

changes in size. One of the advantages of thin layer chromatography over paper chromatography is that corrosive reagents may be used in the former without a noticeable increase in background density.

Several reports have recorded techniques for evaluating substances on paper chromatograms by densitometry of the images formed on sensitized material. Gustafsson, Sundman, and Lindh (6) determined the area of spots of carbohydrates from densitometric measurements on film on which the images appeared bright on a dark background. Sulser (13) employed circular paper chromatography to separate fatty acids and to detect adulteration of rape seed oil. The density of one band compared to another after photographic reproduction was taken as an index of purity of the oil. Only one sample could be assayed at a time. Tyrosine was evaluated on paper chromatograms by reproduction on photocopy paper by Mykolajewycz (11). The bright areas of the images were measured in a densitometer. The recording of thin layer chromatograms on blueprint

paper was reported by Eisenberg (3) and of paper chromatograms by Gordon (5).

In the method presented here, all spots of a plate are photographed simultaneously and no time differences in the reaction of substances with color reagent can occur. This is particularly useful if the spots become intensified with time or if they fade. Many samples can be assayed at one time. Once photographed, a plate can be discarded. The photographs present a permanent record of the experiment, and the quantification may be performed or repeated at any time.

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## Backflushing and Two-Stage Operation of Capillary Columns in Gas Chromatography

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► Backflushing or multicolumn operation has been widely used with packed columns, but not with capillary columns. A capillary column has been successfully backflushed using a unique carrier gas flow switching valve. Also a capillary column has been combined in a two-stage chromatograph with either another capillary column or a packed column. Examples of the separations obtained with these column arrangements are given.

FOR the analysis of dilute hydrocarbon gas mixtures, such as are encountered in vehicle emission and air pollution studies, capillary columns can provide better analyses than packed columns (?). However, for mixtures of wide boiling range, complete separations could not be obtained with a single column. To improve the range of separations and extend the use of capillary columns, two techniques that have been widely used with packed columns—backflushing and two-stage operation—were investigated with capillary columns.

The main consideration in using these techniques with any column system is the valve for switching the carrier gas flow. It must be leak-free and have a small internal volume in relation to the carrier gas flow rate to avoid reducing column efficiency. The valves that have been satisfactorily used with the high flow rates of packed columns have too large an internal volume for use with the low flow rates of capillary columns. In an attempt to overcome this limitation, Fett (3) proposed adding an auxiliary supply of carrier gas at the end of a capillary column to reduce the effective volume of the valve.

Several valves that have recently been developed for process gas chromatographs have very small internal volumes and appear to be suitable as flow switching valves for capillary columns. Crum (2) and Wall (10) have described slider-type valves, and Karasek and Ayers (4) have described a unique diaphragm-type valve. A commercial version of the latter valve has been satisfactorily used by the author for gas sampling (6). This valve is also used in the present work for

backflushing and two-stage operation of capillary columns.

#### EXPERIMENTAL

An F & M Model 609 gas chromatograph, modified (5) for use with capillary columns, was used in this work. A small laboratory oven was added for thermostating a gas sampling valve and a carrier gas flow switching valve at 80° C. Both valves were the same type: a pneumatically operated diaphragm valve (Type D) obtained from the Greenbrier Plant of the Bendix Corp. Leak-free service was obtained by polishing the internal surfaces of these valves (6).

The data for the columns are listed in Table I, A. The capillary columns were obtained from the Perkin-Elmer Corp. The 0.020-inch o.d. columns were used as received in loosely wound coils; the 0.062-inch o.d. capillary column had to be rewound (5) to fit into the F & M column oven and allow the air to circulate around all parts of the column. The packed columns were prepared in the usual manner. The Apiezon C was dissolved in pentane, the solution added to the sieved alumina or silica gel (both from

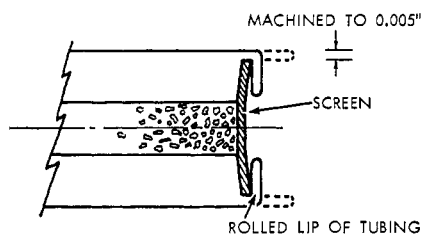


Figure 1. Method of closing ends of column

Burrell Corp.), and the pentane removed by warming the packing. To pack a 0.028-inch i.d. tube, a fine wire was inserted a few inches into the tube to guide the packing and prevent clogging. The packing was retained with 400-mesh screen held in place by a rolled lip on each end of the tube as shown in Figure 1.

The data for the chromatographic experiments are given in Table I. B. Liquid samples were injected using a 1- $\mu$ l. syringe and stream splitting; the gas sample was injected directly into the column using a sampling valve. The gas flows to the detector were chosen to obtain maximum detector response. Hydrogen and auxiliary nitrogen were added to the carrier gas stream at the detector to make a 1 to 1 nitrogen-hydrogen fuel mixture.

#### RESULTS

**Backflushing.** The apparatus arrangement for backflushing a capillary column is schematically shown in Figure 2. A six-port valve was used and is satisfactory for flow switching; however, a four-port valve (if available) would suffice and obviate the

connecting tube. A flow restriction tube prevents flow surges from blowing out the flame of the detector (F.I.D.) when the valve is switched.

This column arrangement was used to obtain the separation shown in Figure 3 for a mixture of 22 paraffins ranging from isopentane to *n*-octane. The lighter components were foreflushed and the heavier components backflushed from the column. The backflushed components were eluted in reverse order to their normal elution, a phenomenon discussed by Villalobos, Brace, and Johns (9).

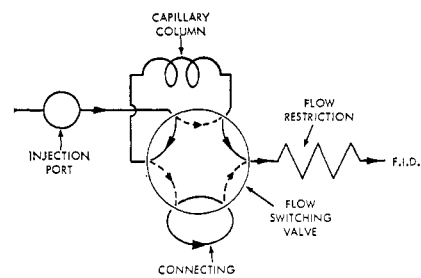


Figure 2. Column backflushing arrangement

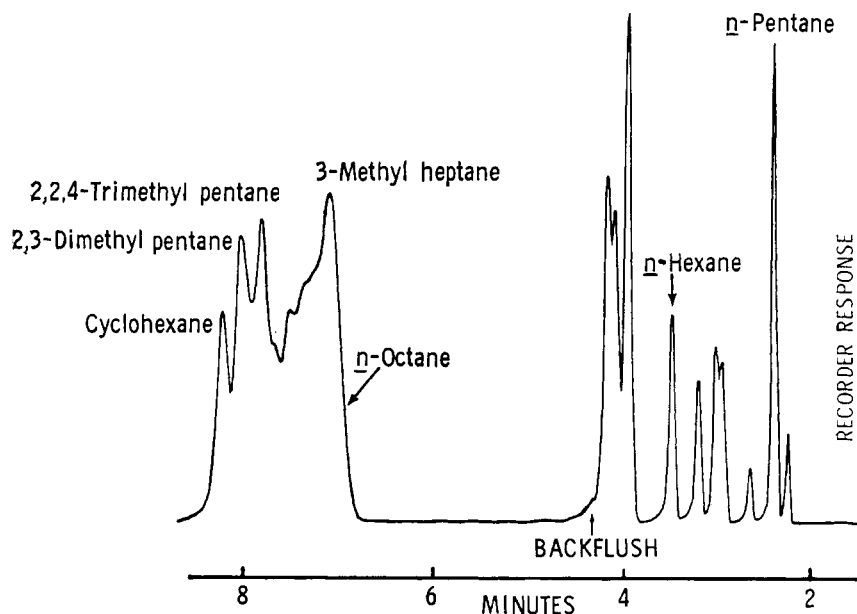


Figure 3. Backflushing heavier portion of  $C_5$  to  $C_8$  paraffin mixture

Table I. Experimental Data

Column No.	A. Column Data			Packed	
	1	2	3	4a	4b
Length, ft.	150	150	150	0.5	1.5
I.d., inch	0.010	0.010	0.010	0.028	0.028
O.d., inch	0.020	0.020	0.062	0.062	0.062
Liquid phase	MBMA <sup>a</sup>	DC 200 silicone	DC 200 silicone	5% Apiezon C	5% Apiezon C
Support	...	...	...	Alumina 80-100 mesh	Silica gel 100-120 mesh
Figure No.	B. Chromatographic Conditions				
	3	5	7		
Sample	$C_5$ to $C_8$ paraffins	$C_8$ to $C_{10}$ aromatics	Gas: $C_1$ to $C_8$ hydrocarbons		
Sample volume, ml.	$2.5 \times 10^{-4}$	$1.5 \times 10^{-4}$	2.15		
Sample split ratio	23.3	22.8	None		
Column No.	2	1 and 2	3 and 4		
Column temp., °C.	25	100	As noted		
Inlet pressure, p.s.i.g.	35	35	59		
Air, ml./minute	620	620	620		
H <sub>2</sub> , ml./minute	35	35	35		
N <sub>2</sub> , ml./minute					
Carrier	4.8	2.8	7.4		
Auxiliary	30	30	28		

<sup>a</sup> 20% Apiezon L, 80% *m*-Bis(*m*-phenoxyphenoxy)benzene.

The advantages of backflushing include not only reducing analysis time by removing higher-boiling components, but also prolonging column life by removing contamination that tends to build up at the column inlet. This consideration is probably more important with capillary than with packed columns, since capillary columns are more expensive and more difficult to prepare. Also, migration of the liquid phase toward the column outlet is more serious in capillary than in packed columns, so that by reversing the carrier gas flow, any net migration can be minimized.

The effect of the valve on the separation efficiency of the capillary column was determined by comparing the separations obtained with and without the valve connected to the same column. Only data on a few of the lighter paraffins were compared, since with the valve the heavier paraffins were backflushed, and without the valve they were temperature-programmed (5).

The retention volume,  $V_R$ , of a component is composed of the holdup volume of the chromatographic system,

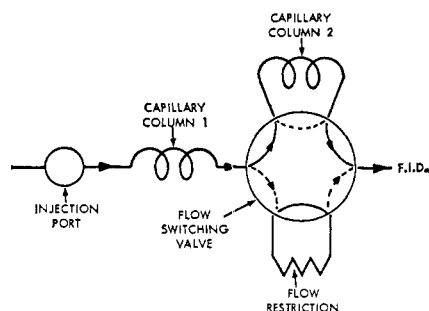


Figure 4. Two-stage arrangement of capillary columns

$V_M$ , and the adjusted retention volume,  $V_R'$ , due to the partitioning of the component between the liquid and gas phases (1).

$$V_R = V_M + V_R'$$

Adding the valve should only increase the value of  $V_M$  (and consequently  $V_R$ ) and not affect  $V_R'$ , so that

$$V_R' = V_{R_1} - V_{M_1} = V_{R_2} - V_{M_2}$$

Subscripts 1 and 2 indicate the absence and presence of the valve, respectively. Transposing terms gives

$$V_{R_2} - V_{R_1} = V_{M_2} - V_{M_1}$$

which is equal to the holdup volume of the valve and should therefore be constant. However, values of  $V_{R_2} - V_{R_1}$  for four hydrocarbons listed in Table II are not constant, but increase with retention volume. This indicates that the valve contributes not only to the holdup volume, but also to the separation process, presumably because of adsorption on the valve and associated fittings.

The calculation of the number of theoretical plates,  $N$ , is commonly used for evaluating column performance (1).

$$N = 5.54(V_R/v)^2$$

where  $v$  is the gas volume equivalent to the peak width at half peak height. However, as Purnell (8) has pointed out,  $N$  is really not a good basis for comparing two systems in which the holdup volumes are changed. Increasing the holdup volume by adding the valve increases the value of  $V_R$ , and therefore the value of  $N$ . On the other hand,  $v$  is also increased, because of adsorption and diffusion in the valve, so that the values of  $N_1$  and  $N_2$  listed in Table II are similar.

The calculation of the resolution,  $R$ , between two components X and Y is a better basis for comparison, since the difference in retention volumes,  $\Delta V_R$ , is used and the holdup volume cancels out.

$$R = \frac{2(\Delta V_R)}{1.7(v_X + v_Y)}$$

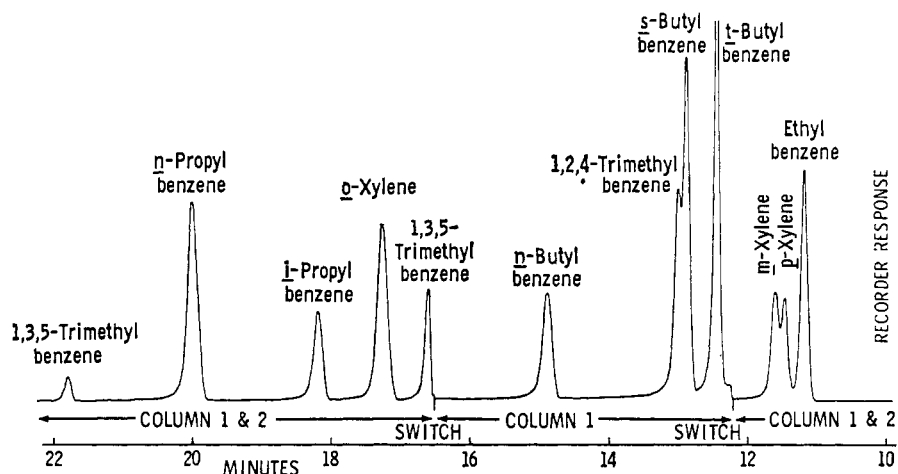


Figure 5. Two-stage separation of aromatic mixture with capillary columns

The term 1.7 was used to convert peak width measurements made at half peak height to the corresponding widths at the base line, the latter being the usual method for calculating  $R$  (1). As listed in Table II,  $R_1$  and  $R_2$  are essentially equal, which indicates that the component resolution was unaffected by the addition of the valve. However, a visual examination of the two chromatograms shows slightly greater peak tailing when the valve was used. In other words, adding the valve increases not only  $V_R$  and  $v$  (leaving  $N$  and  $R$  unchanged), but also contributes to slightly greater peak tailing.

**Two-Stage Operation, I.** Two-stage operation of packed columns has been frequently used for separating wide boiling range mixtures. To investigate this technique with capillary columns, the two column-one detector arrangement shown in Figure 4 was used. Depending upon the position of the switching valve, the carrier gas flows either through columns 1 and 2 in series or bypasses column 2 through a flow-restriction tube.

The separation of a mixture of aromatic hydrocarbons with this column arrangement is shown in Figure 5. The butylbenzenes and 1,2,4-trimethylbenzene were separated with column 1 only (bypassing column 2), whereas the re-

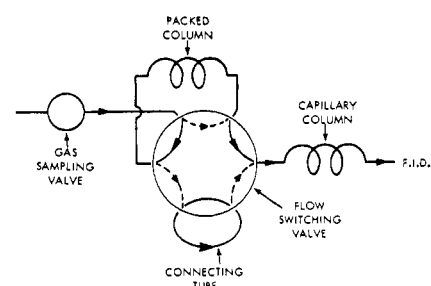


Figure 6. Two-stage arrangement of packed and capillary column

maining components were separated with both columns in series.

The advantages of this column arrangement are twofold. First, analysis time is reduced by letting the higher-boiling components bypass column 2. Secondly, two different types of capillary columns can be used, so that separations not possible with one type can be obtained with the other. Thus the MBMA column was used for partially separating the *m*- and *p*-xylenes.

This arrangement also has a serious disadvantage, as illustrated by the unusual elution of 1,3,5-trimethylbenzene before *o*-xylene. This occurred because the valve was initially switched just after the 1,3,5-trimethyl-

Table II. Effect of Valve on Column Parameters<sup>a</sup>

Hydrocarbon	$V_{R_2} - V_{R_1}$ , ml.	$N_1$	$N_2$	$R_1$	$R_2$
<i>n</i> -Pentane	1.72	11700	11800	8.05	8.00
3-Methylpentane	2.30	13200	12900	2.40	2.46
<i>n</i> -Hexane	2.50	13600	14300	3.75	3.73
Methylcyclopentane	2.79	14100	14300		

<sup>a</sup> Subscripts 1 and 2 indicate absence and presence of valve, respectively.

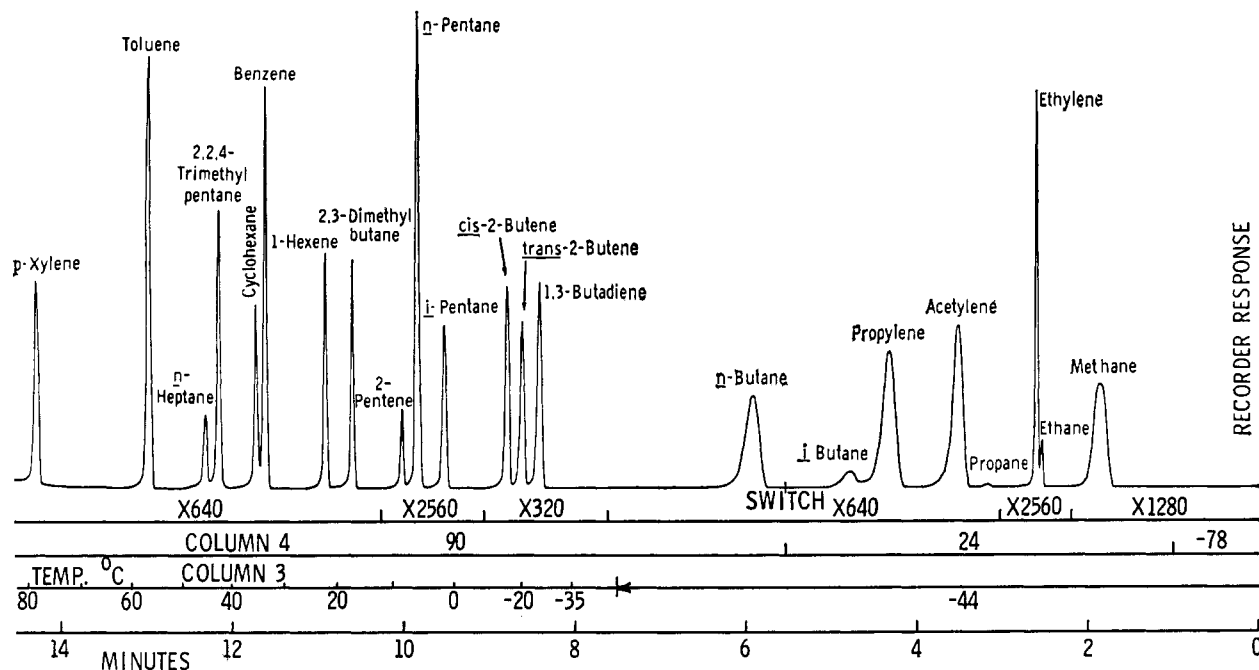


Figure 7. Two-stage separation of hydrocarbon gas mixture with packed and capillary column

benzene had entered column 2. The pressure gradient in the isolated column 2 caused a reverse gas flow that carried most of this compound from the inlet end of column 2 through the valve to the outlet end. When the valve was again switched to reconnect column 2, the carrier gas (now at elevated pressure at the column outlet) rapidly eluted the 1,3,5-trimethylbenzene to give a sharp front to the peak. The remaining components were eluted in the usual order, including a small amount of 1,3,5-trimethylbenzene that had remained at the inlet end of column 2. Thus the disadvantage of not knowing exactly when to switch the valve leads to the possibility of dividing a peak, either when the valve is initially switched or by the shifting of a component when column 2 is isolated.

**Two-Stage Operation, II.** A more useful two-stage arrangement, one combining the advantages of a packed column with those of a capillary column, is shown in Figure 6. The carrier gas always flows first through the packed column, then through the capillary column. Switching the valve only changes the direction of flow through the packed column.

This column arrangement has been advantageously used for analyzing dilute hydrocarbon gas mixtures. Figure 7 shows the separation of a mixture of hydrocarbons that ranged in concentrations from 1 to 228 p.p.m.; 99.8% of the sample consisted of inorganic

gases, chiefly nitrogen and carbon dioxide.

The sample was injected into column 4 (4a and 4b in series) cooled with dry ice. After trapping the hydrocarbons for 1 minute, the column was warmed to room temperature with a water bath to elute the  $C_1$  to  $C_4$  hydrocarbons (except the  $C_4$  olefins). After  $n$ -butane had passed into column 3, the valve was switched and the remaining hydrocarbons in column 4 were backflushed into column 3, where they were separated. Column 4 was heated to 90° C. with a water bath to accelerate the backflushing operation. Column 3 was cooled to -44° C. (with chipped dry ice added to the column oven) to separate the  $C_4$  olefins, then programmed to 80° C. to elute the heavier hydrocarbons rapidly.

A complete separation of all of the hydrocarbons was obtained in one 15-minute run. Alumina was used in the packed column to remove carbon dioxide, which is also trapped and otherwise eluted with ethane and ethylene and affects their separation and detector measurement. The methane peak is broader than usual, because it was not trapped as well as the other hydrocarbons. The separation of the  $C_3$  and  $C_4$  hydrocarbons was improved by passing them through column 3 cooled to -44° C. The heavier hydrocarbons were very effectively separated on column 3 after being trapped and backflushed from column 4. Extremely dilute mixtures have been analyzed by increasing the sample size

to 20 ml. The principal effect of increasing the sample size is poorer trapping of the methane and consequently a much broader peak. This method is now being used for the analysis of automobile exhaust gases.

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