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CYANOSILICONES AS STATIONARY PHASES IN GAS CHROMATOGRAPHY

II. GUM AND RUBBER PHASES ON FUSED SILICA

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SUMMARY

The presence of cyano groups in the stationary phase may provide useful selective interactions during separation. In capillary gas chromatography, the full utility of such interactions will be evident when the columns show high efficiency, good deactivation, high thermal stability and reproducibility in retention characteristics. Columns that fulfil these requirements were prepared. Fused silica capillaries were treated with hydrochloric acid, silanized at a high temperature with cyclic cyanopropylsiloxane and statically coated with cyanopropylsilicone gum, which was finally immobilized by *in situ* cross-linking. Vulcanization is normally inhibited by cyanopropyl groups, but a certain percentage of tolyl groups in the gum made cross-linking possible. The usefulness of the cyanosilicone columns is demonstrated by the separation of several samples such as fatty acid methyl esters and pheromones.

INTRODUCTION

Polar fused silica columns are of importance for the development of chromatographic technology owing to the combination of the inertness and flexibility of fused silica capillaries with useful selective interactions during the separation. For cyanosilicone stationary phases, the degrees of these interactions is dependent on the dipole moment and polarizability of the material that is separated on the column. The potential advantages of the selectivity can, however, be utilized only when the column shows high efficiency, good deactivation and high thermal stability. Columns that fulfil these requirements will make it feasible to increase the separation power available today in applications such as FAME (fatty acid methyl esters), PAC (polyaromatic compounds), pheromones, sugars and non-derivatized drugs.

Commercially available cyano-containing silicone stationary phases, such as OV-225, which has proved to be useful in packed column gas chromatography, have low molecular weight. They can be deposited as an even film on a capillary column wall, but such a film will certainly not be stable at elevated temperatures. The vis-

cosity of polar siloxanes decreases considerably with increased temperature compared with methylsilicones^{1,2}. This condition makes it especially advantageous to stabilize polar silicone stationary phases by *in situ* cross-linking^{3,4}.

In Part I⁵, a procedure was described for the synthesis of gum phases that contained 33, 50 and 95 % cyano substitution on the siloxane backbone. It is well known that cyanopropyl and phenyl substituents in a siloxane inhibit cross-linking⁶; we tried to circumvent the problem by introduction of some vinyl or tolyl substitution in the gums that were to be cross-linked. It was found that cross-linking was highly facilitated by tolyl substitution^{5,7}. Silicone gums having a high degree of cyano substitution (95 %), however, could not be efficiently cross-linked by the above methods.

In this paper, the preparation of fused-silica capillary columns coated with immobilized cyanopropylsilicone phases is described. Three types of cyanosilicone rubber phases are used, having 33, 50 and 75 % cyano substitution. Immobilization is achieved by virtue of some degree of tolyl substitution.

EXPERIMENTAL

Preparation of cyclotetrasiloxanes for chemical modification

Bis(cyanopropyl)cyclotetrasiloxane and cyanopropyl(methyl)cyclotetrasiloxane were synthesized by acid hydrolysis of the corresponding dichlorosilanes (Petrarch Systems, Bristol, PA, U.S.A.) according to a procedure described earlier⁸.

Preparation of cyanosilicone gum phases

Hydrolysis. Prepolymers containing 33% cyano/33% tolyl, 50% cyano/25% tolyl and 75% cyano/12.5% tolyl groups were prepared from bis(cyanopropyl)dichlorosilane, methyl(tolyl)dichlorosilane and dimethyldichlorosilane (Petrarch Systems), respectively.

Prepolymers were prepared by basic reversed hydrolysis through the slow addition of an excess of 6 *N* ammonia solution to the silane mixtures with vigorous stirring at 0°C and under an atmosphere of nitrogen⁹.

Polymerization of the prepolymer. After drying, the prepolymers were further polymerized to gums using sodium silanolate and tetramethylammonium hydroxide as catalysts^{1,10}, the reactions being performed with stirring under an atmosphere of dry nitrogen. The gums were then carefully neutralized, rinsed with water to remove catalyst residues and finally dried under calcium sulphate. Low-molecular-weight material was removed by fractionation in methylene chloride-methanol. Residual silanol groups were capped by reaction with 1,3-divinyltetramethyldisiloxane (Petrarch System) in refluxing acetonitrile for 6 h under nitrogen. Finally, the gum was passed through a second fractionation procedure.

Column preparation

Two types of fused silica of I.D. 0.20 mm were used, obtained from Hewlett-Packard (Avondale, PA, U.S.A.) and Chrompack (Middelburg, The Netherlands). Before coating, most of the capillaries were treated with hydrochloric acid and silanized, but some capillaries were coated directly. For the acid treatment of the fused silica, a solution of 50% analytical-reagent grade concentrated hydrochloric acid (Merck, Darmstadt, G.F.R.) in glass-distilled water was prepared. A plug (*ca.* 10% of

the column length) of this solution was pushed through the capillary by nitrogen. When the plug had left the capillary the capillary ends were carefully sealed, after which the capillary was heat treated at 100 or 150°C. The acid was displaced by one capillary length of dilute hydrochloric acid (pH 3) followed by one capillary length of analytical-reagent grade methanol (Merck). Dehydration was carried out at 260°C for 5 h under a slow stream of nitrogen. The leached fused-silica capillary was then directly dynamically coated with a 20% (w/v) solution of the cyclic cyanosiloxane in methylene chloride at a constant rate of 20 mm/sec in such a way that an even film of the siloxane covered the silica walls. After evaporation of the solvent (6 h), the capillary ends were carefully sealed under vacuum, during which no air was allowed to enter the column. Chemical modification was carried out at 395°C for 2 h, the oven being heated and cooled slowly at 5°C/min. The column end was opened under methylene chloride, thus forming a plug that was used to rinse the column from excess of reagent, after which the column was allowed to dry¹¹.

Of the stationary phases used, three were synthesized in our laboratory and one, Silar 10C, was obtained from Applied Science Labs. (State College, PA, U.S.A.). The home-made silicone gums 33-CN, 50-CN and 75-CN were dissolved in methylene chloride; 3 or 5% of dicumyl peroxide (Merck), calculated from the amount of stationary phase, was added to the coating solution if the phase was to be cross-linked by peroxides. Silar 10C was dissolved in ethyl acetate. Before use, the peroxide was recrystallized from ethanol-water (72:28) and the methylene chloride was distilled over phosphorous pentoxide. Only freshly prepared solutions were used. Coating of the capillaries was performed by the static method, with the capillary in a horizontal position in a water-bath. The coated column was opened in an atmosphere of dry nitrogen, and any possible residues of solvent were then flushed out of the column with dry nitrogen.

Dynamic curing was performed to achieve cross-linking of the silicone gum. The column, containing peroxide, was thus installed in a gas chromatography (GC) oven, programmed from 40 to 170°C at a rate of 5°C/min and maintained at 170°C for 40 min; a slow stream of dry hydrogen (0.1 ml/min) was allowed to pass through the column during the curing. After curing, the column was rinsed with 5 ml of methylene chloride and conditioned to 250°C at 1°C/min.

Using radiation from a high-voltage electron source, we attempted to cross-link the 33% cyanopropyl-substituted phase. A radiation dose of 25 Mrad was used.

RESULTS AND DISCUSSION

Fused silica has a high energy surface, and is considered to be wettable by high-polarity polysiloxanes¹². However, we have experienced considerable variations between batches of silica. Fused silica of good quality was thus wettable for cyanosilicone gums with cyano substitution up to about 75%. Columns prepared from unmodified fused silica show very good efficiency, but they show adsorption of amines and alcohols in nanogram amounts (Fig. 1). To achieve a higher degree of column deactivation, we silanized the surface at high temperatures. Silanization alters the wettability of the fused silica surface and, in order to achieve sufficient wettability for coating with cyanosilicone gums, the silanization was performed with cyanosiloxane concentration of only 0.2 groups/nm². Further, a prerequisite for evenly covering

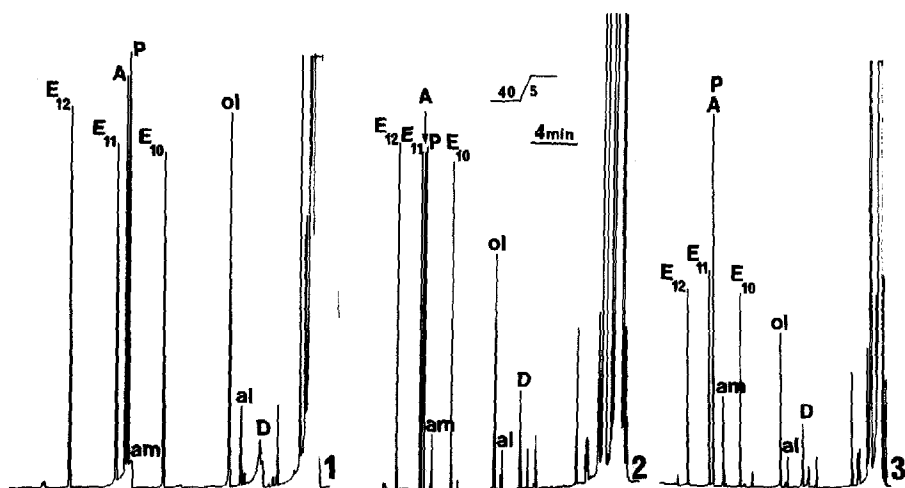


Fig. 1. Gas chromatograms (flame ionization detection, FID) of a Grob test mixture on fused-silica capillary columns coated with 33CN rubber phase. Columns: 1 = no pre-treatment; 2 = HCl-treated and silanized with 50% cyano-substituted siloxane; 3 = HCl-treated and silanized with 100% cyano-substituted siloxane. Peaks: D = 2,3-butanediol; al = nonanal; ol = 1-octanol; A = 2,6-dimethylaniline; P = 2,6-dimethylphenol; am = dicyclohexylamine; E₁₀, E₁₁, E₁₂ = C₁₀-, C₁₁-, C₁₂-acid methyl ester, respectively. Each peak corresponds to *ca.* 1 ng of substance.

substituted cyclic siloxanes¹¹. The desired wettability was not obtained, however, on silanization of untreated fused silica. This may depend on the fact that the concentration of silanol groups in a fused-silica surface is low. Wright *et al.*¹³ found a silanol

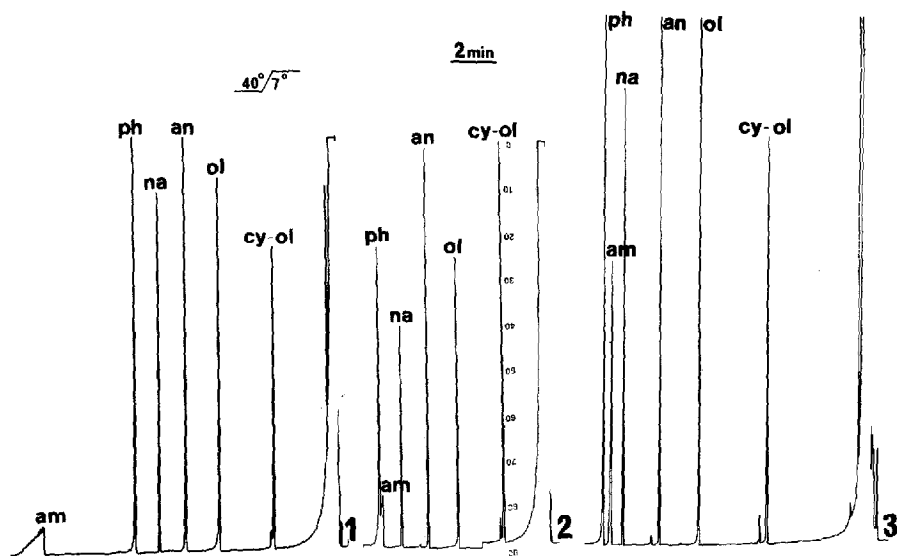


Fig. 2. Gas chromatograms (FID) of a test mixture on different capillary columns coated with a 33CN rubber phase. Columns: 1 = dynamically treated with HCl at 100°; 2 = statically treated with HCl at 100° for 24 h; 3 = statically treated with HCl at 150° for 24 h. Peaks: cy-ol = cyclohexanol; ol = octanol; an = aniline; na = naphthalene; ph = phenol; am = dicyclohexylamine. Each peak corresponds to *ca.* 1 ng of substance.

silanization is that the silanizing reagent is distributed regularly over the capillary surface during the reaction. The reagents used here do not wet non-treated fused silica sufficiently, and have too high boiling points for evaporation; these factors may explain the unsatisfactory results. It was found, however, that satisfactory silanization could be achieved on fused-silica capillaries that had been treated with hydrochloric acid. Great care was taken not to contaminate the fused-silica surface during hydrochloric acid treatment. Different dynamic and static procedures for acid and also base treatment were examined, and we found that an acid treatment at 150°C, with a film of acid on the capillary wall, gives the most reproducible results (Fig. 2).

Two types of cyclic tetrasiloxanes were used for the silanization, having 50 and 100% cyano-substitution, respectively. Equally good results were obtained with both reagents; *cf.*, efficiencies in Table I and deactivation in Fig. 1. It should be pointed out, however, that the 50% cyanosilylated surface can be coated with stationary phases having a maximum of 50% cyano substitution.

No air or solvent residues should be present in the column during silanization, as these will cause destruction of the cyanosiloxane.

The fused-silica columns were statically coated. We have not yet found a solvent that is suitable for static coating with highly polar cyanopropyl(methyl)silicone gums; acetonitrile may be used, but columns of low efficiency are achieved⁵. The presence of some tolyl substitution in a cyanosilicone gum, however, makes it soluble in methylene chloride, which we consider to be an excellent solvent for static coating on account of its moderate heat of vaporization.

Peroxide-initiated cross-linking of cyanosilicone gums must be performed by a dynamic procedure where the column is slowly purged with an inert gas during reaction; some active reaction products are thus rinsed out. After dynamic curing, no change in activity or efficiency caused by the peroxide can be detected, even though as

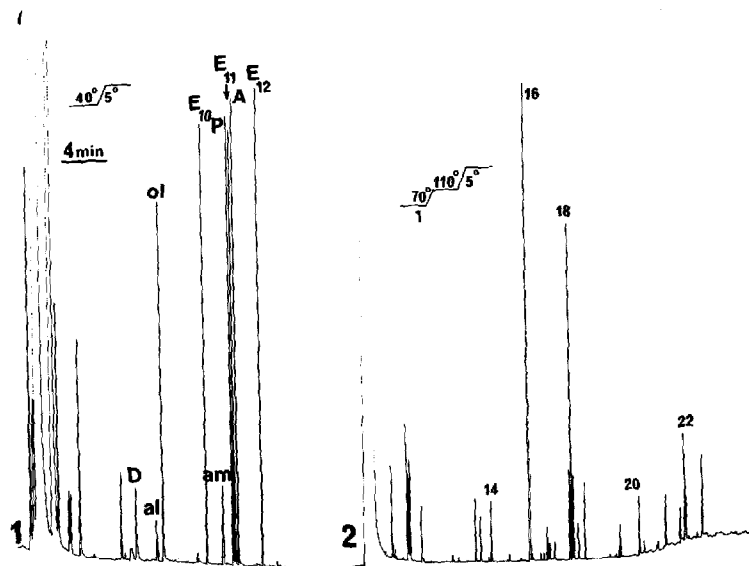


Fig. 3. Gas chromatograms (FID) on a radiation-cured 33CN fused-silica capillary column. 1 = Grob test mixture, peaks as in Fig. 1; 2 = splitless injection of a fatty acid methyl ester extract from a biological testis standard mixture.

TABLE I
CHARACTERISTICS OF SOME FUSED-SILICA COLUMNS COATED WITH CYANOSILOXANES

HETP = Height equivalent to a theoretical plate; MAOT = maximum allowable operating temperature; UTE = utilization of the theoretical best efficiency.

Stationary phase	Substituents	Pre-treatment, type of leaching	Chemical modification	Silicone viscosity	Capacity ratio, <i>k</i> (naphthalene)	HETP (mm)	UTE (%)	Kováts retention index at 90°C		MAOT (°C)
								Octanol	Naphthalene	
33-CN	33% cyanopropyl 33% methyl 33% tolyl	—	—	Rubber	12	0.20	100	1382	1552	275
33-CN		Dynamic, 100°C	100% CN	Rubber	12	0.30	66	1387	1552	300
33-CN		Static, 150°C	100% CN	Rubber	13	0.22	88	1389	1556	300
33-CN		Static, 150°C	50% CN	Rubber	19	0.19	107	1386	1559	300
33-CN		Static, 150°C	50% CN	Gum	18	0.26	78	1391	1555	300
33-CN*		Static, 150°C	50% CN	Rubber	15	0.26	77	1379	1549	300
50-CN	50% cyanopropyl 25% methyl 25% tolyl	—	—	Rubber	6	0.20	94	1438	1637	250
50-CN		Static, 150°C	100% CN	Rubber	11	0.50	40	1485	1661	300
50-CN		Static, 150°C	50% CN	Rubber	21	0.26	78	1448	1629	300
75-CN	75% cyanopropyl 12.5% methyl 12.5% tolyl	—	—	Rubber	13	1.25	16	1659	1847	200
75-CN		Static, 150°C	100% CN	Rubber	17	0.34	54	1647	1817	300
75-CN		Static, 150°C	100% CN	Gum	14	0.36	56	1615	1832	275
75-CN		Static, 150°C	50% CN	Rubber	Droplets					
Silar 10C	100% cyanopropyl	Static, 150°C	100% CN	Fluid	17	0.27	75	1617	1833	275

* Radiation-initiated cross-linking.

much as 3–5% of dicumyl peroxide must be used in order to achieve cross-linking. Bleeding will, however, be increased on peroxide-initiated vulcanization of cyanosilicone coated columns. In an attempt to avoid such an increase in column bleeding, we tried to cross-link the cyanosilicone gums by radiation, a method that has been used for many years in the rubber industry^{14–16}. This procedure has recently been used for curing silicone-coated columns^{17,18}. Curing by radiation has several advantages: first, no chemicals that might cause a reduction in the column performance are introduced; second, reaction takes place in a closed system at room temperature; and third, the capillaries can be tested before vulcanization. Preliminary results indicate that vulcanization of cyanosilicones by radiation is preferable to peroxide-initiated vulcanization. To optimize the dose rate for the different cyanogums, the molecular weight distribution of the gum phase has first to be examined, and all low-molecular-weight material must be removed in order to achieve reproducible results. The relatively high total dose used here, 25 Mrad, gave no detectable change in column performance (Fig. 3).

In the preparation of silicone gums, two fractionation steps were performed in order to remove low-molecular-weight material. Fractionation was thus performed after polymerization and also after end-capping. It was found that the percentage of the stationary phase that was extractable after curing is strongly dependent on the molecular weight distribution of the silicone gum that is to be cured. The fractionation can be improved by the use of size exclusion chromatographic methods and the separation system should be adjusted so that the fraction of interest is collected in the total exclusion volume. In such a separation, trace amounts of alkali residues that originate from the polymerization step can be removed. Alkali residues are known to catalyse silicone decomposition¹⁹.

The principal advantage of cross-linked cyanosilicone stationary phases compared with corresponding silicone gums is their much greater stability. Cross-linking thus leads to much higher maximum allowable operating temperature (MAOT) (Table I). For a conventional silicone phase, film thickness may be a factor that critically affects MAOT. Silar 10C is thus stable to 275°C when applied as a thin film

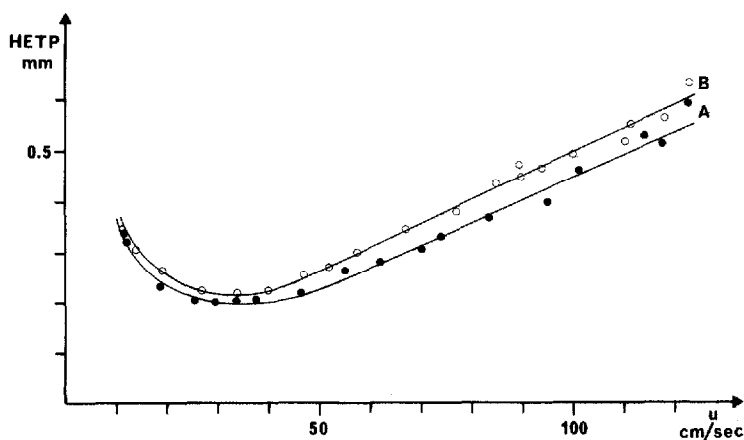


Fig. 4. Van Deemter curves for a 33CN rubber and a corresponding gum on fused silica capillaries. Stationary phase thickness = 0.30 μm . Sample = methylated fatty acid, $\text{C}_{18,1}$, with a capacity ratio of ca. 10 at 180°C. A = 33CN rubber; B = 33CN gum.

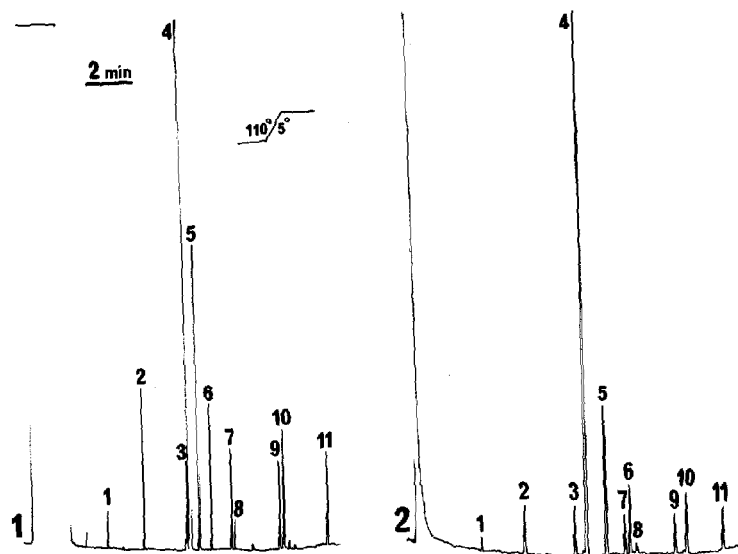


Fig. 5. Gas chromatograms (FID) of a fatty acid methyl ester standard mixture on different capillary columns. Columns: 1 = 50CN rubber on a fused-silica column, deactivated with bis(cyanopropyl)cyclosiloxane; 2 = 75CN rubber on the same type of support as 1. Peaks: 1 = 14:0; 2 = 16:0; 3 = 18:0; 4 = 18:1; 5 = 18:2; 6 = 18:3; 7 = 20:0; 8 = 20:1; 9 = 22:0; 10 = 22:1; 11 = 24:0.

(film thickness, $d_f \approx 0.05 \mu\text{m}$); thicker films of Silar 10C have MAOT of 250°C or less. The thickness of cross-linked stationary phase films does not affect stability in such a determinative way. The columns coated with cross-linked phases presented in this work have a $d_f > 0.20 \mu\text{m}$.

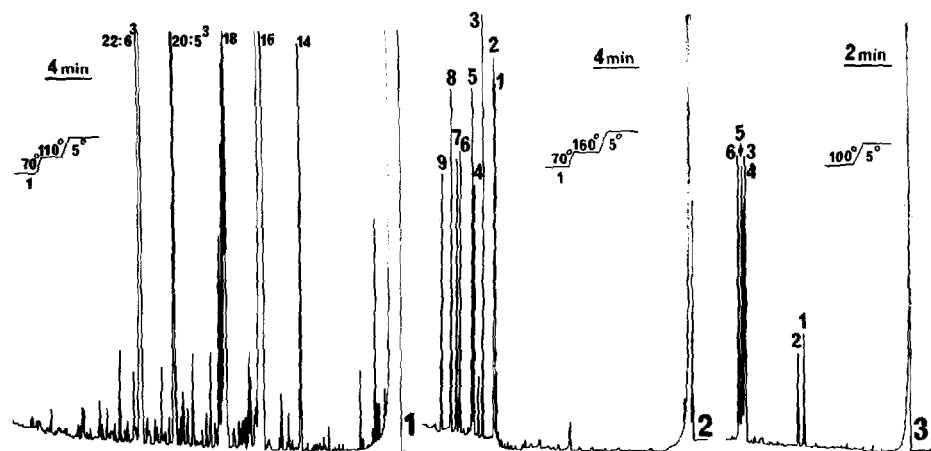


Fig. 6. Gas chromatograms (FID) on different fused-silica capillary columns coated with rubber phases. 1 = Column coated with 50CN rubber; sample, fatty acid methyl esters from a herring liver extract; splitless injection. 2 = Column coated with 33CN rubber; sample, test mixture of underivatized antidepressants. Peaks: 1 = amitriptyline; 2 = trimipramine; 3 = imipramine; 4 = nortriptyline; 5 = desmethylnortriptyline; 6 = desipramine; 7 = protriptyline; 8 = desmethyldoxepine; 9 = chlomipramine. Chromatogram 3 = column coated with 50CN rubber; sample, pheromone test mixture. Peaks: 1 = Z6-12:Ac; 2 = Z9-12:Ac; 3 = E7-14:Ac; 4 = Z7-14:Ac; 5 = Z10-14:Ac; 6 = E10-14:Ac.

Cross-linking of the stationary phase does not influence the retention properties of octanol and naphthalene, as reflected by the Kováts retention index for these substances (Table I). The retention indices also indicate that the cross-linked phase 75CN gives retentions for these compounds similar to those obtained when using Silar 10C.

In the Golay equation, resistance to mass transfer in the liquid phase is expressed by the C_L term. For a given stationary phase, C_L increases with film thickness²⁰. For methylsilicone gums, it was found that C_L increases with increasing size of the solute molecules²¹. Further, C_L may be decreased when temperature is increased²². It may be assumed that C_L should also increase when a stationary phase is immobilized by cross-linking. In a preliminary test, no such increase was observed, however (Fig. 4); in fact, the vulcanized phase shows a lower value of C_{L+G} than the corresponding gum.

Some applications are demonstrated in Figs. 5 and 6.

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