5 sec, respectively. Measurements with a thermocouple (see *Apparatus*) showed that under these con-

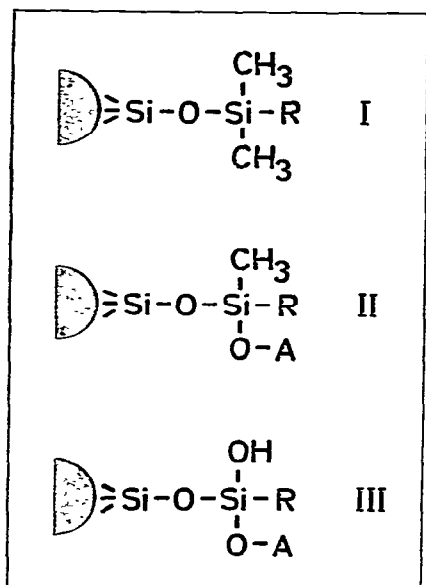


Fig. 2. Schematic formulae of the different structures of CBSPs obtained when mono- (I), di- (II) and tri- (III) functional silanizing agents are reacted with the silica surface. R = Hydrocarbon chain; A = H or Si→.

ditions a temperature of about 495°C was reached in the sample holder. Pyrolysis times longer than 5 sec gave a still increasing temperature. It is thus obvious that the temperature rise time was relatively long and that the pyrolysis of the sample took place during the rise to the set temperature.

Determination of the chain length of the CBSP

Fig. 3 shows the pyrograms of some home-made and commercial CBSPs. Each type of CBSP gave a characteristic pyrogram. The pyrolysis products were a homologous series of predominantly monoalkenes. The formation of alkenes as degradation products from alkanes has been established previously^{16,30,31}. Some workers^{15,19} claim that the Si-O bond is also cleaved at the temperatures used, giving silicon compounds as pyrolysis products. However, such compounds could not be identified as isolated peaks in the pyrograms.

The determination of the chain length of different CBSPs is based on the determination of the chain length of the latest eluted hydrocarbon in the pyrogram, corresponding solely to breaking of the Si-C bond in the CBSP. The determination was accomplished by comparing the retention times with those from a Py-GC-MS run with a C₁₈ CBSP, where the presence and identities of alkenes were confirmed.

Determination of the functionality of the silanizing agent

The fact that the substitution pattern at the silicon atom has a major influence on the bond strength between the silica and the main hydrocarbon chain, as mentioned above, can be utilized to obtain information about the surface structure and the probable functionality (mono-, di or tri-) of the silanizing agent (see Fig. 2). Fig. 4 shows the pyrograms of three different C₁₈ materials synthesized in our labora-

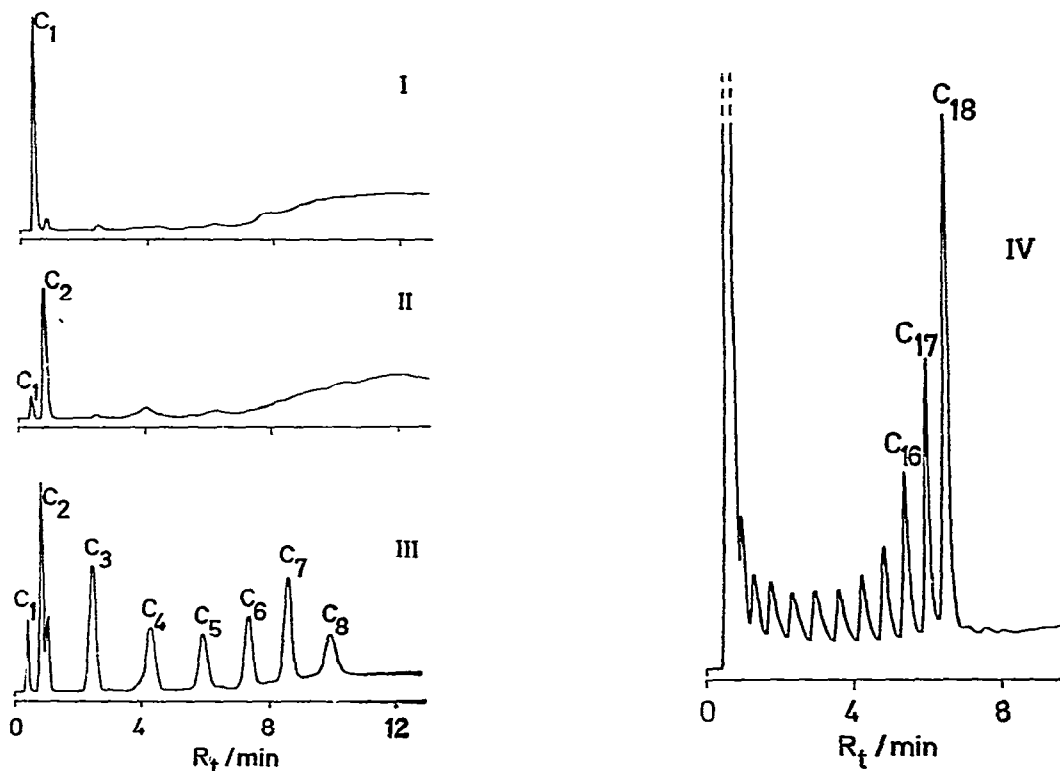


Fig. 3. Pyrograms of CBSPs with different chain length of the hydrocarbon moiety (R). I, R = methyl-, GC system A; II, R = vinyl-, GC system A; III, R = octyl-, GC system A; IV, R = octadecyl-, GC system B. I, II and IV, home-made CBSP; III, LiChrosorb RP-8.

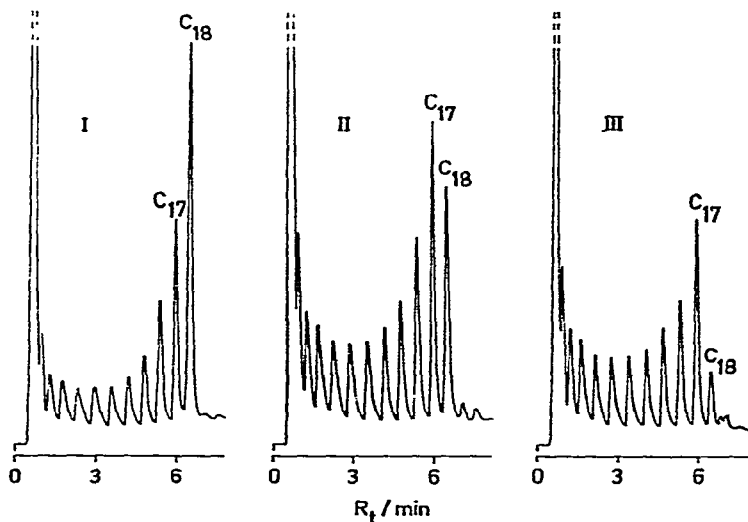


Fig. 4. Pyrograms of three different C_{18} materials. I = ODMCS-silica; II = OMDCS-silica; III = OTCS-silica. GC system B. For explanation see Table I.

tory. As can be seen, the peak height ratios of heptadecene and octadecene vary with the type of silanizing agent.

Table III gives the C_{17}/C_{18} peak height ratios of some C_{18} materials. As demonstrated, the ratio falls into three main groups, which is believed to be due to the different functionalities of the silanizing agents. The deviation of the ratios within the three functionality groups (mono-, di- and tri-) could be attributed to different kinds of original silica and, with di- and trifunctional silanizing agents, to different degrees of post-polymerization. The identity of the silanizing agent was confirmed by the manufacturers in some instances. The precision of the peak-height ratio expressed as relative standard deviation was 6% for measurements during 1 day and 10% for measurements during 3 months.

TABLE III

DETERMINATION OF PROBABLE FUNCTIONALITY OF DIFFERENT C_{18} MATERIALS BY MEANS OF Py-GC

<i>Packing material</i>	<i>Batch</i>	<i>Mean particle diameter (μm)</i>	<i>Peak-height ratio C_{17}/C_{18}</i>	<i>Probable functionality of silanizing agent</i>
ODMCS-silica *	Home-made	5	0.54	Mono
OMDCS-silica *	Home-made	5	1.24	Di
OTCS-silica *	Home-made	5	3.5	Tri
Zorbax ODS	18341-66	6	0.76	Mono
μ -Bondapak	58	10	1.18	Di
Lichrosorb	VV58	10	1.30	Di
Partisil	100130	10	3.1	Tri
Spherisorb	MH 15/175	10	3.6	Tri
Hypersil	GA 613	5	4.7	Tri
Nucleosil	8111	5	5.1	Tri
Nucleosil	9101	5	7.5	Tri
Nucleosil	9122	5	6.8	Tri

* For explanation, see Table I.

Verification of capping of CBSP

The necessity for subsequent treatment of CBSPs with TMCS or HMDS for the capping of residual silanol groups has been discussed. The results from elemental analysis of the CBSPs do not give a sufficiently large increase in the carbon content to verify that capping takes place³². On the other hand, chromatographic tests indicated that capping does occur and improves efficiency^{33,34}.

Experience from work with CBSPs at this laboratory pointed to a considerable difference in performance between different batches of the same phase, especially when chromatographing basic substances such as amines and amides. This is demonstrated in Fig. 5, which shows the results for a mixture of some urea derivatives run on three different batches of Nucleosil C_{18} under identical conditions. It can be seen that not only the retentions but also the order of retention are different. It is believed that this behaviour is due to differences in capping of residual silanol groups.

In order to investigate the value of Py-GC for the verification of capping of CBSPs, two kinds of home-made C_{18} phases, one treated with trimethylchlorosilane (TMCS) and one untreated, were subjected to Py-GC (see Fig. 6). It can be seen that

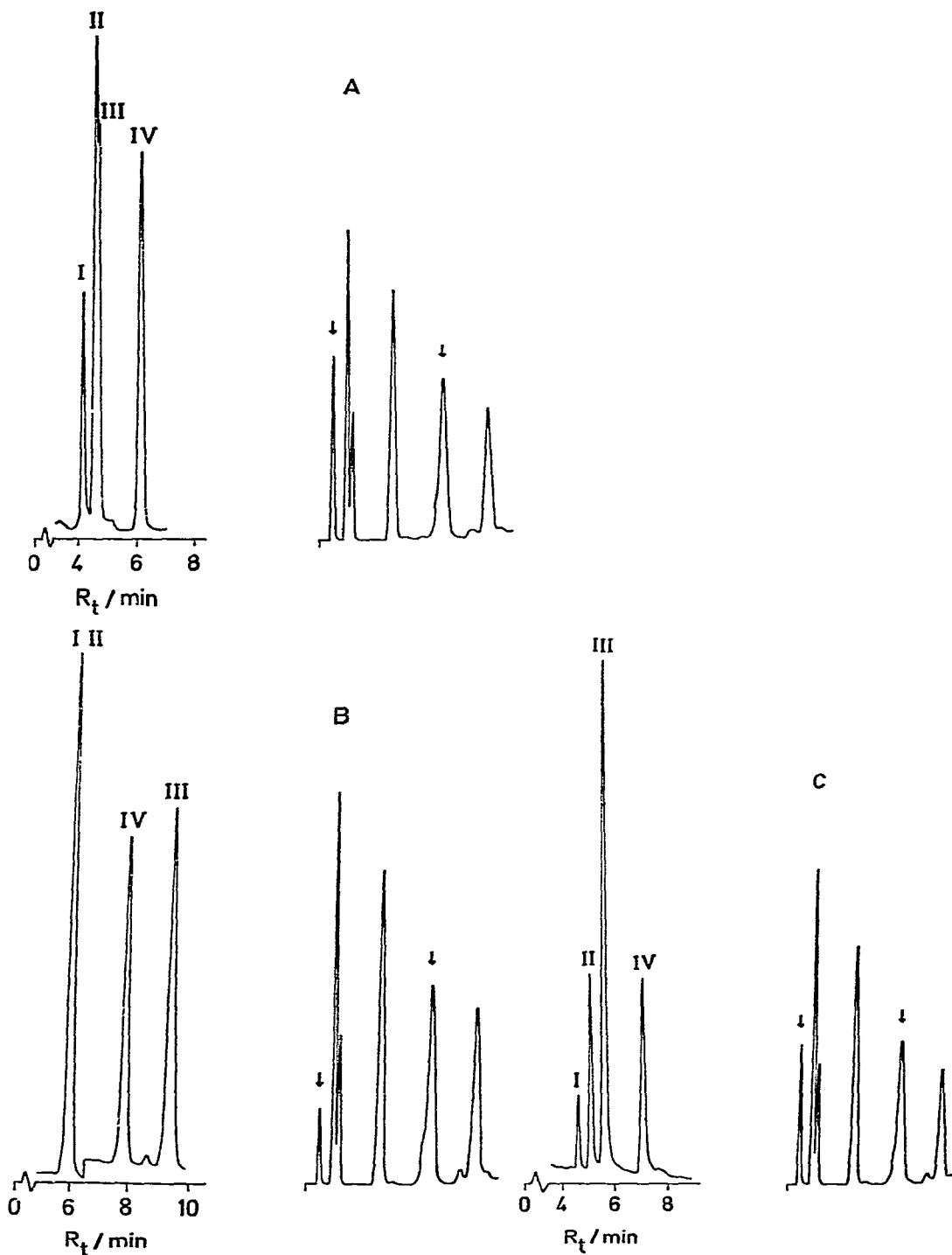


Fig. 5. Liquid chromatogram (left) and corresponding pyrogram (right) of three different batches of Nucleosil C_{18} ($5 \mu m$). A, Batch 8111; B, batch 9101; C, batch 0061. Batch 8111 and 0061 are capped and batch 9101 is not capped. Liquid chromatograms: urea derivatives of 9-(N-methylaminoethyl)anthracene reagent and (I) toluene 2,6-diisocyanate, (II) toluene 2,4-diisocyanate, (III) hexamethylene diisocyanate and (IV) 4,4'-diphenylmethane diisocyanate; eluent, acetonitrile-water (70:30), the aqueous phase containing 3% of triethylamine, pH 3.0; flow-rate, 2 ml/min; column, 200×5 mm Nucleosil C_{18} ($5 \mu m$). Pyrograms: GC system A; the arrows mark the C_1 and C_4 peaks.

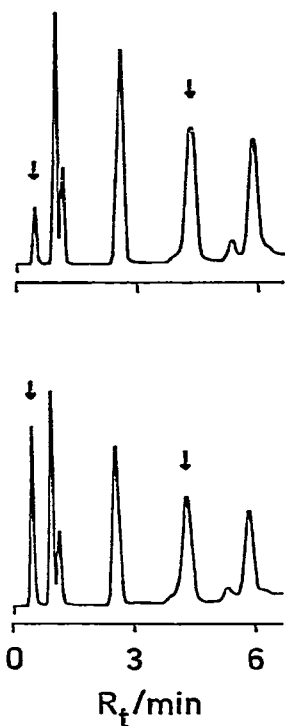


Fig. 6. Pyrograms of OTCS-silica. Top, uncapped material; bottom, capped material. The arrows mark the C_1 and C_4 peaks used for the calculation of the C_1/C_4 peak-height ratios in Table IV. GC system A.

the pyrograms are almost identical, with the exception of the relative height of the methane peak. Thus, it is greater for the capped material. The ratio of the peak heights of methane and propene was taken as a measure of the capping efficiency. Table IV gives the peak-height ratios of the methane/propene peaks for trifunctional capped and uncapped material. Of course, this ratio will vary with the functionality of the silanizing agent. However, as capping is of minor importance for the mono- and difunctional silanizing agents, our investigation has been mainly concerned with the trifunctional one. The precision of this peak height ratio, expressed as relative standard deviation, was 11%.

The three batches of Nucleosil C_{18} mentioned above were pyrolysed (see Fig. 5) and the methane/propene peak-height ratio was determined. The previous investigation (see Table III) indicates that the bonded phase of Nucleosil C_{18} is of the trifunctional type. From Table IV it can be seen that the methane/propene peak-height ratio from batches 9101 and 9122 fits well with an uncapped C_{18} phase of the trifunctional type. Batch 8111 seems to be a capped C_{18} phase of the same type and the third batch (0061) seems also to be capped. These observations were later confirmed by the manufacturer.

In Table IV are also tabulated the carbon contents of some of the pyrolysed phases, determined by elemental analysis. It can be seen that the carbon content

TABLE IV

INVESTIGATION OF CAPPING OF SOME TRIFUNCTIONAL C_{18} MATERIALS BY MEANS OF Py-GC

<i>Packing material</i>	<i>Batch</i>	<i>Mean particle diameter (μm)</i>	<i>Peak-height ratio, C_1/C_4</i>	<i>Elemental analysis, C (%)</i>
OTCS-silica *	Home-made	5	0.40**	17.2
Capped OTCS-silica	Home-made	5	1.60***	18.0
Nucleosil	8111	5	1.22 [§]	14.9
Nucleosil	9101	5	0.49**	13.3
Nucleosil	9122	5	0.42**	13.5
Nucleosil	0061	5	0.99 [§]	13.6
Partisil	100130	10	1.82 ^{§§}	
Spherisorb	MH 15/175	10	0.97 ^{§§}	
Hypersil	GA 613	5	1.36 ^{§§}	

* See table I.

** Not capped.

*** Treated with TMCS.

[§] Capped according to the manufacturer.

^{§§} Probably capped.

varies considerably between the different materials investigated. However, this fact does not reveal whether the material is capped or not. In order to try to verify if a packing material is capped on the basis of carbon elemental analysis, a comparison between the carbon contents of a capped and the same uncapped batch must be made.

In Table IV such a comparison was made for the first two packing materials and a slight increase in carbon content was obtained for the capped material. However, it is questionable if this can be taken as a proof of capping, because of the small difference and the fact that it has been stated that the carbon content does not necessarily increase after capping³².

CONCLUSIONS

Py-GC offers a useful method for the characterization of CBSPs on silica gel. The method is rapid and reasonably reliable and can be used without any pre-treatment of the samples. The method can be employed for studying both the nature of the hydrocarbonaceous phase and the functionality of the silanizing agent. It is also useful for establishing if the phase has been capped or not and the capping efficiency. It is believed that further quantitative investigations could give a more detailed picture of the structure of CBSPs and such investigations are planned for the near future.

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