Use of Hydrolyzed Chlorosilanes for the Preparation of High Resolution Glass Capillary Columns

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Summary

A new procedure for the preparation of high resolution open tubular glass capillary columns is described. This procedure involves the preparation of polysiloxane polymers obtained by alkaline hydrolysis of alkyl chlorosilane. The mixture of polysiloxane polymers is then coated on the wall of previously HCl treated glass capillary columns using a dynamic method. A base-catalysed reaction using gaseous ammonia, applied to the coated polymers leads to a stable chemically bonded stationary phase, with non-polar characteristics. This type of column is easier to prepare than conventional ones and exhibits excellent chromatographic properties, both with regard to their resolution, stability and reproducibility. The flexibility of this method permits the use of other types of commercially available chlorosilanes (i.e. methylphenyl chlorosilane) to prepare polar polysiloxane polymers suitable for analysis of complex biochemical mixtures, such as steroid metabolites.

Introduction

It has often been suggested that chemical bonding should offer a potential answer to many of the difficulties encountered in the preparation of chromatographic systems, especially with regard to the stability of the stationary phase film, which is of prime concern in glass capillary gas chromatography. Several groups have described chemical bonding procedures for the preparation of liquid [1] as well as gas chromatographic [2,3] phases by esterification of various solid supports but the thermal stability of the resulting materials was questionable [1–3]. Bonding of siloxane polymers on to silica particles or porous glass has been reported [4–6] and used for liquid or gas chromatographic separations [7]. These materials were limited to non-selective stationary phases and have been used to prepare low resolution, conventional packed GLC columns [7].

The invaluable advantages of glass capillary systems for high resolution GLC separations of complex biological mixtures and the well recognized problems related to the preparation of corresponding stable columns led us to investigate the potential of chemical bonding of polysiloxane polymers to a conveniently prepared inner capillary glass wall. The principle of such an approach has been presented [8] using chlorosilane hydrolysis "in situ"; however, no chromatographic parameters nor any GLC applications have ever been reported using this method.

We have previously described a new approach to the preparation of non-polar high resolution glass capillary columns coated with highly stable methylpolysiloxane polymer film [9, 10]. The present report discusses the chemical bonding process on the basis of the experimental conditions required for the major steps of the procedure. In addition, the method has been extended to the preparation of glass capillary systems of various polarities. The high resolution GLC columns obtained by this simple and straightforward procedure are remarkable for their stability; their chromatographic performance remained unchanged for a year of continuous use.

Experimental

Glass capillary tubing. A glass drawing machine (Sedere, Paris, France) was used to prepare glass capillary tubing (0.16 to 0.30 mm ID) from pyrex glass tubes (6 mm OD, 2 mm ID: Sovirel, France or/and Schott, FRG), after successive washings with water, methanol, acetone and drying with a nitrogen stream. Lengths of 20–25 m were routinely used for capillary work.

Hydrochloric acid treatment. The capillary tube was filled with gaseous HCl according to *Alexander* [11], sealed at both ends, heated at 370 °C for 18 hours, then flushed with nitrogen.

Polysiloxane polymers. The following initial chlorosilanes were used in this work: dimethyl-dichlorosilane (DMCS: Merck, FRG); diethyl-dichlorosilane (DECS) and methyl-phenyl-dichlorosilane (MPCS; Silar, Scotia Laboratories, USA). To 20 cm^3 chlorosilane in a round bottom flask was added dropwise an excess (about 60 cm^3) 7 mol dm^{-3} aqueous ammonia, at room temperature. The oily polymer formed was left to decant overnight, then washed with distilled water until neutral and the remaining water eliminated by a 15 min centrifugation at 20,000 g. The final oily product was stored in a dark bottle until used.

Coating of the column. A plug of polysiloxane polymer (10–20 % v/v in dichloromethane), about 0.25 column length, was forced through the capillary tube at about 10 cm sec^{-1}, under nitrogen pressure. The nitrogen flow was increased as the plug emerged from the column and thereafter was maintained usually overnight or at least for 2 hours. The column was then filled with gaseous ammonia generated by reaction of solid sodium hydroxide with aqueous ammonia. Both ends of the coated capillary were flame-sealed and the column was heated in a GC oven by programming the temperature from 100 to 320 °C (1 °C min^{-1}) thereafter at 320 °C for 18 hours. The column was then ready for GC use after conditioning by one temperature program cycle from 150 to 300 °C (2 °C min^{-1}).

Gas chromatography (GLC) was carried out using Carlo Erba (models 2400 T and 2200) instruments, equipped with flame ionisation detectors and all-glass solid injection systems as described by *Ros* [12], with a carrier gas (hydrogen) flow between 0.6 and 1.5 $cm^3 min^{-1}$. Gas chromatography-mass spectrometry analyses were performed using an AEI double beam MS-30 mass spectrometer.

Scanning electron microscopy (SEM). Broken portions of capillary tubes were coated under vacuum with a 250 Å palladium-gold layer over a part of their inner surface. This method did not damage the glass surface organization nor introduce any contaminant in the observed samples. SEM observations were performed with a Stereoscan S-4 apparatus (Cambridge, UK).

Biological samples. Hydrolysis and extraction of hormonal urinary steroids and derivatization for GLC studies were carried out as previously described [13].

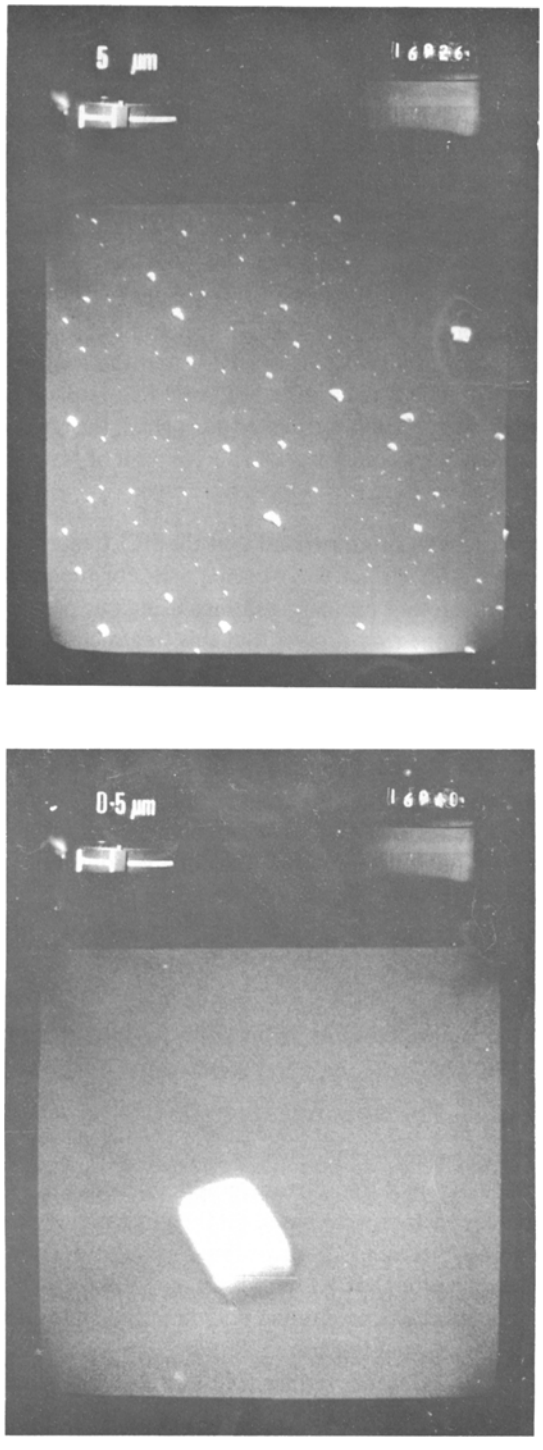

Fig. 1

● Scanning electron micrograph of HCl treated borosilicate glass surface.
(a) G = 2000, (b) G = 20 000 : microcrystal

Results and Discussion

1. HCl treatment of the borosilicate glass

The effect of etching of soda glass by HCl treatment has been investigated in detail [11, 14]. However, it has been suggested that gaseous HCl was without noticeable effect on borosilicate surfaces [11]. Fig. 1 shows a typical aspect of the inner pyrex glass capillary wall after the HCl treatment as described under Experimental. Strikingly regular pattern of crystal particles were distributed all over the treated surfaces. However, the size and distribution of the microcrystals were dependent upon the glass manufacture. Table I gives the average size and abundance of these crystals as evaluated from SEM recordings. However, the density of the crystalline particles was at least 100 times less than in the case of soda glass [14]. Experimental evidence suggested that these particles were indeed a consequence of HCl treatment and that they probably

Table I

	Pyrex Sovirel	Pyrex Schott
% Na_2O	3.5	4
Size of microcrystal	1 × 1.5 μm	1 × 0.6 μm
Density of microcrystals/μm^2	0.2	1

represented NaCl crystals. They could be removed by simple washing with deionized water; the washing water contained 0.5 and 1 ppm NaCl and KCl respectively whereas for similarly treated soda glass, the corresponding washings contained 14 and 1.3 ppm of NaCl and KCl respectively [15].

Since it was demonstrated that the HCl treatment of the pyrex glass surface was a prerequisite for the preparation of satisfactory capillary columns using our procedure [9, 10], one may suggest that this treatment contributes in several possible ways to the overall process: (i) it leads to the formation of microcrystals regularly distributed over the smooth silica surface, (ii) it may increase the number of accessible free hydroxyl groups on the glass surface [16], and (iii) it may promote the formation of Si-Cl bonds. The two latter phenomena may then be involved in the chemical bonding of the polysiloxane polymers on to the glass surface.

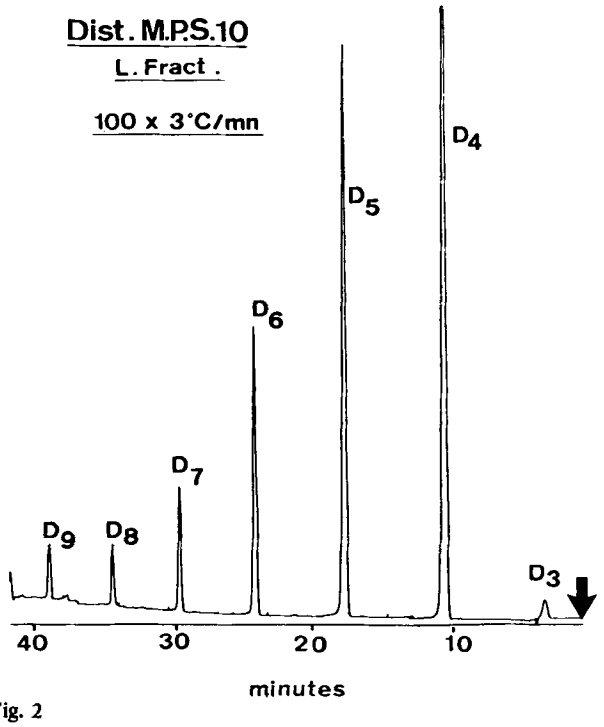

Fig. 2

● GLC analysis of the low boiling fraction of the methylpolysiloxane polymer on a 20 m MPS coated glass capillary column. Temperature programming from 100 °C to 310 °C at 3 °C min^{-1}.

2. Chemical bonding of the siloxane polymers to the glass surface

a. Nature of the siloxane polymers

The experimental conditions chosen in this work for the preparation of the siloxane polymers from the initial dichlorosilanes were based on the pionneering work of *Patenode* et al. [17]. According to this study, aqueous hydrolysis of DMCS under basic conditions yielded polymeric mixtures containing polydimethylcyclosiloxanes (20–50 %) and linear α,ω-dihydroxy-polymethylsiloxanes (50–80 %). Under similar conditions, diethylsilanediol was obtained from DECS [18].

Elementary analysis of our crude polymeric preparation for C, H, O and Si was performed. Table II gives the figures obtained as compared to those given by a commercial methylpolysiloxane OV-101. No detectable difference could be found, especially with regard to the oxygen content of the two polymers. In addition, no trace of Cl or N could be detected in our preparations.

Table II. (MPS = dimethylpolysiloxane)

	C %	H %	O %	Si %
M.P.S.	32,210	7,90	21,665	38,220
OV-101	32,625	8,03	21,125	38,570

The crude oily polymer obtained by hydrolysis of DMCS was fractionnated by distillation into a low ($T_{eb} < 200$ °C, under 2 mm Hg) and a high (residue) boiling fractions. Fig. 2 shows the GLC analysis of the low boiling fraction. The detected components could be identified by GLC using commercial model cyclic dimethyl polysiloxanes and by GLC-mass spectrometry. The main components were rings of 4 to 6 siloxane units (D_4–D_6, Figure 2) and represented about 15 % of our total crude polymeric preparation. The high boiling fraction was submitted to an NMR study (Dr. *R. K. Harris*) which indicated the presence of free hydroxyl groups leading to a chemical shift of $\delta_{Si} = -11.85$. This conclusion was in agreement with the presence of free hydroxyls previously detected by infrared spectroscopy of our crude polydimethylsiloxane preparations [9]. However, the lengths of the polysiloxane chains are unknown at the present time.

From these data, one might suggest that the free hydroxyl groups which could be detected in the components of the high boiling fraction of our polymeric preparations can represent the reacting moiety involved in a chemical reaction leading to the previously demonstrated irreversible attachment of the polymeric phase to the glass surface [9].

b. Extent of irreversible bonding of the siloxane phase to the capillary glass surface

We have previously shown that most of the stationary phase finally retained on the inner capillary glass wall

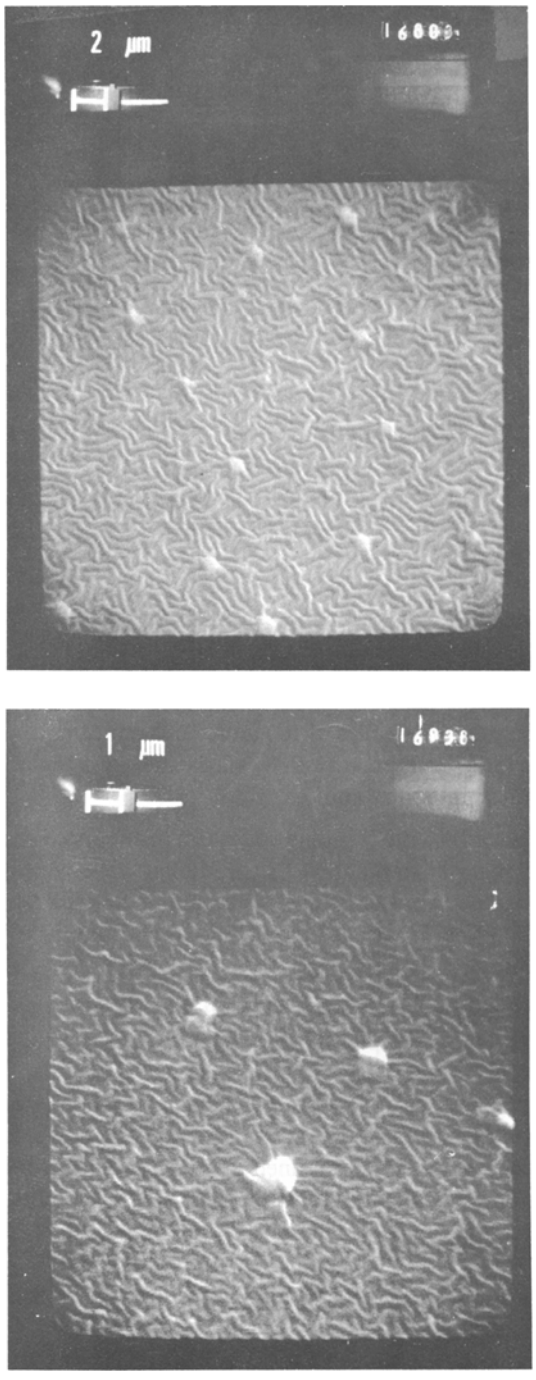

Fig. 3

- Scanning electron micrograph of inner wall of borosilicate glass etched column coated with 20 % methylpolysiloxane solution (MPS).

(a) After coating with polymer (G = 5000).
(b) After completion of whole procedure (G = 10 000).

with our procedure was not extractable with various organic solvents [9]. The extent of irreversible bonding of the siloxane polymer was investigated by measuring the amount of silicon released from finely crushed capillary column samples by extensive extraction for 5 hours with boiling dichloromethane under reflux. Table III summarizes the corresponding data, listing the

Table III. (MPS = dimethylpolysiloxane)

	weight of glass	Si extr. µg/g	Chromatographic properties
Empty Column	1.8 g	26	–
Column + M.P.S.	2.5 g	338	–
Column + M.P.S. 320 °C	2.3 g	326	Very Poor
Column + M.P.S. + NH_3 320 °C	2.7 g	35	Very Good

Table IV

	n° 83 without HCl		n° 84 according to method presented		n° 85 silanized before coating	
	a	b	a	b	a	b
K' (nC_{24})	17.4	5.4	15.5	12.2	7.3	1.75
N	39.300	19.136	73.800	65.000	49.160	31.600
N/m	1574	760	2960	2700	2460	1580
HETP 10^{-3} m	0.63	1.30	0.34	0.37	0.41	0.64
TZ $(nC_{24}-nC_{26})$	24.62	16.7	33.05	31.2	25.74	14.9

a: parameters obtained before washing the column with 5 cm^3 CH_2Cl_2
b: parameters obtained after washing the column with 5 cm^3 CH_2Cl_2

amount of silicon released per g of glass, after each step of our column preparation procedure. The amount of phase extracted from the final column was close to the control values (HCl treated glass). The slight difference observed might be attributed to the release of cyclic siloxane moieties, present in the crude polymer and which are not involved in chemical bonding. By contrast, the simply coated polymer was almost totally recovered by extraction, even after heating (in the absence of NH_3). It can thus be concluded that heating the coated capillary in the presence of gaseous ammonia appears the crucial step required to initiate an irreversible bonding of the siloxane to the glass surface.

c. Chemical bonding process

The only potential reactive groups which could be detected in our polysiloxane polymer preparations were free hydroxyls. With regard to the glass surface, hydroxyl as well as exchangeable chlorine moieties may be involved in a chemical coupling reaction with the polymers. Several experiments were run in order to evaluate the possible mechanism of the coupling reaction (Table IV).

The participation of hydroxyl moieties of the glass surface was demonstrated after thorough silanization of the HCl treated capillary (HMDS/TMCS 5:1, 24 h, 220°C), prior to the coating step. The resulting columns exhibited fair chromatographic parameters. But an important part

of the coated stationary phase was removed by a single washing with dichloromethane, as suggested by the resulting chromatographic parameters (Table IV). These observations demonstrated that previous inactivation of free hydroxyl groups glass by silanization impaired the irreversible polymer bonding process.

The implication of chlorine (possibly included in Si-Cl moieties on the glass surface) was suggested by the inhibition of the irreversible polymer bonding following glass treatment by sodium methanolate.

Omission of the HCl treatment of the glass impaired the quality of the resulting capillary columns.

Silanization of our polysiloxane preparations before coating greatly reduced its irreversible bonding to the capillary wall. Although direct evidence is not available, it can be concluded that the mandatory HCl treatment may result in both free hydroxyl and exchangeable chlorine becoming accessible on the glass surface. It may be suggested that available OH of the siloxane polymers can condense into (O-Si) bonds by dehydration and HCl elimination respectively. This is in agreement with the fact that these processes will be favored at high temperature in the presence of NH_3.

d. Scanning electron microscopic observations

As shown in Fig. 3, the aspect of the inner capillary glass wall appeared similar after polymer coating (3a) and after completion of the irreversible bonding process by heating in the presence of NH_3 (3b). These observations did not provide any information concerning the bonding mechanism; however they showed a very satisfactory result with regard to the homogeneity of the final phase film. The smooth and regular distribution of the polymer surface was preserved after prolonged contact with NH_3 at high temperature (3b).

3. Capillary columns of various polarity: separation of biological mixtures

As previously stated, the reported procedure can easily be extended to prepare glass capillary columns irreversibly coated with stationary phases of various polarities. Starting from DMCS, DECS and MPCS respectively, three different chromatographic systems were obtained and investigated with regard to their potential applicability in the separation of biological mixtures.

The retention indices obtained with these columns for several major human urinary steroid metabolites as methyloxime-trimethylsilylether derivatives (MO-TMS) are listed in Table V. The chromatographic properties of the non-polar methyl polysiloxane (MPS) have been previously examined and found similar to those of the commercially available polydimethylsiloxanes [9, 10]. The polydiethylsiloxane (EPS) exhibited also a non-selective character, and appeared less sensitive than MPS to the stereochemistry of the steroid nucleus, as suggested by the relative retention behaviour of the $5\alpha/5\beta$ isomeric pairs in the androstane series on both systems. The poly methylphenyl siloxane (MPPS) appeared to be an interesting phase of medium polarity, leading to a selective retention of oxygenated structures such as free steroidal ketones.

Table V

	MPS	EPS	MPPS
Androsterone	2501	2556	2792
Etiocholanolone	2520	2554	2806
Dehydroepiandrosterone	2563	2623	2908
11-Keto-androsterone	2603	2656	2983
11-Keto-etiocholanolone	2614	2643	2976
11-OH-androsterone	2697	2715	2816
11-OH-etiocholanolone	2714	2710	2928
Pregnanediol	2757	2778	2887
Pregnanetriol	2789	2783	2860
Tetrahydrodeoxycortisol	2863	2838	2975
Tetrahydrocortisone	2960	2947	3104
Tetrahydrocortisol	3021	2980	3073
Allo-tetrahydrocortisol	3034	3000	3086
Cortolone	3045	3010	3141
β Cortolone	3074	3033	3148

Typical separation profiles of model steroid mixtures on non-polar (EPS) and polar (MPPS) glass capillary systems are illustrated in Figs. 4 and 5.

It can be seen that the bleeding was more pronounced for the MPPS phase than for the EPS (or MPS as well) system. However, satisfactory base lines were obtained after a period of continuous use of these columns and several temperature programming cycles.

These glass capillary systems have been in continuous service for the urinary steroid separations in our clinical laboratory for over a year. Fig. 6 shows the GLC profile given by the neutral urinary steroid extract from a 30 year old patient with a congenital adrenal hyperplasia due to a 21 hydroxylase defect as obtained using a non-polar MPS system. This example represents the typical GLC conditions used with these capillary columns, routinely including repeated temperature program cycles up to 300–320 °C without noticeable loss in chromatographic performances over a one year period.

Conclusion

The simple and straight forward procedure for the preparation of open tubular glass capillary columns using chemically bonded polysiloxane polymers as previously described [9, 10] has been in routine use in our laboratories for the past year. Satisfactory high resolution separations of biological mixtures such as hormonal steroids have regularly been performed. These capillary systems could stand repetitive temperature programs up to 320 °C and appeared remarkable for their stability and reproducibility. However, a drawback may be represented by an adsorption of polar compounds as evidenced by a marked tailing exhibited by structures with underivatized hydroxyl groups. This could be overcome by the use of convenient volatile derivatives as, for example, TMS or oxime-TMS in the case of hormonal steroids or prostaglandins which could then

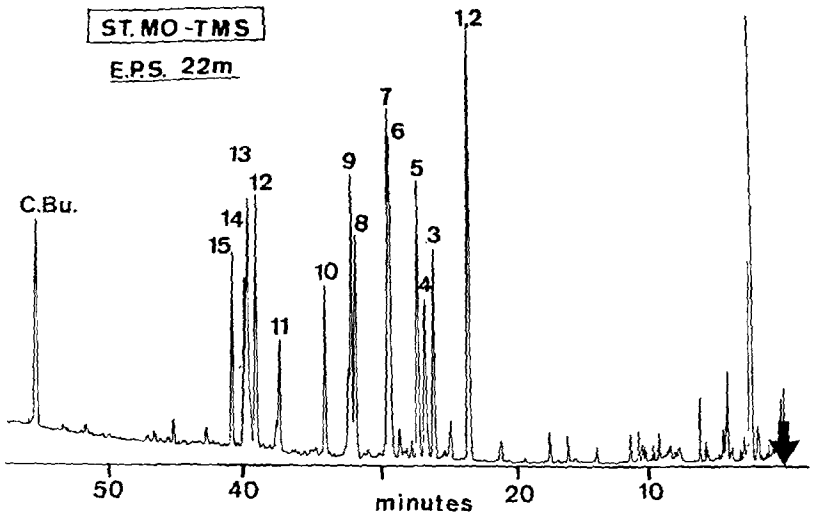

Fig. 4

- GLC separation on ethylpolysiloxane-coated open tubular column of steroid mixture as MO-TMS derivatives.
Temperature programming from 200 to 290° at 2 °C min^{-1}.
Peaks: 1 = Androsterone, 2 = Etiocholanolone, 2 = DHA, 4 = 11-Keto-etiocholanolone, 5 = 11-Keto-androsterone, 6 = 11-OH-etiocholanolone, 7 = 11-OH-androsterone, 8 = Pregnanediol, 9 = Pregnanetriol, 10 = Tetrahydrodesoxycortisol, 11 = Tetrahydrocortisone, 12 = Tetrahydrocortisol, 13 = Allo-tetrahydrocortisol, 14 = Cortolone, 15 = β Cortolone, CBu = Cholesteryl butyrate (internal standard).

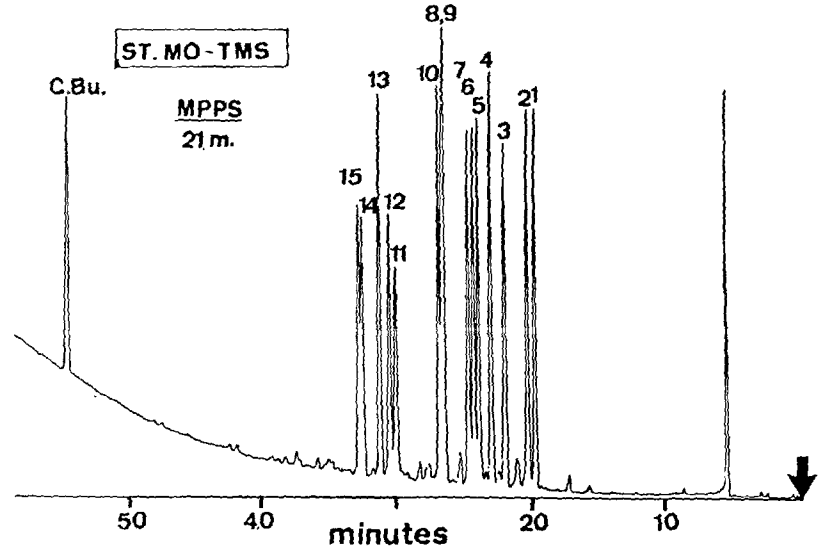

Fig. 5

- GLC separation on methylphenylpolysiloxane-coated open tubular column. Mixture and analytical conditions as in Fig. 4.
Peaks: 1 = Androsterone, 2 = Etiocholanolone, 3 = Pregnanetriol, 4 = Pregnanediol, 5 = DHA, 6 = 11-OH-androsterone, 7 = 11-OH-etiocholanolone, 8 = Tetrahydrodesoxycortisol, 9 = 11-Keto-etiocholanolone, 10 = 11-Keto-androsterone, 11 = Tetrahydrocortisol, 12 = Allo-tetrahydrocortisol, 13 = Tetrahydrocortisone, 14 = Cortolone, 15 = β Cortolone, CBu = Cholesteryl butyrate (internal standard).

be analysed without noticeable loss in the sub-nanogram range. We are currently investigating the possible causes of this adsorption problem in order to extend the applicability of these capillary systems.

The major breakthrough of our procedure may be its flexibility which allowed the preparation of stable glass capillary systems of various polarities by using different monomeric chlorosilanes. This is currently being extend-

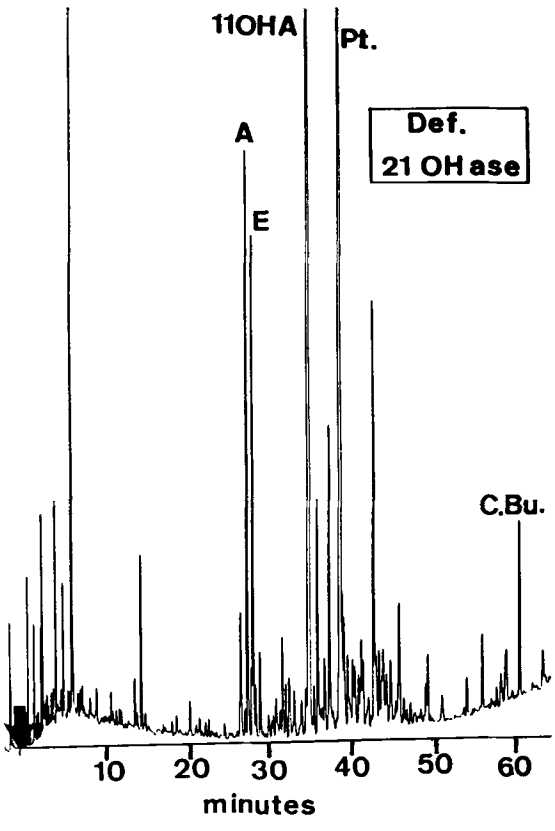

Fig. 6

- GLC analysis of neutral urinary steroid metabolites as MO-TMS derivatives in a case of 21 hydroxylase defect. Analytical conditions as Fig. 4. Peaks: A = Androsterone, E = Etiocholanolone, 11-OH-A = 11-Hydroxyandrosterone, Pt = Pregnanetriol, CBu = internal standard.

ed to the preparation of a range of chemically bonded stationary phases, with the aim of "tailor-made" properties as required for separations of specific biological mixtures.

Acknowledgements

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