SUPPORT-COATED OPEN TUBULAR COLUMNS

IV. SELECTED APPLICATIONS*

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In our previous publications on support-coated open tubular columns[1-3], the advantages of these columns as compared with classical, wall-coated open tubular columns were discussed in detail. As outlined there, the β-value (the ratio of the volumes of the gas and liquid phase in the column) of the new column type is decreased; thus, the number of theoretical plates required for the separation of a given peak-pair—particularly for early peaks—is also decreased. At the same time, due to the increased liquid phase volume, the sample capacity of support-coated open tubular columns is significantly increased as compared with the classical, wall-coated open tubular columns; in fact, the sample capacity of the new columns is of the same order of magnitude as that of the standard 1/8 in. O.D. (0.085 in. I.D.) packed columns.

In the present paper, the influence of sample size on peak resolution is explored in more detail. In addition to this, the minimum detectability of systems incorporating wall-coated and support-coated open tubular columns is compared and the relative performance of support-coated and conventional packed columns is investigated. Finally, a few practical applications are shown.

INSTRUMENTATION

All analyses reported here were carried out with the Model 880 gas chromatograph of the Perkin-Elmer Corporation. The instrument was equipped with a flame ionization detector and connected to a Leeds & Northrup Speedomax "G" 1 or 5 mV recorder. With a 1 mV recorder, this particular system corresponded to a full scale pen deflection of $2.1 \cdot 10^{-12}$ A at attenuation $\times$ 1.

The columns used were prepared from 0.020 in. I.D. copper tubes in the usual way, as discussed in our earlier publications[1-3]. Helium was used as the carrier gas. The flow rates reported were measured at column outlet and ambient temperature. The average linear gas velocities ($\bar{u}$) were calculated with help of the following equation:

$$\bar{u} = \frac{L}{t_M} \qquad (1)$$

where L is the column length (cm) and t_M the retention time of methane (sec).

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Samples were injected with help of Hamilton 1 µl syringes. As discussed in our previous paper[2], support-coated columns could permit direct sample injection into the column. However, for convenience, we generally split the injected sample with a low split ratio (1/10–1/20). The sample volumes listed in the figures and in the text always refer to the actual sample entering the column.

SAMPLE SIZE *vs*. PEAK RESOLUTION

The best way to illustrate the influence of increased sample amounts on peak resolution is to select certain peak pairs representing a difficult separation and to investigate how the actual separation will change with increased sample size. For this study, we have selected two test mixtures.

Our first test mixture consisted of hexene-1 and *n*-hexane, in about 1:1 ratio and it also contained an impurity which emerged—under the conditions used—between the two major peaks. The calculated composition of the sample was:

Hexene-1:   50.2 vol. %
Impurity: 0.8 vol. %
n-Hexane: 49.0 vol. %.

This sample was analyzed on a 150 ft long support-coated column prepared with squalane liquid phase, at 100°C, with a carrier gas flow of 2.76 ml/min ($\bar{u}$ = 20 cm/sec). The respective partition ratio values were measured as 1.52, 1.67 and 1.73. The relative retention (α) of the two major peaks is 1.138 while the relative retention calculated for the second major peak and the impurity peak is 1.036.

Fig. 1 shows the chromatograms obtained with various sample sizes. The volume of the total sample entering the column and the recorder attenuation are
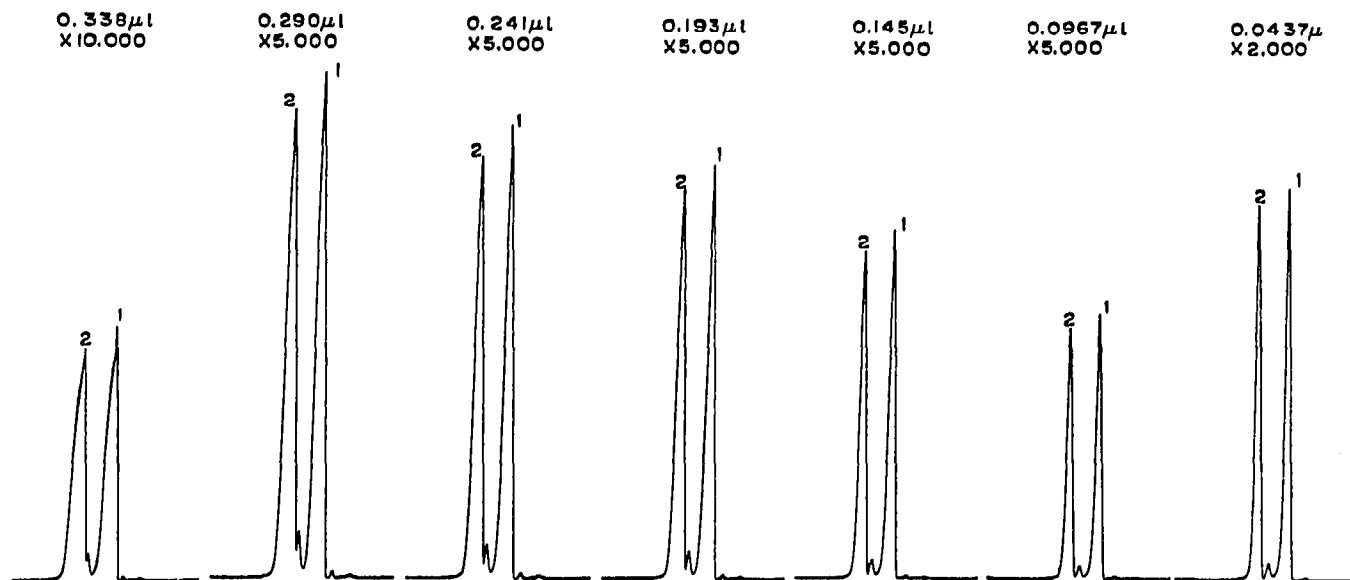

Fig. 1. Chromatograms of a sample containing (1) hexene-1 and (2) *n*-hexane and an impurity, obtained with various sample sizes. Column: 150 ft. × 0.020 in. I.D. support-coated open, tubular, prepared with squalane liquid phase. Column temperature: 100°. Carrier gas (He) flow rate at column outlet: 2.76 ml/min. The numbers above the peaks refer to the total sample volume entering the column and the recorder attenuation used.

listed above each chromatogram. As seen, the separation of the small peak from the second major peak is quite good up to about 0.15 μl total sample volume but the small peak can still be seen even at 0.388 μl total sample.

Fig. 2 plots the values of peak heights, peak area, and resolution, against the volume of a single component entering the column, for the two major peaks (α = 1.138). The resolution (R_h) was calculated from the peak width at half height (w_h):

$$R_h = \frac{2 \, \Delta t}{w_{h1} + w_{h2}} \tag{2}$$

where Δt is the distance of the two peak maxima. Values calculated in this way are by a factor of 1.7 larger than the resolution values (R) calculated from the peak width at base, in the usual way[4]:

$$R_h = 1.7 \, R \tag{3}$$

In Fig. 2, dotted lines indicate the values corresponding to base line separation ($R = 1.5$) and about 85 % separation ($R = 1.0$).
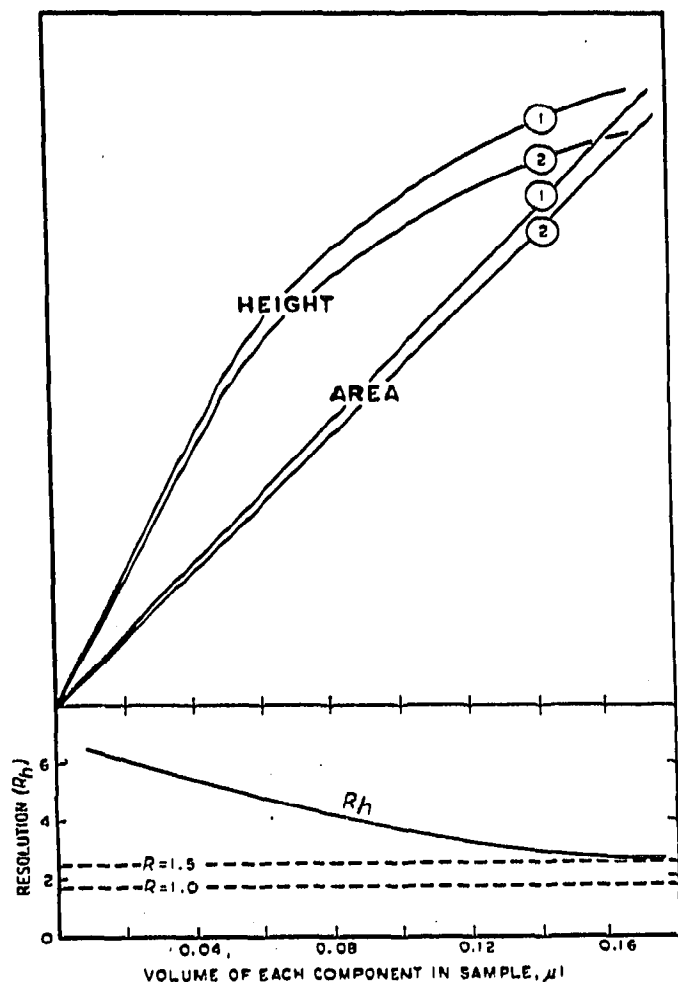

Fig. 2. Plot of the values of peak heights, peak area and resolution against the volume of each component, for the chromatograms shown in Fig. 1.

As seen in Fig. 2, the peak area values are linear over the whole range investigated while the peak heights start to be non-linear at about 0.06 μl (single component). This simply means that for quantitative purposes, one should use peak area rather than peak height above this level. Such an observation can be found for any column above a certain point. Base line separation or better could be obtained up to about 0.16 μl of a single component.

The second test mixture consisted of 3-methylhexane and cyclohexane with a respective concentration of 51.8 and 48.1 vol. %. This sample was analyzed on a 150 ft. long support-coated column prepared with DC-550 phenylsilicone oil liquid phase, at 75°, with a carrier gas flow of 2.7 ml/min ($\bar{u}$ = 17.7 cm/sec). At the given temperature, the two peaks represent a relative retention (α) of 1.079. The values of the partition ratio are 3.70 and 3.99, respectively.

Fig. 3 shows the chromatograms obtained with various sample sizes. The volume of the total sample entering the column and the recorder attenuation are listed above each chromatogram. As seen, the first peak starts to become skewed at about 0.3 μl total sample volume but even there, the separation is still quite good.

Fig. 4 plots the values of peak heights, peak area (for the second peak) and resolution against the volume of a single component entering the column, for the chromatograms shown in Fig. 3. Again, the peak heights start to become non-linear at about 0.06 μl of a single component. The area of the second peak was linear in the whole range investigated; since the first peak skewed significantly with higher sample load, we did not calculate its area. Base line separation or better could be obtained up to about 0.12 μl of a single component.

MINIMUM DETECTABILITY

For the investigation of the minimum detectability, we used ASTM-grade (99.5 % + vol. %) n-heptane as a typical sample because this analysis is the best illustration for a case where high column performance and the determination of trace impurities has to be combined. The problems involved in this analysis were discussed in detail in our previous paper[2].

Fig. 5 shows the chromatogram of an ASTM-grade n-heptane sample, obtained on a 300 ft. × 0.010 in. I.D. classical wall-coated open tubular column prepared with squalane liquid phase, at 37° using a 1 mV recorder with attenuation × 1. We have added 600 p.p.m. cyclohexane and 100 p.p.m. each of cyclopentane and 2-methylpentane to the sample in order to have an indication of the concentration levels of the individual peaks and to be able to calculate minimum detectability. For this analysis, we have introduced $13.2 \cdot 10^{-3}$ μl sample into the column which is about a factor of 5 larger than specified in the original ASTM-method[5]. As a comparison, Fig. 6 shows the analysis of the same sample, on a 150 ft. × 0.020 in. I.D. support-coated open tubular column prepared with squalane liquid phase, at 85°. The volume of sample introduced into the column was now 0.21 μl.

Table I lists the comparative data for these two chromatograms. A peak with 5 mm height obtained on a 1 mV recorder at attenuation × 1 was taken as the minimum detectable limit. As shown, this value was as low as 0.4 p.p.m. with the support-coated column, under the conditions listed.
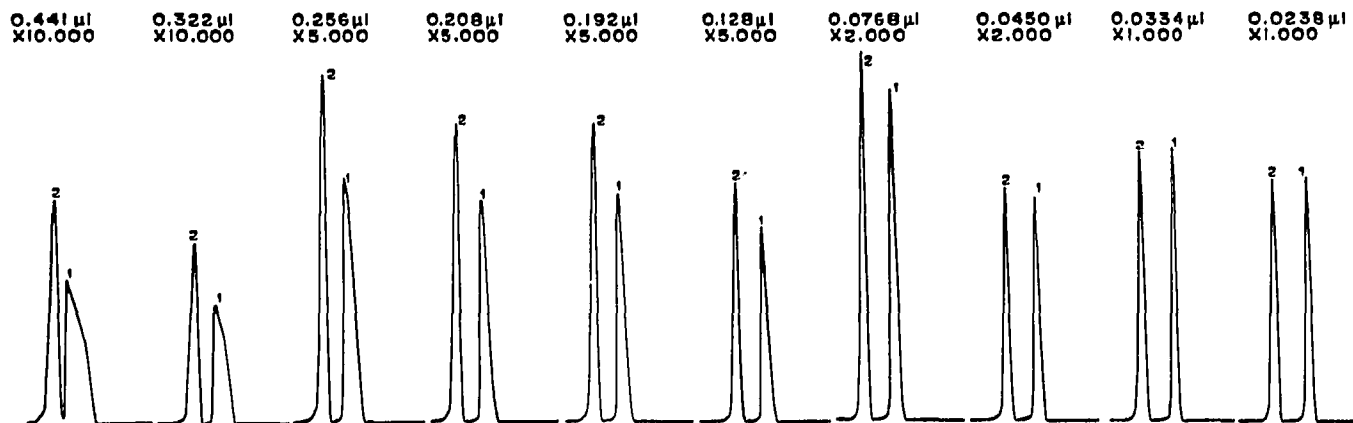
Fig. 3. Chromatograms of a sample containing (1) 3-methylhexane and (2) cyclohexane, obtained with various sample sizes. Column: 150 ft. × 0.020 in. I.D. support-coated, tubular, prepared with DC-550 phenylsilicone oil liquid phase. Column temperature: 75°. Carrier gas (He) flow rate at column outlet: 2.7 ml/min. The numbers above the peaks refer to the total sample volume entering the column and the recorder attenuation used.
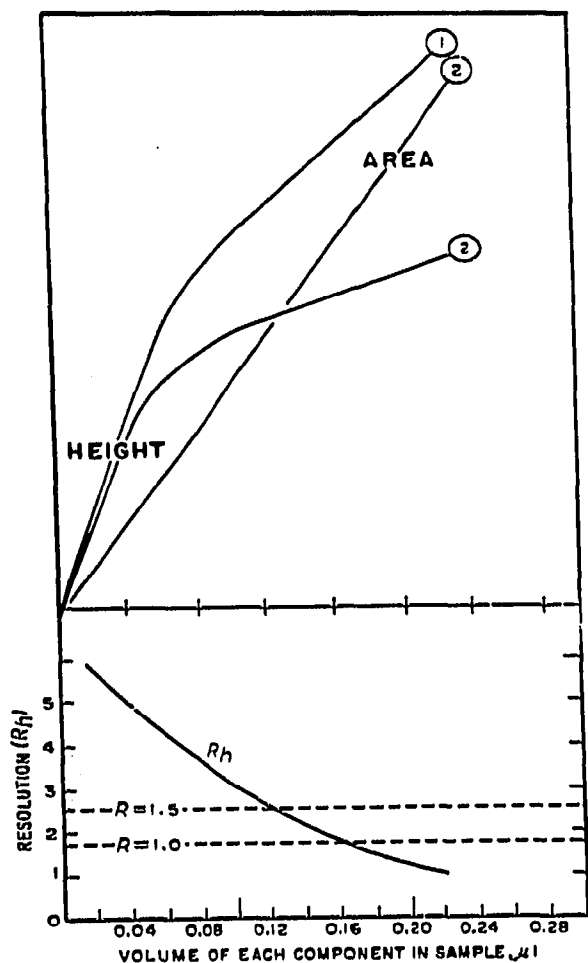

Fig. 4. Plot of the values of peak heights, peak area and resolution against the volume of each component, for the chromatograms shown in Fig. 3.
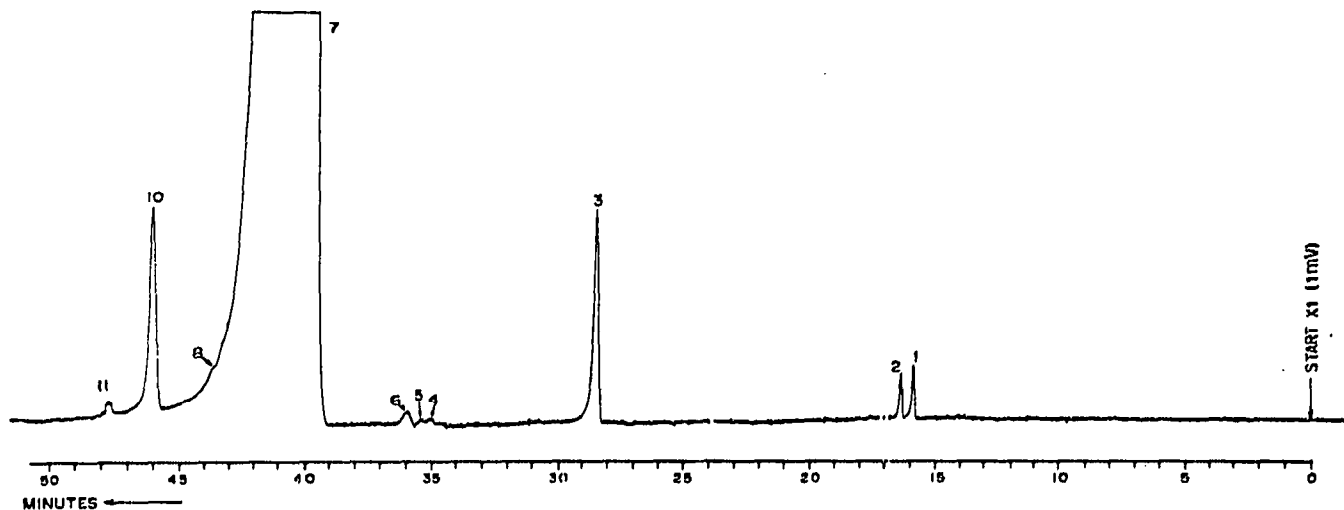
Fig. 5. Analysis of an ASTM-grade *n*-heptane, I. Column: 300 ft. × 0.010 in. I.D. standard wall-coated open tubular. Liquid phase: squalane. Column temperature: 37°. Carrier gas (He) flow rate at column outlet: 1 ml/min. Sample volume: $13.2 \cdot 10^{-3}$ μl. 1 mV recorder. Peaks: 1 = cyclopentane (100 p.p.m.; added), 2 = 2-methylpentane (100 p.p.m.; added), 3 = cyclohexane (600 p.p.m.; added), 7 = *n*-heptane.
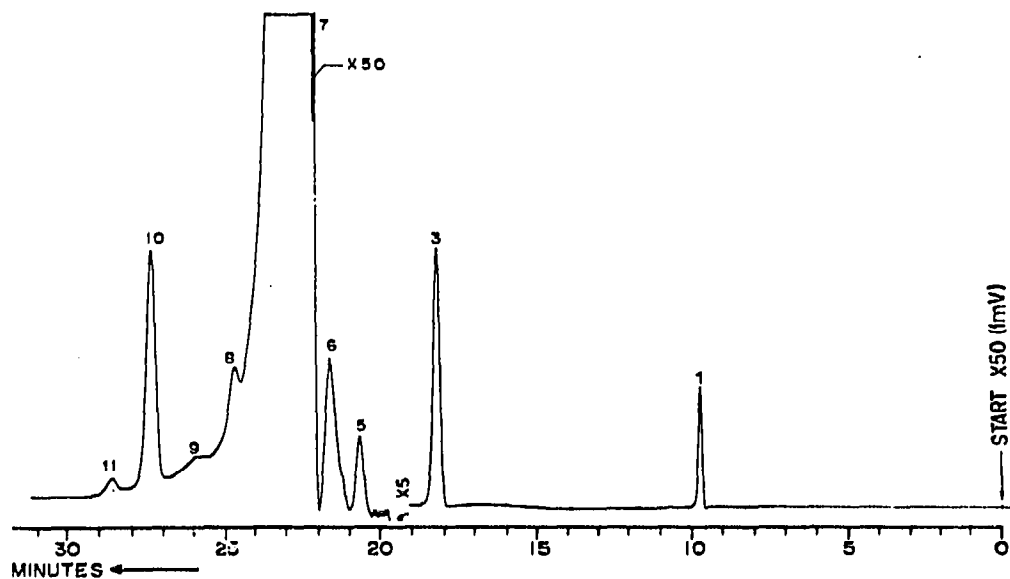

Fig. 6. Analysis of an ASTM-grade *n*-heptane, II. Column: 150 ft. × 0.020 in. I.D. support-coated open tubular. Liquid phase: squalane. Column temperature: 85°. Carrier gas (He) flow rate at column outlet: 3 ml/min. Sample volume: 0.21 μl. 1 mV recorder. Peaks: 1 = cyclopentane + 2-methylpentane (100 p.p.m. each; added), 3 = cyclohexane (600 p.p.m.; added), 7 = *n*-heptane.

TABLE I

COMPARATIVE DATA FOR THE CHROMATOGRAMS SHOWN IN FIGS. 5 AND 6

		Open tubular column	
		Wall-coated	Support-coated
Column length	(ft.)	300	150
Column internal diameter	(in.)	0.010	0.020
Sample volume	(10^{-3} μl)	13.2	210
Column temperature	(°C)	37	85
Carrier gas (He) flow rate	(ml/min)	1.0	3.0
Total number of theoretical plates for cyclo-hexane		116,160	43,540
HETP for cyclohexane	(mm)	0.79	1.05
Minimum detectable limit (cyclohexane) in the sample	(p.p.m.)	18.5	0.4
Retention time of the last peak (No. 11)	(min)	47.7	28.7
Concentration of cyclohexane in the carrier gas at column outlet	(p.p.m.)	4	40

Table I also gives the concentration of cyclohexane in the carrier gas at column outlet. The relatively larger concentration with the support-coated column is advantageous if concentration-sensitive detection devices are used.

COMPARISON WITH PACKED COLUMNS

Until now, support-coated columns were only compared with the standard wall-coated open tubular columns. Below, a comparison with packed columns follows. Since the sample capacity of the usual 1/8 in. O.D. (I.D. 2.2 mm) packed columns and the support-coated open tubular columns is about the same order of magnitude, we will primarily investigate comparative data concerning analysis time, the necessary carrier gas inlet pressure, and column performance.

For this comparison, we took the support-coated column used for the analysis shown in Fig. 6, the conditions of which are listed in Table I. This column is characterized by a β-value of 21.9 and by a specific permeability (B_0) of $583 \cdot 10^{-7}$ cm^2. The partition ratio for cyclohexane was 4.66 and 43,540 theoretical plates were obtained for this peak; this value corresponds to an effective plate number of 29,490. As discussed by DESTY et al.[6], the correlation between the number of theoretical plates (n) and effective plates (N) is expressed by the following equation:

$$N = n \left(\frac{k}{k+1}\right)^2 \tag{4}$$

The number of effective plates is a value which permits a direct comparison of column performance. If one obtains a certain effective plate value on a column, then for a similar separation, the same effective plates are necessary on any column, regardless of its type, prepared with the same liquid phase.

According to DAL NOGARE AND CHIU[7], the optimum β-value for a packed column with 80–100 mesh diatomaceous-earth support is 19. If this column is used at 85°, the value of the partition ratio for cyclohexane is 5.37, on squalane liquid phase.

Thus, the number of theoretical plates on this column corresponding to 29,490 effective plates will be 41,490. Assuming an HETP of 0.6 mm for this column, its length can be calculated as 24.89 m.

Let us assume an outlet flow rate of 40 ml/min for the packed column with 1/8 in. O.D. and 2.2 mm I.D. Using a value of 0.40 for the interparticle porosity (ε) this corresponds to an outlet velocity (u_0) of 43.85 cm/sec. According to DAL NOGARE AND JUVET[8], the specific permeability of a packed column with 80–100 mesh Chromosorb support is $1.96 \cdot 10^{-7}$ cm^2. If the column is operated at 85° with helium carrier gas (viscosity at 85°:$2.2 \cdot 10^{-4}$ poise), the carrier gas inlet pressure (p_i) can be calculated from the KOZENY–CARMAN[9] equation:

$$B_o = 2\,\eta\varepsilon L\,\frac{p_o}{p_i{}^2 - p_o{}^2}\,u_o \tag{5}$$

where η is the carrier gas viscosity, L the column length and p_0 is the column outlet pressure (in this calculation, it is assumed as atmospheric pressure). Knowing the inlet pressure and the compressibility correction factor, the average linear gas velocity can be calculated. On the other hand, from the column length (L), the average gas velocity ($\bar{u}$), and the partition ratio (k), the retention time of a certain peak (t_R) can be obtained:

$$t_R = \frac{L}{\bar{u}}\,(1 + k) \tag{6}$$

In a similar way, we also calculated the retention time of peak No. 11 which is the last peak on the chromatogram.

A packed column with an HETP of 0.6 mm represents very high efficiency, and columns used in practice usually have a higher HETP value. Therefore, we also carried out the same calculation but now for a column with an HETP value of 1 mm. Table II summarizes the values obtained for the support-coated open tubular column and the two packed columns.

As listed in Table II, the retention time of the last peak was only 28.7 min on the support-coated open tubular column while it would be 64.5 and 137.9 min on the packed columns, with respective HETP values of 0.6 and 1.0 mm. The (absolute) carrier gas inlet pressure with the support-coated column was only 1.41 atm; its respective values would be 9.95 and 12.82 atm on the two packed columns.

Finally, we would also like to compare true column performance. As discussed in detail by DESTY et al.[6], the true column performance should be expressed as *the number of effective plates produced in unit time*:

$$\frac{N}{t_R} = \frac{\bar{u}}{h}\,\frac{k^2}{(1 + k)^3} \tag{7}$$

where h is the HETP of the respective peak.

As listed in Table II, for the cyclohexane peak, this value was found as 1630 with the support-coated open tubular column while only 730 and 340 respectively with the two packed columns (expressed as effective plates/min).

TABLE II

COMPARATIVE DATA ON SUPPORT-COATED OPEN TUBULAR AND PACKED COLUMNS PREPARED WITH SQUALANE LIQUID PHASE

| | | Support-coated open tubular column | Packed column | |
			No. 1	No. 2
Column length	(m)	45.720	24.894	41.490
Column internal diameter	(mm)	0.50	2.2	2.2
Column temperature	(°C)	85	85	85
Partition ratio of cyclohexane		4.66	5.37	5.37
Partition ratio of peak No. 11		7.94	9.15	9.15
HETP of the cyclohexane peak	(mm)	1.05	0.60	1.00
Number of theoretical plates for the cyclohexane peak		43,540	41,490	41,490
Number of effective plates for the cyclohexane peak		29,490	29,490	29,490
Carrier gas flow rate at column outlet	(ml/min)	3.0	40.0	40.0
Carrier gas outlet velocity	(cm/sec)	28.93	43.85	43.85
Carrier gas average velocity	(cm/sec)	23.78	6.53	5.09
Carrier gas inlet pressure (abs.)	(atm)	1.41	9.95	12.82
Carrier gas outlet pressure (abs.)	(atm)	1.00	1.00	1.00
Pressure drop along the column	(atm)	0.41	8.95	11.82
Compressibility correction factor		0.822	0.149	0.116
Specific permeability	(10^{-7} cm^2)	586	1.96	1.96
Retention time of cyclohexane	(min)	18.14	40.47	86.54
Retention time of peak No. 11	(min)	28.7	64.5	137.9
Effective plate number/retention time	(min^{-1})	1,630	730	340

EXAMPLES FOR APPLICATIONS

We would like to show four chromatograms on the practical application of support-coated open tubular columns to the analysis of complex samples.

Fig. 7 demonstrates the analysis of a gasoline fraction. A 50 ft. long support-coated column with DC-550 phenylsilicone oil liquid phase was used for this analysis and the carrier gas flow rate was as high as 20 ml/min. The volume of the sample entering the column was 0.2 μl.

Fig. 8 illustrates the analysis of a peppermint oil sample on a 50 ft. long support-coated column prepared with Carbowax 1540 poly(ethylene glycol) liquid phase. The individual peaks in this chromatogram were identified in an integrated GC-MS system[10].

Finally, Figs. 9 and 10 show the separation of C_6–C_9 saturated isomeric hydrocarbons on a 150 ft. long support-coated open tubular column with squalane liquid phase. Two samples were analyzed, the first containing mainly the C_7 while the second the C_8 isomers; the column temperatures were 90 and 110° respectively. These higher temperatures were again made possible by the low β-value; on classical wall-coated open tubular columns prepared with squalane liquid phase, similar samples have to be analyzed at 20–50°, resulting in a much longer analysis time.

We calculated the retention index value[11,12] of each peak and compared these values with the data published recently by the Data Subcommittee of the English Gas Chromatography Discussion Group[13]. Based on this comparison, most peaks
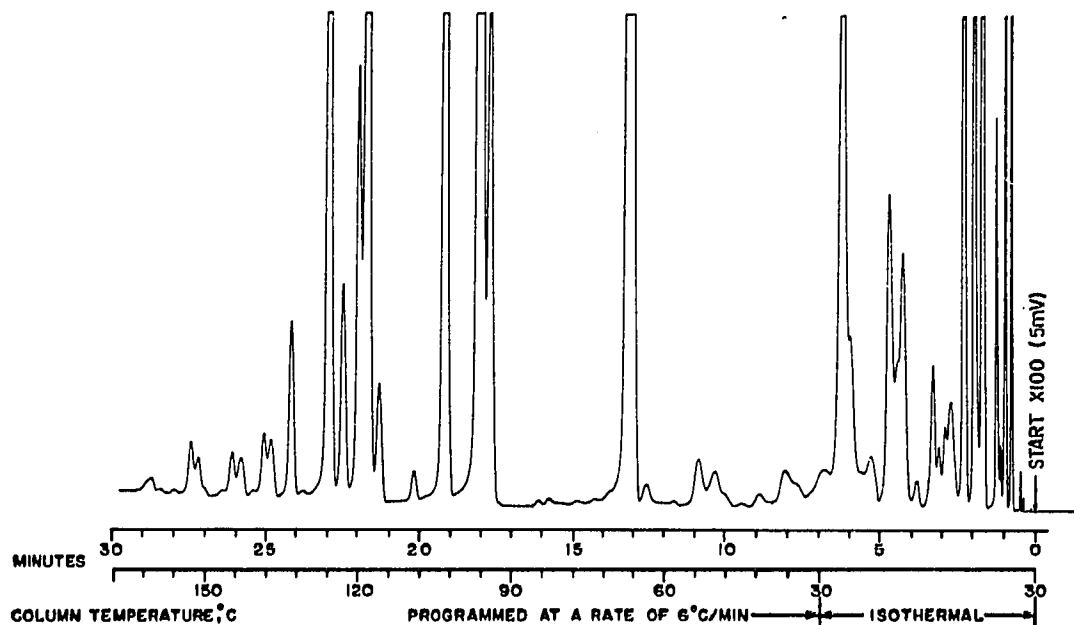
Fig. 7. Analysis of a gasoline fraction. Column: 50 ft. × 0.020 in. I.D. support-coated open tubular. Liquid phase: DC-550 phenylsilicone oil. Carrier gas/flow (He) rate at column outlet: 20 ml/min. Sample volume: 0.2 μl; 5 mV recorder.
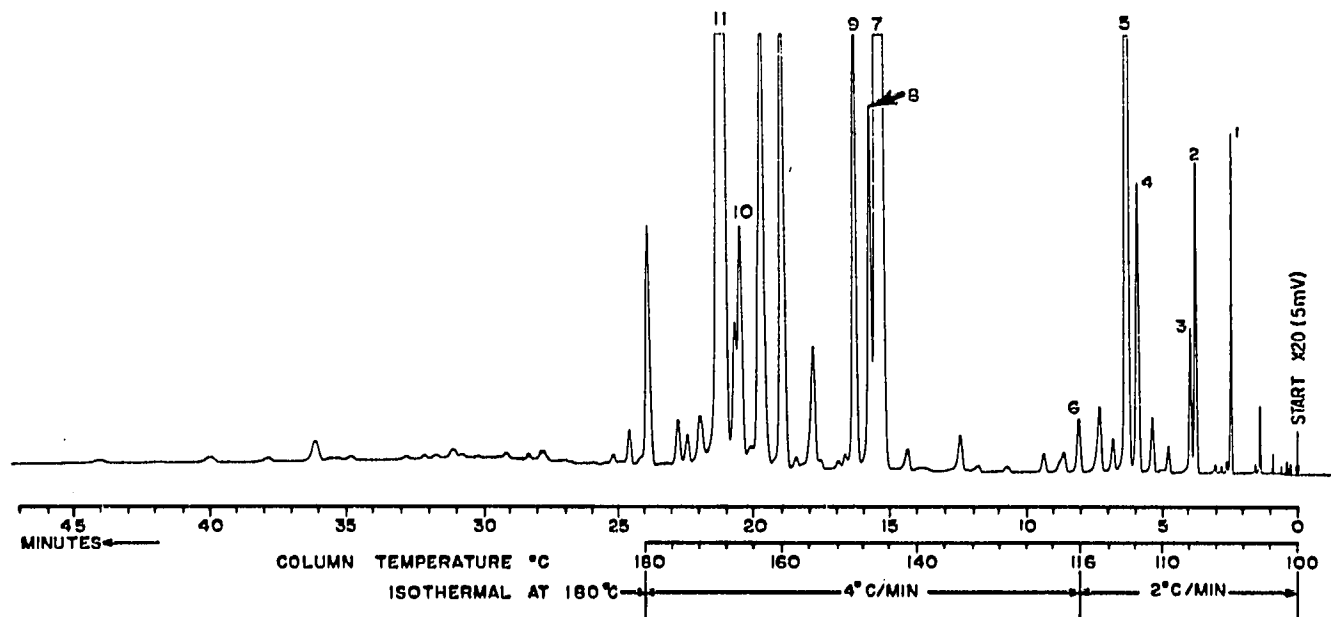

Fig. 8. Analysis of a peppermint oil sample. Column: 50 ft. × 0.020 in. I.D. support-coated open tubular. Liquid phase: Carbowax 1540 poly(ethylene glycol). Carrier gas (He) flow rate at column outlet: 3 ml/min. Sample volume: 0.02 μl. 5 mV recorder. Peaks: 1 = α-pinene; 2 = β-pinene; 3 = myrcene (?); 4 = limonene; 5 = eucalyptol; 6 = p-cymene; 7 = menthone; 8 = menthofurane; 9 = isomenthone; 10 = caryophyllene; 11 = menthol.
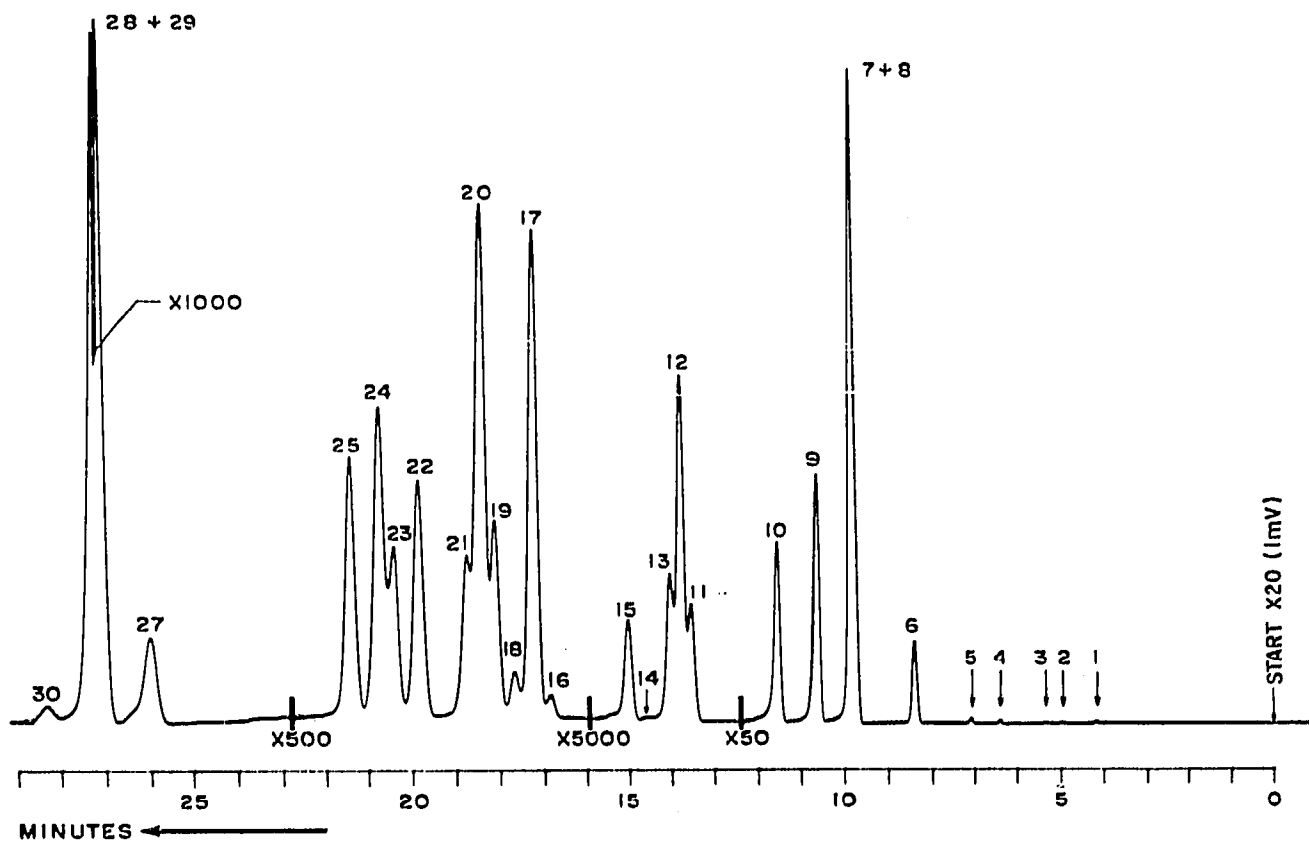
Fig. 9. Analysis of saturated isomeric hydrocarbons, I. Column: 150 ft. × 0.020 in. I.D. support-coated open tubular. Liquid phase: squalane. Column temperature: 90°. Carrier gas (He) flow rate at column outlet: 2.64 ml/min. Sample volume: 0.1 μl. 1 mV recorder. For peak identification, see Table III.
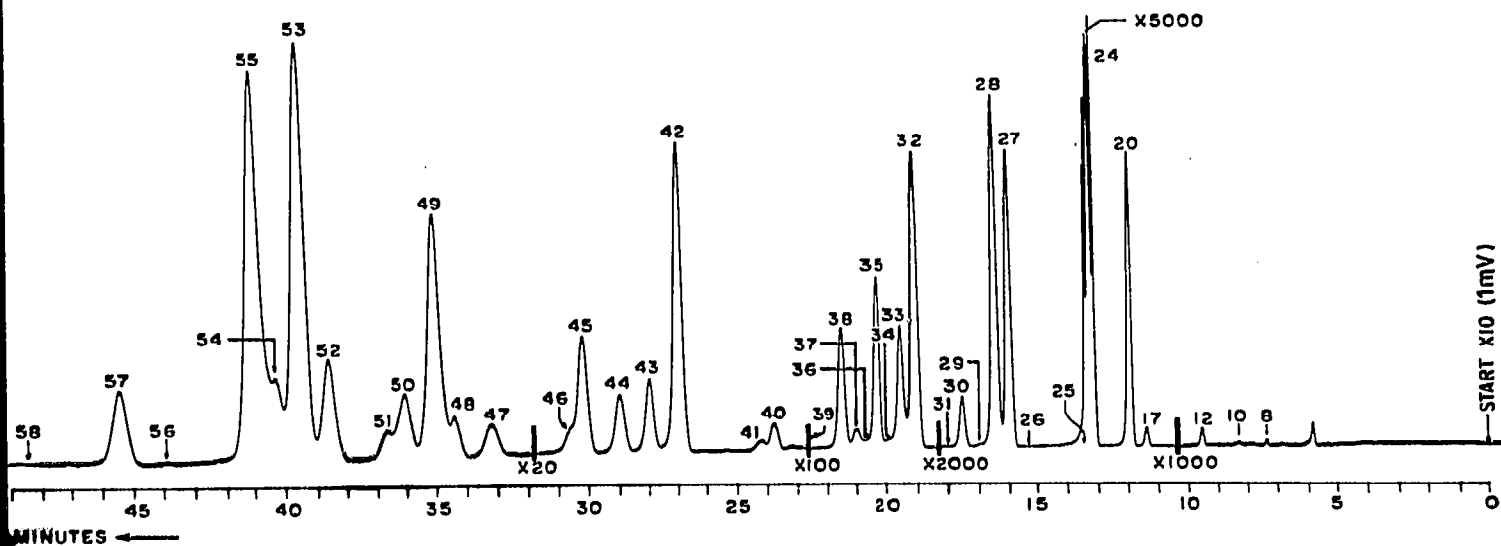

Fig. 10. Analysis of saturated isomeric hydrocarbons, II. Column as in Fig. 9. Column temperature: 110°. Carrier gas (He) flow rate at column outlet: 2.7 ml/min. Sample volume: 0.05 μl. 1 mV recorder. For peak identification, see Table III.

could be identified tentatively. Table III lists the retention index values of the individual peaks and the corresponding substances, based on this tentative identification.

TABLE III

RETENTION INDEX VALUES OF THE PEAKS IN FIGS. 9 AND 10 AND THEIR TENTATIVE IDENTIFICATION

Peak No.	Substance	Retention index at	
		90°C	110°C
5	n-Pentane	500	500
6	2,2-Dimethylbutane	536	—
7	2-Methylpentane 2,3-Dimethylbutane	569	572
8	Cyclopentane		—
9	3-Methylpentane	585	—
10	n-Hexane	600	600
11	2,2-Dimethylpentane	628	—
12	2,4-Dimethylpentane	631	633
13	Methylcyclopentane	633	—
14	?	640	—
15	2,2,3-Trimethylbutane	645	—
16	3,3-Dimethylpentane	664	—
17	2-Methylhexane	667	669
18	Cyclohexane	671	—
19	2,3-Dimethylpentane	675	—
20	3-Methylhexane	678	678
21	Dimethylcyclopentane isomer (?)	680	—
22	3-Ethylpentane	688	—
23	Dimethylcyclopentane isomer (?)	693	—
24	2,2,4-Trimethylpentane	695	696
25	n-Heptane	700	700
26	2,2-Dimethylhexane	—	723
27	2,5-Dimethylhexane	728	731
28	2,4-Dimethylhexane		736
29	Methylcyclohexane 2,2,3,3-Tetramethylbutane	735	741
30	2,2,3-Trimethylpentane	741	767
31	3,3-Dimethylhexane	—	751
32	2,3,4-Trimethylpentane	—	761
33	2,3-Dimethylhexane	—	765
34	2-Methylheptane	—	
35	2-Methyl-3-ethylpentane 4-Methylheptane	—	772
36	2,3,3-Trimethylpentane	—	
37	3-Methylheptane 3,4-Dimethylhexane 3-Ethylhexane	—	777
38	2,2,5-Trimethylhexane	—	781
39	3-Methyl-3-ethylpentane	—	789
40	2,2,4-Trimethylhexane (?)	—	797
41	n-Octane	800	800
42	(?)	—	817
43	(?)	—	823
44	(?)	—	828
45	(?)	—	835
46	(?)	837	837
47	(?)	—	849

TABLE III (continued)

Peak No.	Substance	Retention index at	
		90°C	110°C
48	(?)	—	855
49	(?)	—	858
50	(?)	—	862
51	(?)	—	864
52	(?)	—	872
53	(?)	—	876
54	(?)	—	879
55	(?)	—	881
56	(?)	—	891
57	(?)	—	896
58	n-Nonane	900	900

SUMMARY

It was demonstrated that with support-coated open tubular columns, critical separations (base line resolution) can be achieved even with sample volumes as high as 0.15 µl of a single component. The minimum detectable limit with such a column was as low as 0.4 p.p.m., about a factor of 50 lower than with a standard wall-coated capillary column. Comparison with long packed columns of comparable sample capacity showed that their utilization makes high inlet pressures necessary while the time of analysis is increased many times. The relative performance of support-coated open tubular columns—expressed as effective plates per time—is much higher than even the best packed column.

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