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CAPILLARY GAS CHROMATOGRAPHY OF *s*-TRIAZINES

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SUMMARY

In order to achieve better resolution and lower detection limits for *s*-triazines, glass capillary columns made of soft soda-lime glass and etched with gaseous hydrogen chloride have been introduced. Columns with non-polar (OV-101, SE-30) mixed (Carbowax 20M + SE-30) and polar stationary liquids (Carbowax 20M) were used. The thickness of the ultra-thin film columns was determined by weighing the capillaries before and after coating of the capillary with stationary phase and conditioning, and varied from 0.05 to 0.5 μm. From the capacity ratios of *s*-triazines and *n*-alkanes measured in sections of an originally long dynamically coated column after it had been cut into several equal parts, the constancy of the film thickness along the whole column was judged. The film thickness was not uniform and increased along the whole column.

It was found that glass capillary columns with non-polar stationary phase are not very suitable for the analysis of *s*-triazine herbicides, as tailing occurs with some of them. Symmetrical peaks and the separation of 17 from 18 analysed *s*-triazines were achieved on glass capillary columns coated with Carbowax 20M.

INTRODUCTION

s-Triazines are nitrogen-containing substances with pharmaceutical, chemosterilant, industrial and particularly herbicidal importance. The analytical methodology of triazine herbicides has been reviewed by Gysin and Knüsli[1] and Stammbach *et al.*[2]. For several years, particular emphasis has been placed on the versatility of chromatographic procedures that relate to the establishment of criteria of purity and the separation and identification of these substances and their metabolic and degradation products from environmental residue sources and from tissues. General chromatographic procedures have been reviewed by Fishbein[3] and gas chromatography (GC) by Cochrane and Purkayastha[4].

The GC separation of *s*-triazines has been performed exclusively on packed columns with stationary phases of various polarity. It appears that non-polar phases, most intermediate phases and the various mixed polarity phases do not resolve adequately mixtures of the most common herbicides (atrazine, simazine, propazine). This resolution was accomplished by using the more polar stationary phases.

The aim of this work was to introduce the use of glass capillary columns in the analysis of *s*-triazines using non-polar (OV-101, SE-30), mixed (SE-30 + Carbowax 20M) and polar (Carbowax 20M) stationary phases. Glass capillaries are often used for the separation of complex mixtures of environmental samples, but have been little utilized in herbicide analysis.

EXPERIMENTAL

The drawing of capillaries from glass tubes and the composition of glass have been described previously[5].

The capillaries were made of soda-lime glass (Unihost and PN glasses; Jablonec Glass Works, Jablonec, Czechoslovakia). The initial tubes were 0.7–1.5 m long, with O.D. 7–8 mm and I.D. 2–3 mm, from which capillaries up to 130 m long with O.D. 0.7–0.8 mm and I.D. 0.2–0.3 mm were drawn. The inner diameters of the glass capillary columns were measured from the known length and volume of the capillaries[5].

Surface roughening

Surface roughening was performed by etching the column wall with gaseous hydrogen chloride. Gaseous hydrogen chloride, generated from dry sodium chloride and sulphuric acid, was forced through the capillary. Passage of hydrogen chloride through one end of the capillary was continued for several hours, then the capillary was turned and hydrogen chloride was introduced from the other side. The passage of hydrogen chloride was also continued in this direction for several hours. The amount of hydrogen chloride present in the capillary was determined by using the test of Alexander *et al.*[6]. Usually more than 95% of the capillary volume was filled with hydrogen chloride. Both ends of the filled capillary were sealed using a microburner and the column was heated in a thermostat with forced air circulation for 24 h at 623 °K.

Coating procedures and column conditioning

Dynamic coating procedure. The capillaries were coated dynamically using 0.15–3.5 ml of a solution of the stationary phase in chloroform or *n*-hexane at a flow-rate of 0.3–0.5 cm/sec.

Ultra-thin film capillary columns were prepared by a modified mercury plug method proposed by Schomburg and Husmann[7]. The column was filled with the coating solution (0.15–0.3 ml) by suction, followed by a 5-cm mercury plug. The vacuum was disconnected and the mercury plug was pushed through the capillary at a constant velocity by means of compressed nitrogen. Some columns were filled completely with coating solution by nitrogen pressure and afterwards the mercury plug was pushed through the capillary at a constant velocity (0.025 cm/sec) by means of compressed nitrogen.

Static coating procedure. Capillaries were coated by the static method according to the version of the static method introduced by Bouche and Verzele[8]. A 0.25–0.75% solution of the stationary phase in chloroform was degassed by the following procedure. The stationary phase solution was diluted with pure solvent to more than twice its volume, then gently boiled down to the original volume. After cooling to room

temperature, the solution was immediately used for filling the column by suction. One end of the filled column, which was plunged into aqueous sodium silicate, was warmed in order to expel some drops of the coating solution. As the capillary cooled a small amount of sodium silicate solution entered the column. After drying overnight, the seal became hard and the opposite end was connected to a vacuum at room temperature.

The columns were conditioned with temperature programming at a rate of 4 °K/min from 323 to 513 °K and maintained isothermally at 513 °K for 30 or 60 min. The column efficiency was measured in terms of the number of theoretical plates.

Gas chromatography

Carlo Erba Model 2300 and 2350 gas chromatographs equipped with flame-ionization detectors and stream splitters were used. Glass capillary columns were led directly to the splitter and connected to the detector via a metal capillary by means of shrinkable PTFE tubing. The retention times were measured with a stop-watch. The characteristics of the capillaries are given in Tables I and II.

TABLE I

CHARACTERISTICS OF NON-POLAR COLUMNS

Property	*Column No.*							
	1		*2*		*3*		*4*	
Stationary phase	OV-101		SE-30		SE-30		SE-30	
Coating method	Dynamic		Static		Static		Static	
Concentration of stationary phase solution (%)	29.00		0.25		0.50		0.75	
Velocity of Hg plug in dynamic method (cm/sec)	0.44		—		—		—	
Length (m)	69.7		25.1		22.6		24.8	
Internal diameter (mm)	0.24		0.24		0.23		0.23	
Total volume (ml)	3.12		1.10		0.94		1.06	
Temperature (°K)	473		473		473		473	
Linear carrier gas (nitrogen) flow-rate (cm/sec)	17.03		13.01		15.46		11.15	
Capacity factors (k) and number of theoretical plates per metre (n)								
	k	n	k	n	k	n	k	n
C_{16} *n*-alkane	0.90	5837	1.46	5557	—	—	1.61	5042
C_{18} *n*-alkane	1.88	3340	3.02	3788	2.39	5410	3.54	3444
C_{20} *n*-alkane	3.89	3128	6.21	3052	4.90	3109	6.92	2954
Propazine	1.48	2101	2.25	3822	1.83	2782	2.65	2492
Ipazine	1.66	2082	2.55	3522	2.08	2259	2.99	2064
Prometryn	2.61	1116	3.94	3054	3.19	1920	4.71	1704

Film thickness measurement

The thickness of the stationary liquid film on the capillaries was measured by weighing the capillaries before and after the coating procedure and conditioning[9] (the time dependence of constancy of weight was measured). A semimicro balance in a balance room at a constant temperature was used. The film thickness (t) was calculated from the known weight (w) and density (d) of the stationary liquid and the inner radius (r) and length (L) of the capillary according to the equation

$$t = \frac{w/d}{2\pi r L}$$

TABLE II

CHARACTERISTICS OF POLAR COLUMNS

Property	*Column No.*					
	5	*6*	*7*	*8*	*9*	*10*
Stationary phase	Carbowax 20M	Carbowax 20M	Carbowax 20M	Carbowax 20M	Carbowax 20	Carbowax 20M
Coating method	Dynamic	Dynamic	Dynamic	Static	Static	Static
Concentration of stationary phase solution (%)	10.00	10.00	10.00	0.25	0.50	0.75
Velocity of Hg plug in dynamic method (cm/sec)	0.15	0.45	0.025	—	—	—
Length (m)	27.3	67.0	65.0	19.1	19.8	18.5
Internal diameter (mm)	0.23	0.24	0.25	0.26	0.26	0.26
Total volume (ml)	1.09	3.03	3.06	1.00	1.03	0.97
Temperature (°K)	473	473	473	473	473	473
Linear carrier gas (nitrogen) flow-rate (cm/sec)	19.23	22.03	19.36	26.9	26.6	26.2
Capacity factors (k) and number of theoretical plates per metre (n)						
	k n	k n	k n	k n	k n	k n
C_{24} *n*-alkane	0.53 624	1.76 806	1.16 1420	2.42 758	2.1 388	16.10 462
C_{28} *n*-alkane	1.44 289	6.72 518	4.44 614	8.53 480	7.47 206	30.13 450
C_{30} *n*-alkane	— —	12.88 387	8.54 554	15.91 442	14.74 199	— —
Ipazine	0.84 2568	2.37 2237	1.39 6762	4.12 4789	3.92 2420	13.04 959
Propazine	1.90 2364	4.28 2141	2.46 4950	7.71 4041	7.15 2309	23.70 759
Atraton	2.32 2222	4.97 2029	2.82 3755	9.34 3483	8.79 2125	26.32 695
Prometryn	3.08 2171	6.48 1927	3.71 2882	11.48 2784	10.52 1898	34.31 687

The film thickness on capillary columns coated by the static method was established from the concentration of the stationary phase[9].

The capacity ratios were measured in sections of originally long columns that had been cut into six equal parts. From the measured capacity ratios of *s*-triazines and *n*-alkanes it was possible to judge whether the film thickness was constant along the whole column length.

Scanning electron microscopy

The amount and size of sodium chloride crystals as well as spreading of the stationary phase along the whole column length was investigated by scanning electron microscopy (SEM). Micrographs were taken using a JEOL JSM-U3 instrument (Jeol, Tokyo, Japan) in cooperation with Mr. M. Švec (Dionyz Štur Institute of Geology, Bratislava, Czechoslovakia). The inner surfaces of capillaries were coated with a layer of gold or aluminium of thickness 150–200 Å and kept over silica gel.

Chemicals

The solvents used (chloroform, dichloromethane, acetone and *n*-hexane) were of analytical-reagent grade and were distilled at least once prior to use.

The common names and systematic names of the *s*-triazines are given in Table III. The *s*-triazines used in the experiments were of 98% or greater overall purity. Solutions of the *s*-triazines in chloroform were used for analysis.

TABLE III

s-TRIAZINES

The numbers of the *s*-triazines are used in Figs. 1–13.

Number	*Common name*	*Systematic name*
1	—	2-Chloro-4-amino-6-*tert.*-butylamino-*s*-triazine
2	Ipazine	2-Chloro-4-diethylamino-6-isopropylamino-*s*-triazine
3	—	2-Chloro-4,6-bis(*tert.*-butylamino)-*s*-triazine
4	Trietazine	2-Chloro-4-diethylamino-6-ethylamino-*s*-triazine
5	Prometon	2-Methoxy-4,6-bis(isopropylamino)-*s*-triazine
6	Terbuton	2-Methoxy-4-ethylamino-6-*tert.*-butylamino-*s*-triazine
7	Propazine	2-Chloro-4,6-bis(isopropylamino)-*s*-triazine
8	Terbutylazine	2-Chloro-4-ethylamino-6-*tert.*-butylamino-*s*-triazine
9	Secbumeton	2-Methoxy-4-ethylamino-6-isobutylamino-*s*-triazine
10	Atraton	2-Methoxy-4-ethylamino-6-isopropylamino-*s*-triazine
11	Simeton	2-Methoxy-4,6-bis(ethylamino)-*s*-triazine
12	Atrazine	2-Chloro-4-ethylamino-6-isopropylamino-*s*-triazine
13	Prometryn	2-Methylthio-4,6-bis(isopropylamino)-*s*-triazine
14	Terbutryn	2-Methylthio-4-ethylamino-6-*tert.*-butylamino-*s*-triazine
15	Simazine	2-Chloro-4,6-bis(ethylamino)-*s*-triazine
16	Ametryn	2-Methylthio-4-ethylamino-6-isopropylamino-*s*-triazine
17	Desmetryn	2-Methylthio-4-methylamino-6-isopropylamino-*s*-triazine
18	Simetryn	2-Methylthio-4,6-bis(ethylamino)-*s*-triazine

RESULTS AND DISCUSSION

The analysis of substances that contain nitrogen atoms in the molecule on capillary columns is subject to several problems that have not yet been solved. In preliminary experiments on the separation of *s*-triazines on metal capillaries we were unsuccesful with both non-polar and polar stationary liquids. We found that capillaries with a high-quality non-active surface coated with non-polar stationary phases without any adsorption properties or columns coated with certain polar stationary liquids are required.

Because of the high inertness of the wall of the glass capillary column, we attempted to analyse *s*-triazines on glass capillary columns.

Non-polar stationary phase columns

The separation of a mixture of *s*-triazines and C_{16}–C_{20} *n*-alkanes on a capillary column dynamically coated with OV-101 stationary phases at 473 °K is shown in Fig. 1 (column No. 1). It can be seen that the peaks of the *n*-alkanes and some peaks of the *s*-triazines are symmetrical. The tailing depends on the structure of the *s*-triazines and is connected with the adsorptive properties of the surface. Fig. 2A shows that methoxy-*s*-triazines give very unsymmetrical peaks on OV-101 liquid phase (atraton, peak 10), and methylthio-*s*-triazines less unsymmetrical peaks (ametryn, peak 16). Chloro-*s*-triazines (atrazine, peak 12; simazine, peak 15; propazine, peak

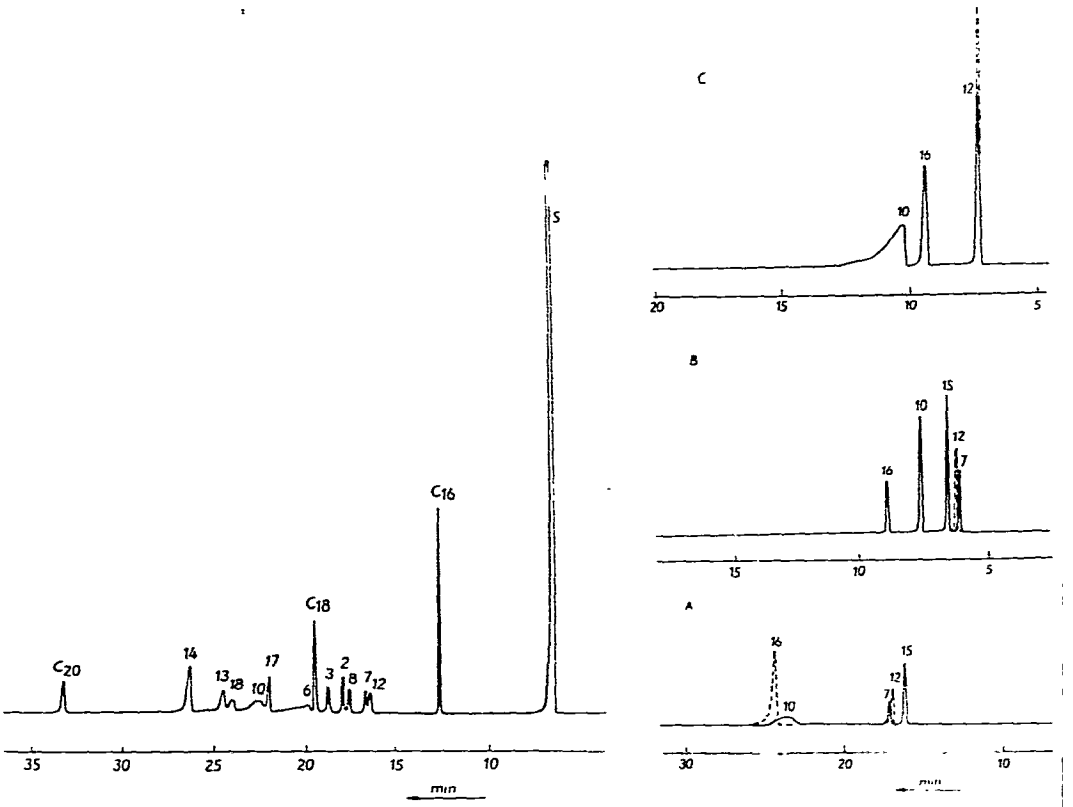

Fig. 1. Separation of *s*-triazines and C_{16}–C_{20} *n*-alkanes on an OV-101 glass capillary column (No. 1). Numbering of *s*-triazines in all figures as in Table III.

Fig. 2. Separation of *s*-triazines on OV-101 columns at 473 °K. Peaks: 7 = propazine; 10 = atraton; 12 = atrazine; 15 = simazine; 16 = ametryn. A, OV-101 column; linear carrier gas (nitrogen) flow-rate, 17.03 cm/sec. B, Deactivated OV-101 column with 0.5% Carbowax 20M; linear carrier gas flow-rate, 26.53 cm/sec. C, Deactivated OV-101 column after heating for a longer time above 503 °K; linear carrier gas flow-rate, 22.7 cm/sec.

7) give relatively symmetrical peaks. The highest non-symmetry of methoxy-*s*-triazines could be explained by the adsorption properties of the SiOH groups (from *s*-triazines preferentially methoxy-*s*-triazines could form hydrogen bonds). After deactivating the active centres with a polar substance (0.5% Carbowax 20M) before coating the capillary with the stationary phase, the tailing of methoxy-*s*-triazines on OV-101 columns was reduced (atraton, peak 10 in Fig. 2B). The tailing of methoxy-*s*-triazines reappears after heating of such deactivated OV-101 columns for a long period at about 503 °K. Chloro-*s*-triazines maintained relatively good peak symmetry (Fig. 2C). The characteristics and chromatographic conditions of these OV-101 columns are not included in the tables.

The properties of the glass of capillary columns coated with a non-polar stationary phase depend, in addition to other factors, on the film thickness of the stationary phase. We determined by weighing the thickness of the stationary phase

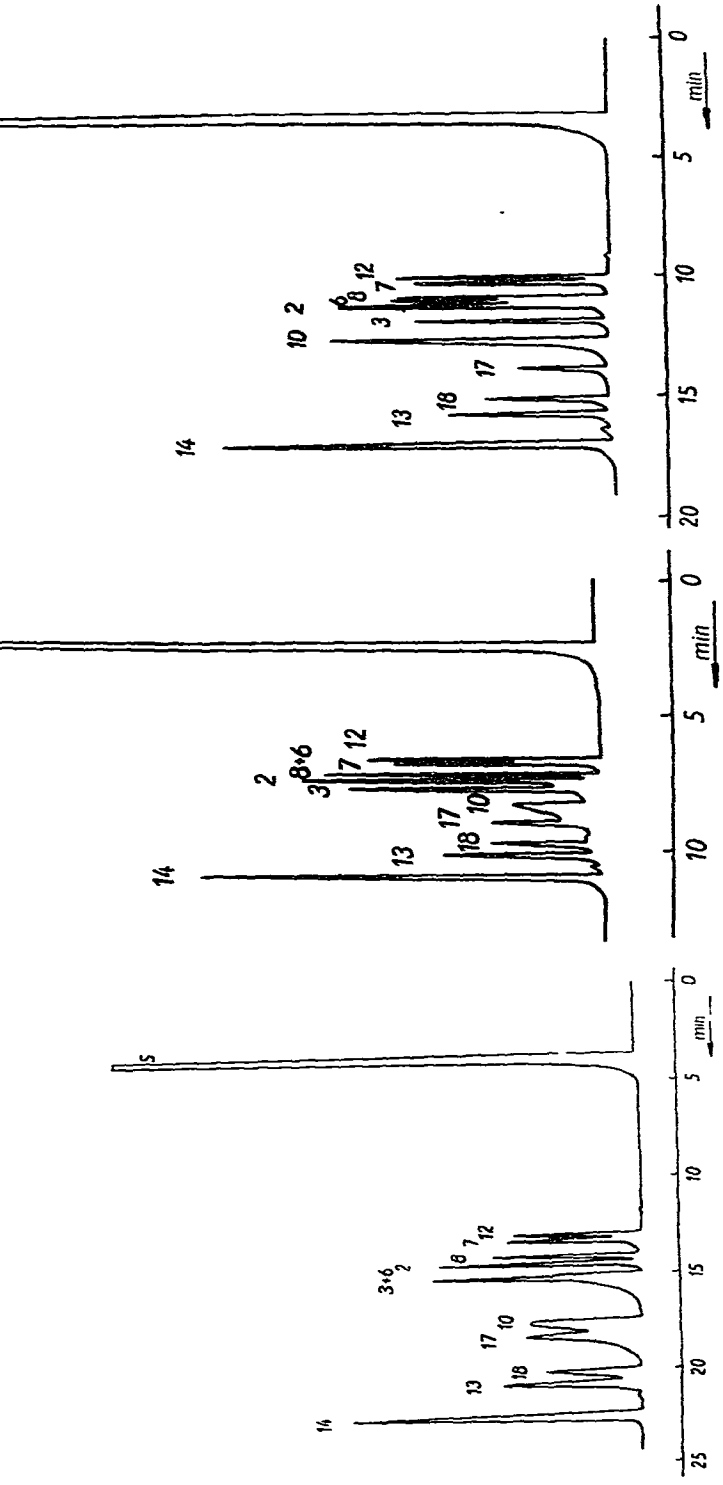

Fig. 3. Separation of *s*-triazine mixture on statically coated SE-30 (0.25%) column (No. 2).

Fig. 4. Separation of *s*-triazine mixture on statically coated SE-30 (0.5%) column (No. 3).

Fig. 5. Separation of *s*-triazine mixture on statically coated SE-30 (0.75%) column (No. 4).

on ultra-thin film dynamically coated capillaries and it was found to vary from 0.05 to 0.50 μm according to the concentration of the coating solution.

We also analysed *s*-triazines on capillaries statically coated with a 0.25–0.75% solution of SE-30 stationary phase. Even on these columns with a thicker film of stationary phase (0.2–0.8 μm) the asymmetry of the methoxy-*s*-triazine peaks was not eliminated (Figs. 3–5).

It can be said in general that glass capillary columns coated with non-polar stationary phases are not very suitable for the analysis of *s*-triazines as tailing occurs with some of them. Even columns with a deactivated etched surface are not the most convenient as overlapping of some of the *s*-triazine peaks occurs and after prolonged use of such columns at higher temperatures the deactivating agent disappears.

Polar and mixed stationary phase columns

The problems that occurred in the separation of *s*-triazines on columns coated with non-polar stationary phases were eliminated by using columns coated with polar stationary liquids. From preliminary experiments it was shown that for the analysis of *s*-triazines the most suitable polar stationary phase is Carbowax 20M.

The separation of 18 *s*-triazines on a dynamically coated Carbowax 20M column at 473 °K is shown in Fig. 6 (Column No. 5). The peaks of all analysed *s*-triazines are symmetrical.

The constancy of the film thickness along the whole length of the column was judged from the capacity ratios of *s*-triazines and *n*-alkanes measured in sections of a

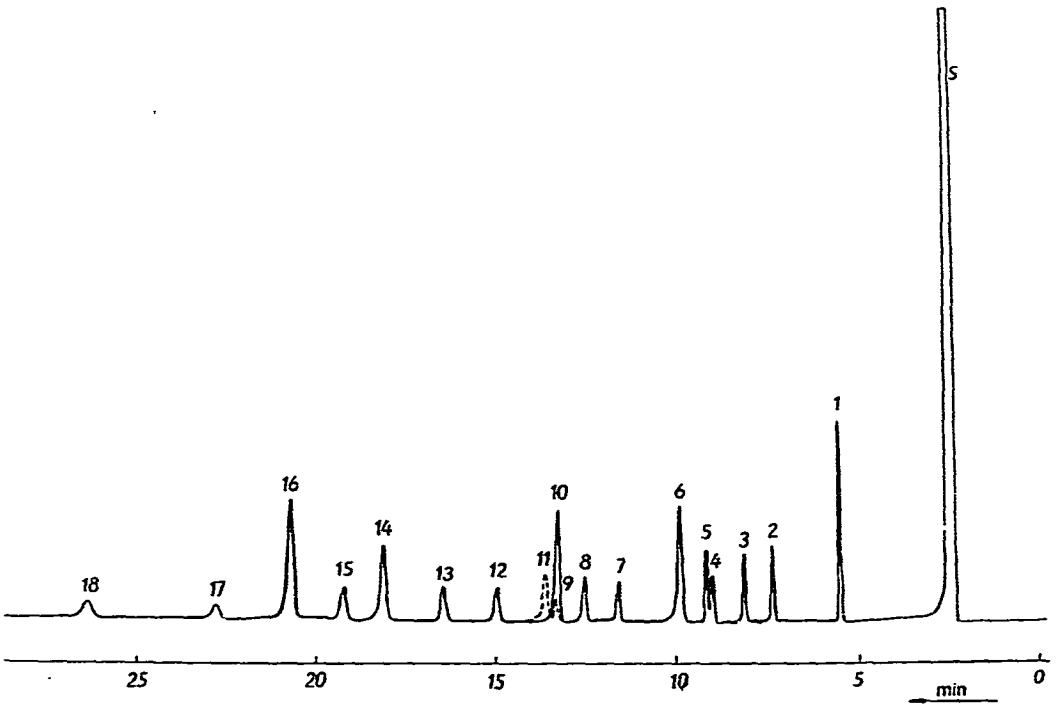

Fig. 6. Separation of *s*-triazine mixture on dynamically coated Carbowax 20M column (No. 5).

67-m long column (No. 6) after it had been cut into six equal parts (Fig. 7). The results show that the film thickness along the column is not uniform. With the plug method of coating, the first 10 turnings of the 67-m-long capillary column were filled with stationary phase (10% Carbowax 20M) which was then pushed through the capillary at a constant velocity (0.45 cm/sec) except for the last 10 turnings. A buffer column was not connected to the end of the column.

On comparison of these results with those obtained by measuring the capacity ratios of *s*-triazines and *n*-alkanes in sections of a 65-m long column (No. 7) completely filled with stationary phase (10% Carbowax 20M) and pushed through the capillary at a constant velocity of 0.025 cm/sec, no essential differences were found (Fig. 8),

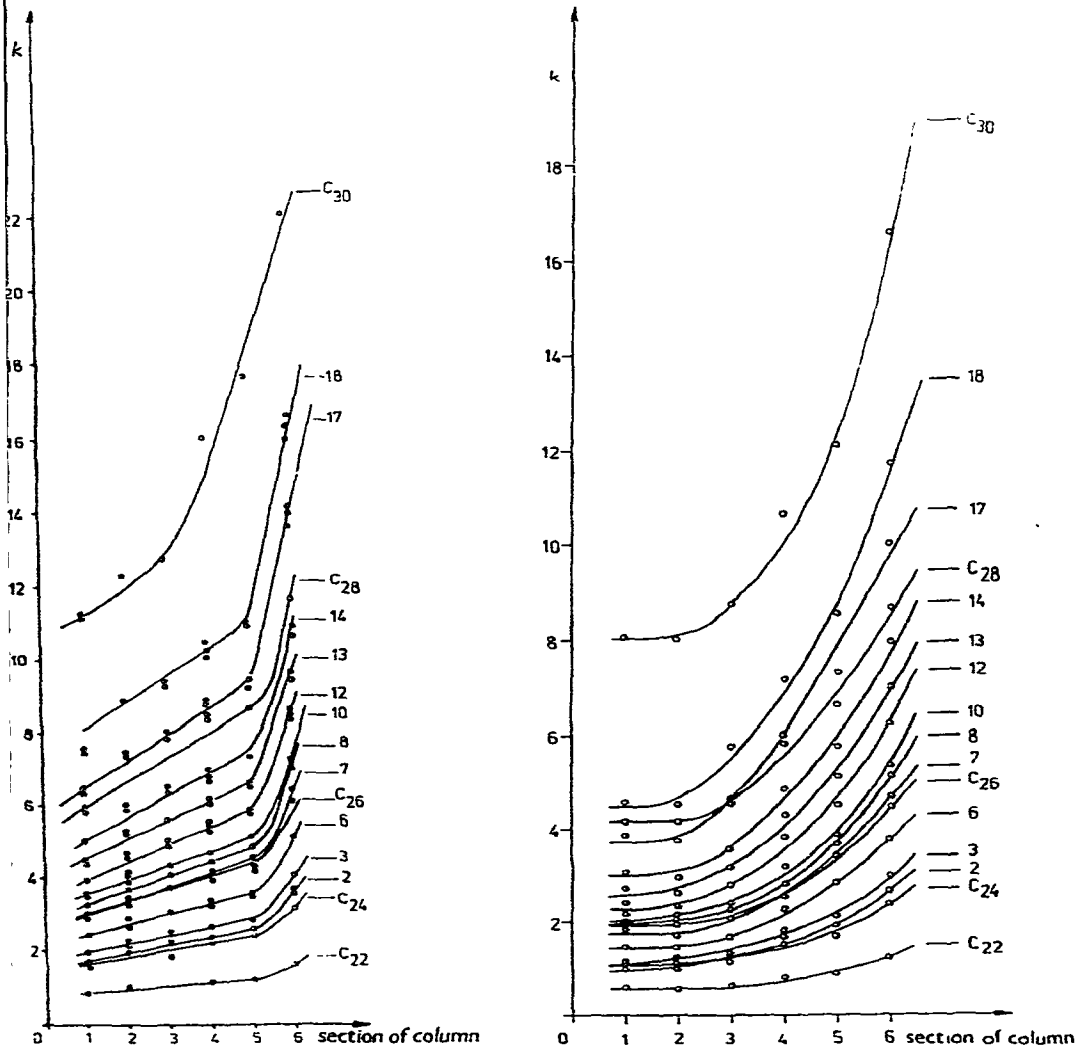

Fig. 7. Capacity ratios of *s*-triazine and *n*-alkanes measured in sections of column No. 6.

Fig. 8. Capacity ratios of *s*-triazines and *n*-alkanes measured in sections of column No. 7.

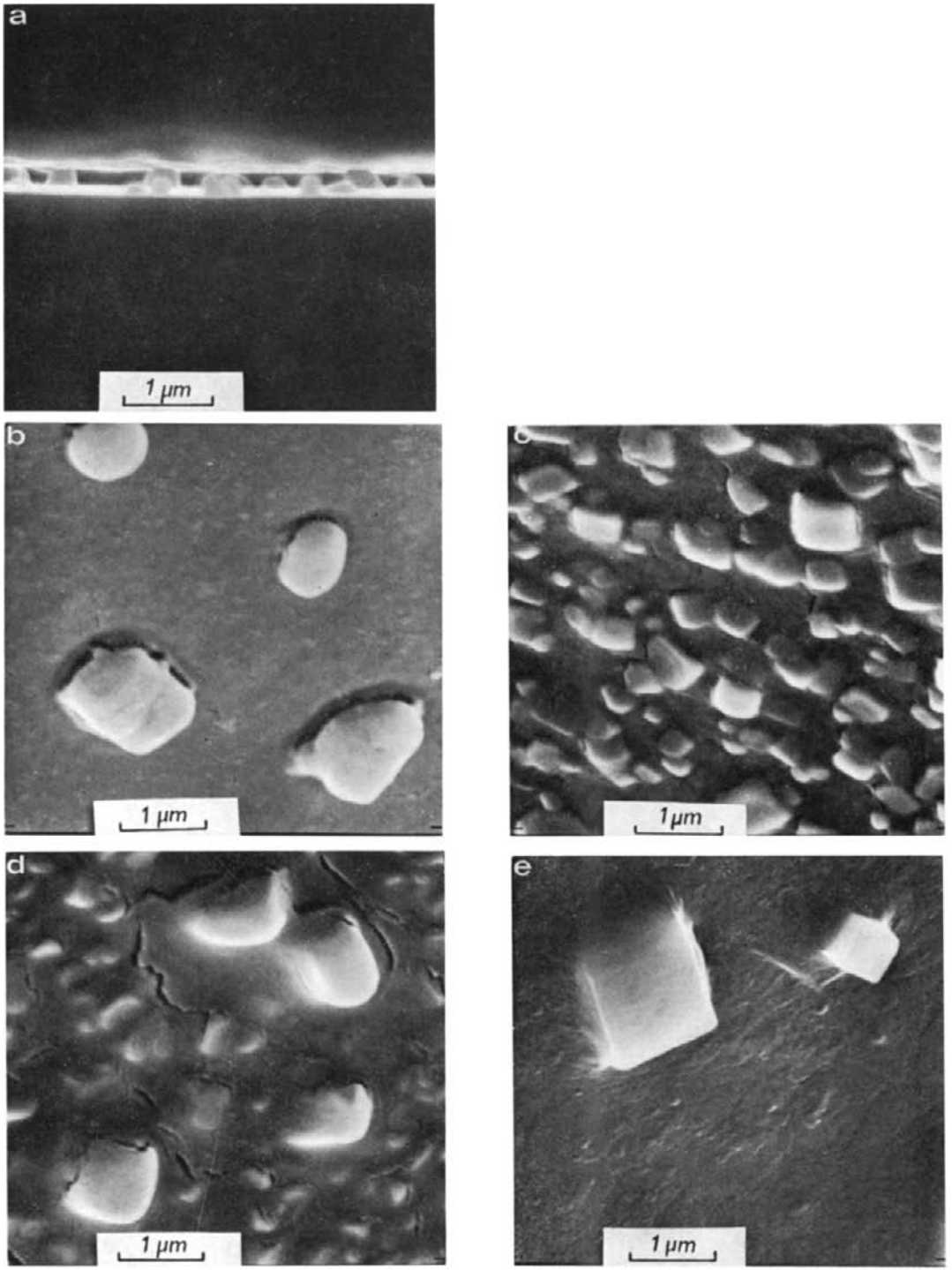

Fig. 9. SEM pictures of inner wall of dynamically coated Carbowax 20M column No. 6 (a, b, c and d, coated with a layer of gold; e, coated with a layer of aluminium). (a) Part of cross-section of the column; (b), micrograph taken in the first section of the column; (c), micrograph taken in the middle of the column; (d), micrograph taken in the last section of the column; (e), micrograph taken in the first section of column.

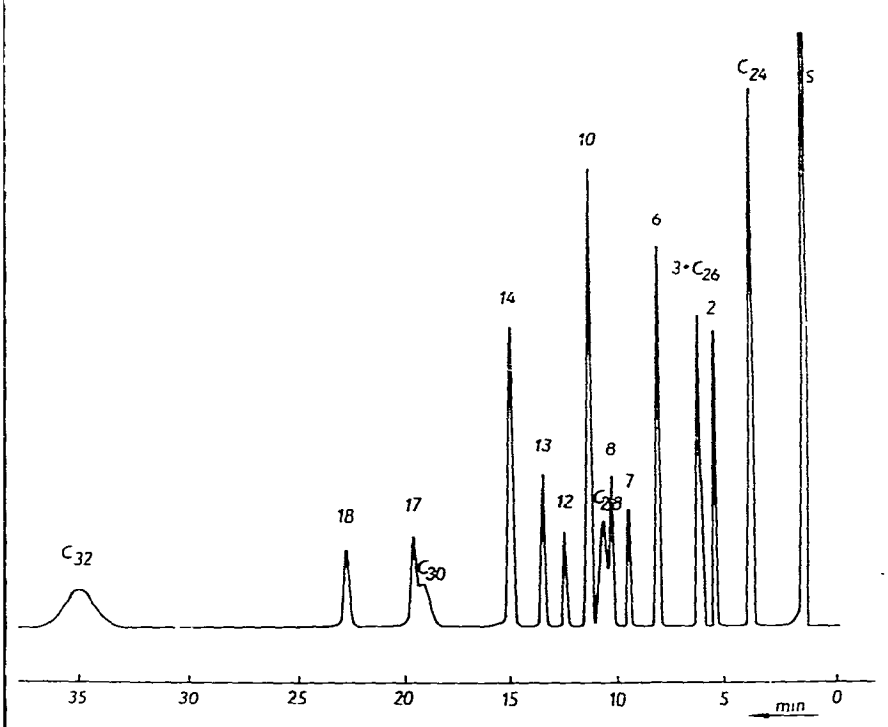

Fig. 10. Separation of *s*-triazines and *n*-alkanes on statically coated Carbowax 20M (0.25%) column (No. 8).

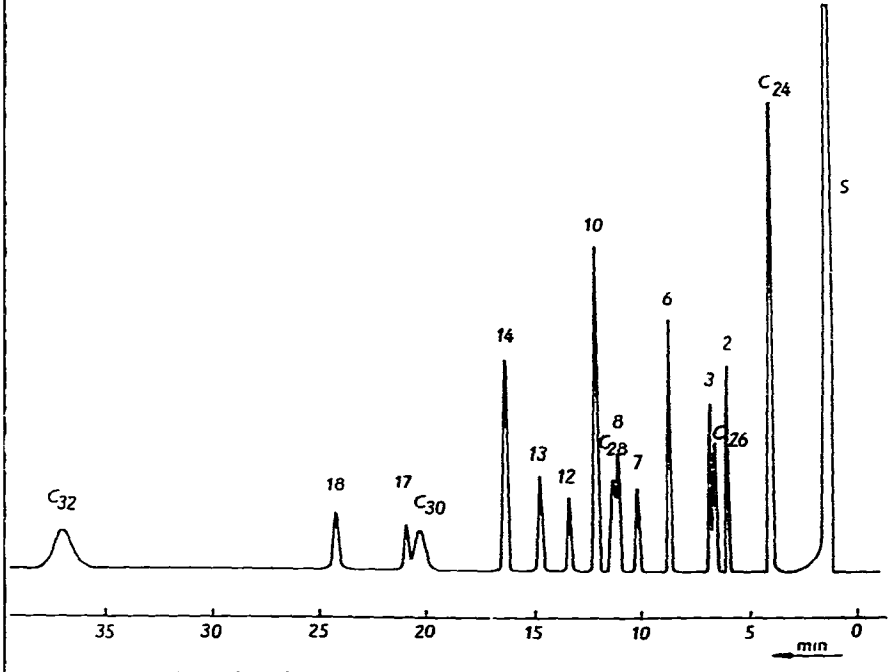

Fig. 11. Separation of *s*-triazine and *n*-alkanes on statically coated Carbowax 20M (0.5%) column (No. 9).

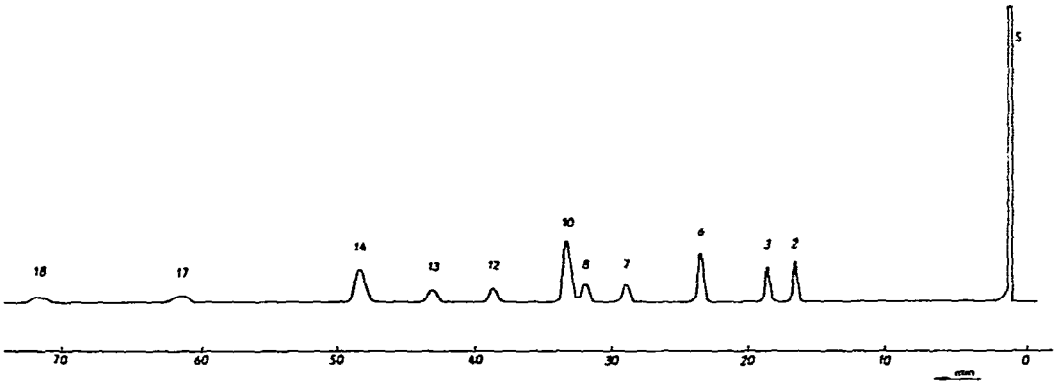

Fig. 12. Separation of *s*-triazines on statically coated Carbowax 20M (0.75%) column (No. 10).

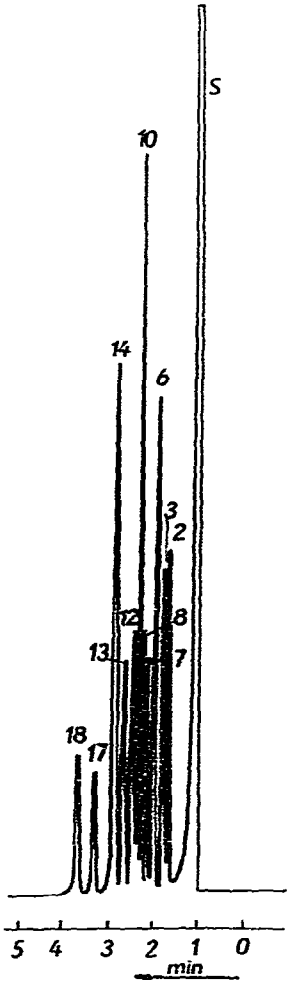

Fig. 13. Separation of *s*-triazines on dynamically coated SE-30 + Carbowax 20M column at 473 °K with a linear carrier gas flow-rate of 13.3 cm/sec.

except that the capacity ratios of the corresponding substances on column No. 7 were lower, which was probably caused by the lower flow-rate of the stationary phase in the coating procedure.

The amount and size of the sodium chloride particles and the spreading of the stationary phase on the glass surface were studied by SEM. As Carbowax 20M is a solid at room temperature, micrographs of the inner glass capillary wall coated with Carbowax 20M were obtained from different parts of a 67-m long column (No. 6). It was found that after coating the inner surface of capillaries with a layer of aluminum (Fig. 9e) better SEM pictures were obtained than when gold was used for coating (Fig. 9b, c and d). From Fig. 9b, c and d it can be concluded that at the beginning and at the end of the column there are rather large sodium chloride crystals, while in the direction of the centre of the column from each end the amount of sodium chloride crystals gradually increased and their size became smaller. As gaseous hydrogen chloride was introduced from both ends of the column during etching of the columns and the heat treatment was continued for a long period, according to Franken *et al.*[9] large sodium crystals are grown rather than smaller crystals.

The surface of Carbowax 20M at the beginning of the column is relatively smooth and towards the end of the column the film of Carbowax 20M appears thicker and its surface is damaged at various positions. An increase in the film thickness along the whole column, which was concluded from the capacity ratios of *s*-triazines and *n*-alkanes measured in sections of columns, was also indirectly proved by SEM (Fig. 9b, c and d).

The influence of columns with thicker films prepared by the static coating procedure (0.25–0.75% solutions of Carbowax 20M) on the separation of *s*-triazines and shape of their peaks was investigated. Columns coated with 0.25% and 0.5% solutions of Carbowax 20M gave a good resolution of the s-triazines studied and their peaks were symmetrical (Figs. 10 and 11, columns Nos. 8 and 9). On columns with 0.75% of Carbowax 20M the peaks of *s*-triazines were broader (Fig. 12). The efficiency of the columns decreased with an increasing concentration of Carbowax 20M in the coating solution (Table II, columns Nos. 8, 9 and 10).

We attempted to carry out a rapid analysis of *s*-triazines (in 4 min) on a mixed stationary phase of SE-30 + Carbowax 20M in the ratio 1:1 (3% of each) at 473 °K. The analysis was performed on a dynamically coated 16-m long column with an ultra-thin film (Fig. 13).

Details of the analytical aspects of the GC of *s*-triazines on glass capillary columns will be published elsewhere.

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