Glass Open-Tubular Capillary Columns with Chemically Bonded Methyl-Phenyl Siloxane Stationary Phases of Tailor Made Polarity

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Summary

The previously described method yielding apolar, chemically bonded methyl polysiloxane glass capillary columns was extended to the production of capillary systems of controlled polarity. The approach involved prior synthesis of a reactive methyl-phenyl polysiloxane polymer by copolymerisation of a mixture of dimethyl and diphenyl chlorosilanes. The polymer was then chemically bonded to the capillary glass surface to yield remarkably stable, high resolution analytical systems which were shown to be particularly well suited to the separation of biochemical mixtures at the subnanogram level. The principle of copolymerisation of differently substituted silanes confers to the method a considerable flexibility which could be used to devise polar capillary systems tailor made for a given analytical problem.

Manufacture of long life thermostable glass capillary columns coated with polar stationary phase has been a challenging problem in gas chromatography (GC) during recent years. One of the key difficulties recognized is the critical wettability of the glass surface which hinders the production of stable coatings, especially with polar polymers. Most research groups in the field have made use of commercially available polymers as coating material and the aim has generally been to increase the specific coating surface of the capillary wall. This has been attempted by two main approaches: (i) physico-chemical treatment of the glass surface, either by etching [1, 2], dehydration [3] or whisker growth [4], leading to open tubular systems which have facilitated coating (WCOT) with a limited number of commercial polymers;
(ii) stabilization of the stationary phase layer by dispersion over microparticles as a colloidal coating suspension, to obtain porous layer systems (PLOT) which have been used for the preparation of a larger variety of polar GC columns [5–11].

We have previously proposed a different approach involving the preparation of a reactive siloxane polymer which could then be chemically bonded to a capillary glass surface [12]. Remarkably stable, high-resolution, apolar GC columns were obtained and have been satisfactorily used for analysis of biological mixtures [13, 14].

In this paper, we report the extension of this principle to the preparation of chemically bonded stationary phases, using methyl-phenyl siloxane polymers prepared in the laboratory. A wide range of polymers with controlled polarity could be obtained. This confers a considerable flexibility to this approach and introduces the possibility of polar columns tailor-made for a given analytical problem. GC parameters of the resulting capillary systems are reported, in relation to some chemical properties of the siloxane polymers constituting the stationary phase. The polar capillary columns obtained are shown to be particularly well suited to the analysis of complex biological mixtures such as steroid metabolites with excellent qualitative and quantitative performance.

Material and Methods

Dimethyldichlorosilane (DMCS), diphenyldichlorosilane (DPCS) were from SILAR Laboratories, Inc., Watervliet, NY (USA).

Commercially available methyl-phenyl polysiloxane stationary phases were from Applied Science Laboratories (USA).

1. Preparation of siloxane polymers

The previously described procedure [14] for preparation of siloxane polymers by hydrolysis of homologous (either dimethyl or methyl-phenyl) chlorosilanes was used, starting from a mixture of two differently substituted chlorosilanes. DMCS and DPCS in the desired proportion usually for a total volume of 20 cm^3 were mixed with constant stirring. To this mixture was added dropwise about 60 cm^3 7 mol dm^{-3} NH_3 at room temperature. Disappearance of acidic gas emission indicated the end of the reaction. A polymer of variable viscosity (depending upon the starting proportion of DPCS) resulted and was decanted and washed with distilled water until neutral. Each partition step was completed by a brief centrifugation. Handling of highly viscous preparations was facilitated by dilution with dichloromethane. The polymer was finally collected after a 20,000 g centrifugation for 15 min and stored in dark bottles at 5–10 °C.

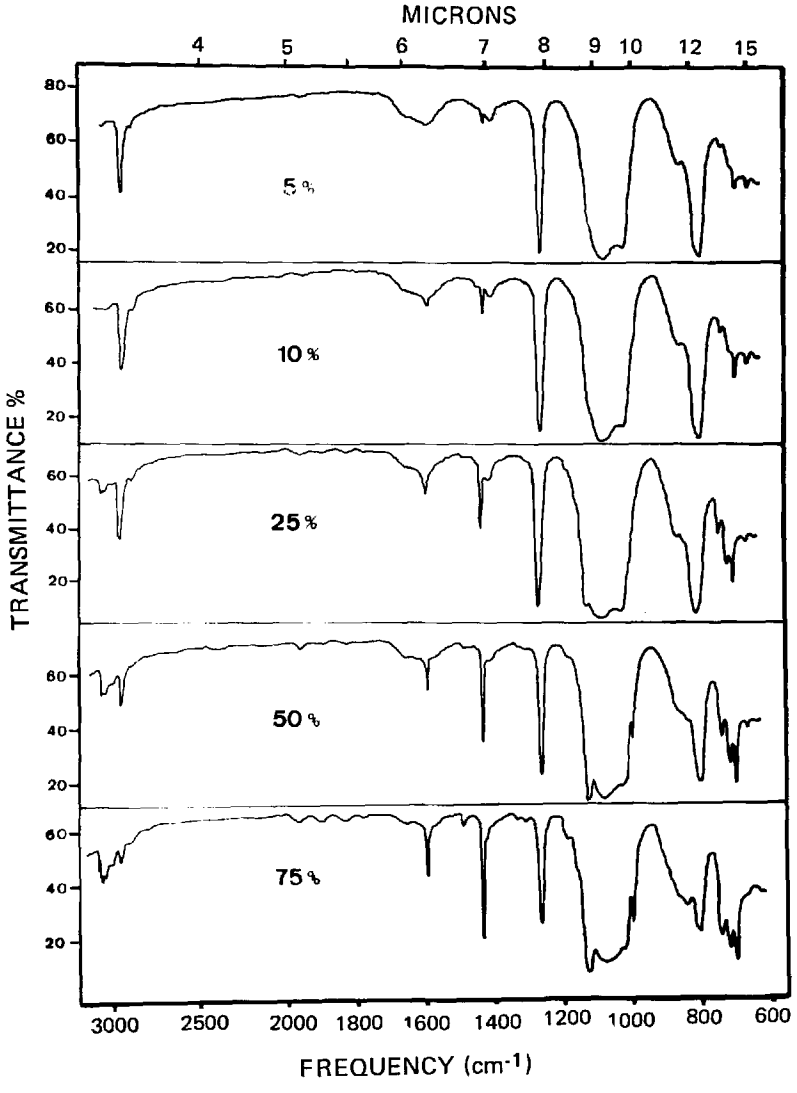

Fig. 1

● Infra-red spectra of synthetic methyl-phenyl-polysiloxanes with increasing phenyl-to-methyl ratio.

2. Glass capillary preparation

Pyrex (Schott, Mainz, Germany) glass tubes (6 mm OD, 2 mm ID) were introduced in a glass drawing machine (Sedere, Paris, France) to yield coiled capillary tubes of 0.20–0.25 mm ID. This diameter was evaluated on several pieces of each capillary with a microscope equipped with a micrometer. The glass capillary (20 to 25 m long), was then filled with gaseous HCl according to *Alexander* [1], sealed at both ends and heated in an oven at 380 °C for 2 hours.

3. Coating and irreversible bonding of the polymer to the capillary

After flushing the capillary with a nitrogen stream, the polymer solution (10 % in dichloromethane) was introduced as a plug (about 1/4 of the column length) and pushed through the tube under a slight nitrogen pressure. As the polymer plug emerged, the nitrogen flow was increased and maintained for 18 hours. The dry column was then filled with gaseous ammonia [13, 14], sealed and introduced into a GC oven at 70 °C. The temperature was raised at 0.4 °C/min up to 180 °C and then maintained for 24 hours.

After flushing with a nitrogen stream, the column was placed in the gas chromatograph and conditioned by programming the temperature from 100 to 320 °C at 2 °C/min. After 24 hours at 320 °C, the column was ready to use.

4. Infrared spectra were recorded using a Perkin-Elmer model 521 spectrometer; the polymer was disposed between two NaCl pellets sufficient to yield a light transmission of 10–60 %.

5. Gas chromatographic procedures using a dry injection device and all ancillary techniques including steroid derivative preparation were as previously described [13, 14].

Results and Discussion

1. Copolymerisation of dimethyl and diphenyl-silanes

A series of siloxane polymers were prepared with starting chlorosilane mixtures containing increasing ratios of DPCS to DMCS (5 : 95 to 75 : 25). The resulting polymers were analysed by infrared spectroscopy and the corresponding spectra are given in Fig. 1. All spectra exhibited a

1441 cm^{-1} band attributed to the Ph-Si bonds [17] as well as the 1025 cm^{-1} and 1080 cm^{-1} bands generated by the Si-O-Si bonds of linear polysiloxanes [15]. In addition, the intensity of two absorption bands progressively increased as the content of DPCS in the starting mixture was raised. These could be attributed to the Ph-Si (1125 cm^{-1}) and aromatic C = C (1632 cm^{-1}) bonds, respectively [16]. These spectra were qualitatively similar to those given by the methyl-phenyl-siloxanes observed by others [16]. It may thus be concluded that the hydrolysis conditions used with mixtures of DMCS and DPCS led to the copolymerisation of the differently substituted silanes since phenyl substitution increased with increasing proportion of DPCS in the reaction mixture.

In order to check if the amount of phenyl substitution in the resulting methyl-phenyl polysiloxane (MPPS) could be accurately defined by the DPCS/DMCS starting ratio, the IR data were used in a quantitative fashion, slightly modified from *Lady* et al. [17]. This method linearly relates the Ph/Me ratio in the polymer to the ratio

$$\frac{\text{OD at 1441 cm}^{-1}\text{ (Ph)}}{\text{OD at 1266 cm}^{-1}\text{ (Me)}}.$$

To assess the quantitative validity of the method, we recorded the IR spectra of methyl phenyl siloxanes with commercially defined phenyl substitution, i.e. OV-1 (0 %); OV-7 (20 %); OV-11 (35 %); OV-17 (50 %); OV-22 (65 %). The corresponding figures are given in Table I and were plotted as shown in Fig. 2. A linear relationship was indeed obtained, with a regression coefficient of 0.986 and an ordinate at the origin very closed to the theoretical zero value (0.014). When applied to our synthetic polymers, the resulting figures (Table I) and the corresponding plot (Fig. 2) showed that the ratio of DPCS to DMCS in the starting mixture directly governed the Ph/Me ratio in the resulting polysiloxane structure. In fact, the linear relationship between the experimental DPCS/DMCS ratio and the IR Ph/Me ratio was better (r = 0.998) than with commercially available polymers. However, the average straight lines calculated in both cases by the least squares method could be superimposed (Fig. 2). These data indicated that DMCS and DPCS participated equally well in the hydrolysis-polymerisation process to yield a Ph-Me-siloxane-polymer directly reflecting their respective concentration. However, this may not hold if the polymerisation method is to be extended using differently substituted starting chlorosilanes.

Table I. Quantitative evaluation of the phenyl substitution, by IR spectroscopy in the MPPS resulting from the co-polymerization of DMCS and DPCS, and in the commercial methyl-phenyl polysiloxanes.

Phenyl ratio	5	10	20	25	35	50	65	75
T 1441 cm^{-1} / T 1266 cm^{-1}								
MPPS	0.07	0.150	–	0.400	–	0.670	–	1.10
Commercial MPPS	–	–	0.460	–	0.544	0.832	0.891	–

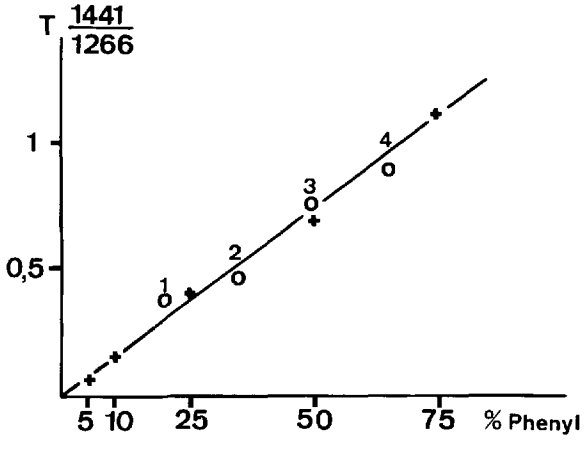

Fig. 2

• Linear relationship between percentage of DPCS in DMCS-DPCS starting mixture, before polymerisation and transmittance obtained at 1441 cm^{-1} and 1266 cm^{-1}.
+ – methyl-phenyl-polysiloxanes synthetized by copolymerisation of two chlorosilanes.
o – methyl-phenyl-polysiloxanes of commercial origin.

In the case of phenyl-methyl-siloxanes, the polarity of the polymer obtained (i.e. the ratio Ph/Ph + Me substituents) by our method can thus be accurately controlled within a wide range of phenyl substitution. This confers a remarkable flexibility in the design of a stationary phase ideally adapted to a given analytical problem.

2. *Chromatographic properties of chemically-bonded polar capillary columns*

a) Chromatographic parameters

Tetracosane and hexacosane were used as test compounds with isothermal separations at 220 °C and the major chromatographic characteristics calculated for the columns coated with MPPS ranging from 5 to 75 % phenyl substitution (Table II). These values compared favorably with the parameters reported by others using classical approaches with non-chemically bonded stationary phases in the manufacture of their capillary columns [3, 9, 18, 19]. One may notice that the theoretical plate number decreased as the polarity increased. However, we never observed less than 2,000 theoretical plates per meter in all cases and even these columns were quite satisfactory in yielding good overall gas chromatographic separations.

The response of our GC systems in relation to the mobile phase velocity was examined for each manufactured columns. Fig. 3 gives the van Deemter curves obtained with two capillary columns of widely differing polarity (25 and 75 % phenyl substitution, respectively). The optimum gas velocity did not greatly differ for the two systems; on average, it was found to be between 30 to 40 cm/sec.

Table II. Chromatographic parameters obtained for glass capillary columns irreversibly coated with MPPS polymers of increasing polarity, obtained by increasing degree of phenyl substitution. Temperature 220 °C. Reference compounds: tetracosane and hexacosane.

% Ø	5	10	20	25	50	75
L (m)	19	20.5	20	22	26	18.5
k'(nC_{24})	7.7	8.6	6	5.9	5.8	3.5
α (C_{24}–C_{26})	1.94	1.92	1.93	1.94	1.91	1.86
N	59770	53950	53832	56080	65000	38430
N/m	3145	2630	2691	2550	2500	2080
HETP (mm)	0.32	0.36	0.37	0.39	0.40	0.46
U_{opt}(cm s^{-1})	36	42	32	36	36	34
TZ	28	24	27	28	27	20

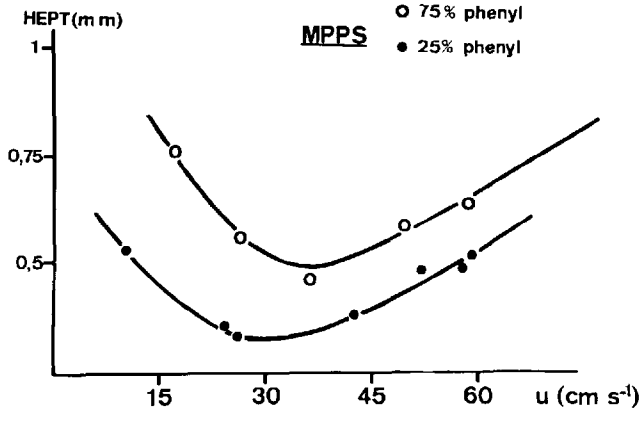

Fig. 3

Van Deemter curves obtained with glass capillary columns, chemically bonded to two synthetic methyl-phenyl-polysiloxanes of widely differing phenyl substitutions.

b) Quantitative analysis and adsorption problem

The resolution and sensitivity of glass capillary analysis require careful evaluation of the quantitative recovery of the samples processed through the system. If the method is to be used in biochemical analysis, especially with fragile and/or polar structures, quantitative results at the ng (and pg) level would be desirable. Fig. 4 shows a calibration curve obtained using non-derivatized progesterone used as a test steroid and analysed on a 20 % phenyl-substituted chemically-bonded siloxane column. The response was satisfactorily linear in the range 1–40 ng steroid injected. Thus, there was no appreciable loss of sample by adsorption at this level; however, a slight loss of progesterone was observed below 1 ng, although 600 pg of the compound was detectable. This adsorption phenomenon was obviated even in the pg range when polar oxygenated steroids were derivatized which indeed represents the general practical analysis conditions. In this case, no appreciable adsorption impaired the detection of C-19 steroid (e.g. androsterone) as methyloxime-trimethylsilyl derivatives in the 400–1000 pg range.

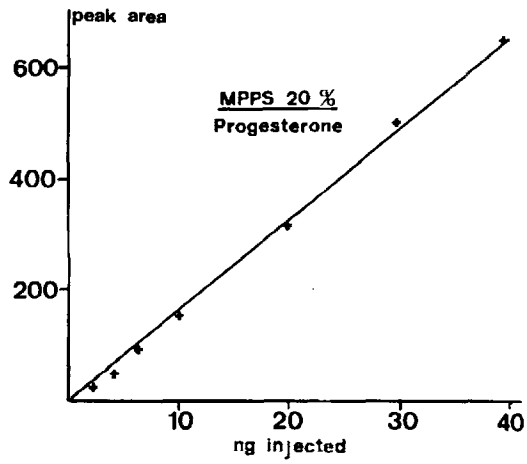

Fig. 4

• Calibration curve obtained for progesterone on glass capillary column with chemically bonded 20 % phenyl MPPS polymer.

The adsorption phenomenon has been a well recognized general problem in gas chromatography, especially in the case of analysis of polar hydroxysteroids. In the classical approaches using physical glass capillary coating as the basic principle, several procedures have been proposed to obviate this difficulty, such as silanization of the glass surface [20, 21], use of triethanolamine [22], carbowax [23, 24] or a surfactant [25]. In the development of our chemical bonding procedure, these methods were evaluated; however, all of them were found either ineffective or impaired the bonding process of the phase to the glass surface. A careful study of the heating conditions during the chemical bonding process led to the conclusion that a slow rate of temperature increase (0.4 °C/min) up to less than 200 °C led to the best results as far as adsorption was concerned. This may be explained by a smooth glass-polymer bonding reaction taking place under these conditions, as opposed to the additional intramolecular reactions which may occur in the polymer at higher temperatures (> 300 °C).

3. Application to the separation of biological mixtures

Thanks to the flexibility of the procedure, it was possible to select a synthetic stationary phase of controlled polarity, according to the analytical problem to be tackled. An example of a difficult separation which was resolved in this way is illustrated in Fig. 5. This concerned the separation of the two anomeric forms of glucose as 1-methyl,3-tosyl, 2,4,6-trimethyl-silylether derivative. The two isomers could not be separated on a GC column packed with a mixed phase system widely used in carbohydrate analysis (Fig. 5). The continuous polarity range of our methyl-phenyl-siloxane capillary systems permitted the selection of the best conditions for resolution. One of those was given by a 50 % phenyl substituted polymer which gave a wide separation between the two isomeric glucoses

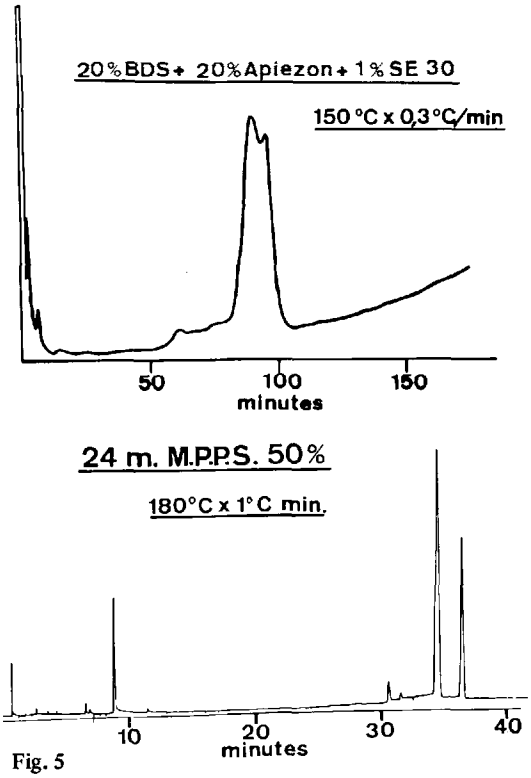

Fig. 5

• Gas chromatographic analysis of glucose anomers as 1-methyl, 3-tosyl-2,4,6-TMS.

Upper panel: separation obtained with conventionally packed column.

Lower panel: separation obtained using 24 m, 50 % phenyl-MPPS, chemically-bonded glass-capillary column.

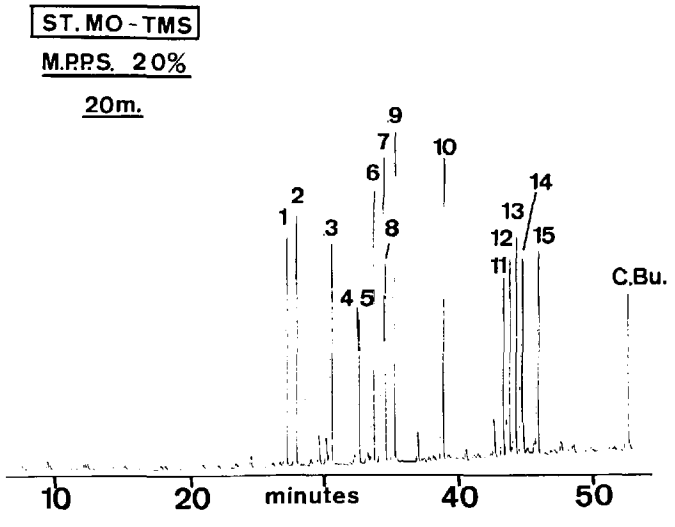

Fig. 6

• Gas chromatographic separation of mixture of steroids as MO-TMS, on 20 m, 20 % phenyl MPPS chemically-bonded glass-capillary column. Temperature programmed at 2 °C/min from 180 °C to 300 °C.

Peaks: 1 = Androsterone; 2 = Etiocholanolone; 3 = Dehydro-epiandrosterone; 4 = 11-Keto-Androsterone; 5 = 11-Keto-Etiocholanolone; 6 = 11-OH-Androsterone; 7 = 11-OH-Etiocholanolone; 8 = Pregnanediol; 9 = Pregnanetriol; 10 = Tetrahydrodesoxycortisol; 11 = Tetrahydrocortisone; 12 = Tetrahydrocortisol; 13 = Allo-Tetrahydrocortisol; 14 = Cortolone; 15 = β-Cortolone; CBu = Cholesteryl butyrate (Internal Standard).

(Fig. 5). In addition, the analysis time was greatly shortened with our capillary system.

Fig. 6 shows that these methyl-phenyl-siloxane, chemically-bonded capillary columns were found highly satisfactory for the analysis of biological steroid metabolite mixtures, both from the qualitative and the quantitative points of view. This type of separation has been in routine use in our laboratory for physio-pathological investigations of hormonal steroids in humans. The remarkable thermal stability of these capillary systems led to a low bleeding level during repeated temperature programming up to 320 °C (Fig. 6). Several of these columns have been in daily use in our laboratory under these conditions for nine months without loss of their chromatographic properties.

Conclusion

It has been previously demonstrated that polysiloxane stationary phases could be irreversibly bonded to glass surfaces to yield gas chromatographic capillary columns of high resolution and remarkable stability. The method used prior synthesis of a reactive siloxane polymer from a homologous silane monomer and further bonding of the polymer to the capillary glass surface [13, 14]. The present work extends the method to the synthesis of siloxane polymers from a mixture of two differently substituted silanes. This approach yielded a simple and straightforward procedure for the manufacture of highly stable glass capillary systems of accurately controlled polarity. The method is thus highly flexible and can be applied to devise tailor-made capillaries, suited to a particular analytical problem. These GC systems appeared very suitable for both qualitative and quantitative analysis of complex biochemical mixtures.

The overall advantages of this approach should help widen greatly the potential applicability of glass-capillary gas chromatography.

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