Journal of Chromatography A, 657 (1993) 103–109
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 213

Characterization of capillary column stationary phases by statistical analysis of retention data☆

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(First received December 10th, 1992; revised manuscript received April 14th, 1993)

ABSTRACT

Characterization of the stationary phases of gas chromatographic capillary columns was attempted by statistical analysis of retention data obtained from test mixtures regularly used in the laboratory. The method was applied to columns belonging to two groups: the first group included those coated with two methyl silicone-based stationary phases, SE-30 (OV-1) and SE-54, while columns in the second group contained FFAP, AT-1000 and Carbowax 20M, three polyethylene glycol-based phases. The relative retention of a single pair of components allowed a complete classification of the two phases of the non-polar group, whereas the polar columns were difficult to classify even using discriminant statistical techniques.

INTRODUCTION

The so-called "column test mixtures" allow a simple quality control method for capillary columns that is very useful for both GC users and manufacturers. They provide information about efficiency and deactivation, and can serve to measure the phase ratio [1,2]. Some attempts have been made to use these mixtures as an indicator of column polarity. The typical elution order of Grob's mixtures I and II [3–5] has been proposed as an aid to determining the type of stationary phase in a column; but since these mixtures are analysed in programmed temperature mode, parameters related to geometrical properties of columns can change the elution pattern. For similar phases, such as SE-30 and SE-54 columns, mixture II provides almost identical elution patterns.

McReynolds constants [6] are based on the determination of the retention indices of several probe compounds, and on the use of a squalane (reference) column. Since they were developed mainly for the characterization of the stationary phases in packed columns, the probe compounds are very volatile and their retention indices are often difficult to determine with accuracy in capillary columns, especially in those with a very high phase ratio. Other methods have been proposed for phase characterization, based on thermodynamic criteria [7,8], selectivity index [9], relative retentions [10] and other measures [11].

In the present work we applied statistical analysis to the characterization of the polarity of capillary columns, using test mixtures in the isothermal mode. Capacity ratio (k) values from several groups of both non-polar and polar columns were used as starting data. The quantitative values assigned in the column characterization are a general measure of the phase chromatographic behaviour and can also be used for phase identification. Our main objective was to assess the possibility of differentiation between columns coated with stationary phases of slightly different polarity, independently of their tube material, deactivation grade and phase ratio.

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☆ Presented at the *21st Scientific Meeting of the Spanish Group of Chromatography and Related Techniques*, Granada, October 21–23, 1992.

0021-9673/93/$06.00

MATERIALS AND METHODS

A total of 56 capillary columns, coated with non-polar silicone (SE-30, OV-1 and SE-54) or polyethylene glycol (PEG)-based (Carbowax 20M, FFAP and AT-1000) stationary phases, described in Table I, were tested. They were either prepared in our laboratory (using the static method) or purchased from different suppliers (Alltech, Deerfield, IL, USA; Varian, Walnut Creek, CA, USA; Teknokroma, Barcelona, Spain). These columns had been used in many analytical applications and their dimensions, phase ratios and deactivation grades were very different.

Test mixtures were prepared with reagents of analytical grade dissolved in dichloromethane (all from Merck, Darmstadt, Germany). The components in each mixture (Table II) were selected to include, in a close elution range for columns of each phase group, several chemically different compounds. Mixtures were injected into columns of the non-polar group at 100°C, and at 160°C into columns of the polar group.

Retention data from columns in Table I were collected over several years, when each column was tested. Different gas chromatographs [Hewlett-Packard (Palo Alto, CA, USA) Model 5890; Perkin-Elmer (Norwalk, CT, USA) Models 900 and 3920; Varian, Model 3300; Carlo Erba (Milan, Italy) Models 4130 and 5160] and recorders were used. Carrier gas was always nitrogen at a flow-rate near the Van Deemter optimum in every case. Injections were made in split mode, with split ratios varying between 30:1 and 150:1.

Capacity ratios (k) were obtained from retention time and dead time measures for the components in each test mixture (eight components for the non-polar mixture, five for the polar mixture). Relative retentions $r_{x,y}$ were calculated for all possible pairs of components x,y (28 pairs for the non-polar mixture, ten for the polar mixture). These relative values were used in the characterization in order to avoid possible errors in the determination of film thickness and column internal diameter.

Discriminant analysis was carried out by using the BMDP program package [16]. Stepwise discriminant analysis (program 7M in the BMDP package) calculates the combination of variables that best predicts the group to which a case belongs: in each step, the variable having the highest F value is added to the combination. Mean values, standard deviations and coefficients of variation for each variable were also calculated by the same program.

RESULTS AND DISCUSSION

Methyl silicones (OV-1, SE-30 and others) are very commonly used in capillary GC. Incorporation of a small proportion of phenyl groups (about 5% in SE-54) leads to improved properties at low temperatures [17], although the level of substitution is not enough to provide any worthwhile selectivity [18].

Seventeen columns prepared with SE-30 or OV-1 were considered to belong to a single group (group A1), since these phases are considered to be equivalent [17,18]; McReynolds constants (Table III) are almost identical for the two phases. Eight columns prepared with SE-54 were included in group A2.

When the $r_{x,y}$ non-polar data set from groups A1 and A2 was submitted to stepwise discriminant analysis, a 100% correct classification was obtained with the quotient between capacity ratio of dodecane and capacity ratio of naphthalene [k(C12)/k(N) variable], which showed the highest F value. Although a combination of more variables calculated by discriminant analysis provides an even better separation between SE-30 and SE-54 groups, the use of one or two variables is enough to show the difference between both groups. Fig. 1 plots the non-polar columns using as variables k(C13)/k(E10) and k(C12)/k(N).

The most common poly(ethylene oxide) or PEG-based phases are the Carbowax group. Carbowax 20M is the trade name of a PEG polymer with an average molecule mass between 15 000 and 20 000; although extensively used, it has the disadvantage of batch-to-batch variability [22]. The reaction product of Carbowax 20M with nitroterephthalic acid has better properties for the elution of free acids, and is usually called free fatty acid phase (FFAP) [23]. A purified

TABLE I

CHARACTERISTICS OF THE COLUMNS USED IN THIS STUDY

d_f = Film thickness; N = no; Y = yes.

Phase	Tubing	I.D. (mm)	Length (m)	d_f (μm)	Leaching[a]	Dehydration[b]	Deactivation[c]	Cross-linked
SE-30	Pyrex	0.2	22	0.17	Y	Y	N	N
SE-30	Pyrex	0.2	24	0.1	Y	Y	TMSI	N
SE-30	Pyrex	0.2	16	0.12	Y	Y	D4	N
SE-30	Pyrex	0.2	19	0.1	Y	Y	D4	Y
SE-30	Pyrex	0.2	12	1.4	Y	Y	D4	Y
OV-1	Pyrex	0.2	6.5	0.25	Y	Y	D4	Y
OV-1	Pyrex	0.2	24	0.1	Y	Y	D4	N
OV-1	Pyrex	0.35	22	0.3	N	N	N	N
OV-1	Silica	0.22	24	0.3	N	N	TMSI	Y
OV-1	Silica	0.22	22	0.14	N	N	TMSI	Y
OV-1	Silica	0.22	25	0.14	N	N	TMSI	N
OV-1	Silica	0.22	25	0.15	N	N	N	N
OV-1	Silica	0.33	22	0.08	N	N	N	N
OV-1	Silica	0.25	10	0.2	N	N	N	Y
OV-1	Silica	0.25	5	0.2	N	N	N	Y
OV-1	Silica	0.1	5	0.1	N	N	N	Y
Methylsilicone	Silica	0.2	10	–	(Supplier: Alltech)			
Methylsilicone	Silica	0.33	4.5	–	(Supplier: Varian)			
SE-54	Soda-lime glass	0.2	20	0.08	Y	Y	HMDS + DPTMDS	N
SE-54	Soda-lime glass	0.2	20	0.16	Y	Y	HMDS + DPTMDS	N
SE-54	Pyrex	0.2	43	–	Y	Y	HMDS + DPTMDS	Y
SE-54	Pyrex	0.2	18	0.05	Y	Y	TMSI	Y
SE-54	Silica	0.2	25	0.2	N	Y	N	N
SE-54	Silica	0.25	10	0.2	N	Y	N	Y
SE-54	Silica	0.2	23	0.15	N	Y	N	N
SE-54	Silica	0.32	37	0.32	(Supplier: Teknokroma)			
AT-1000	Pyrex	0.2	15	1.0	Y	Y	GPTMS	Y
AT-1000	Silica	0.22	18	1.1	Y	Y	GPTMS	Y
AT-1000	Silica	0.22	9.5	0.3	Y	Y	GPTMS	Y
AT-1000	Silica	0.22	9.5	0.3	Y	Y	GPTMS	Y
AT-1000	Silica	0.22	9.0	0.3	Y	Y	GPTMS	Y
AT-1000	Silica	0.22	10	0.3	Y	Y	GPTMS	Y
FFAP	Silica	0.25	10	0.37	Y	Y	GPTMS	Y
FFAP	Silica	0.25	9	0.37	Y	Y	GPTMS	Y
FFAP	Silica	0.25	10	0.37	Y	Y	GPTMS	Y
FFAP	Silica	0.25	10	0.37	Y	Y	GPTMS	Y
FFAP	Silica	0.25	8	0.37	Y	Y	GPTMS	Y
FFAP	Silica	0.25	9	0.37	Y	Y	GPTMS	Y
FFAP	Silica	0.32	11	1.6	Y	Y	GPTMS	Y
Carbowax 20M	Silica	0.22	20	0.17	N	N	N	N
Carbowax 20M	Silica	0.22	20	0.19	N	N	N	N
Carbowax 20M	Silica	0.2	23	0.2	N	N	GPTMS	Y
Carbowax 20M	Silica	0.25	22	0.3	Y	Y	GPTMS	Y
Carbowax 20M	Pyrex	0.2	–	0.4	Y	Y	N	Y
Carbowax 20M	Pyrex	0.2	18	0.2	Y	Y	GPTMS	Y
Carbowax 20M	Pyrex	0.2	21	0.2	Y	Y	GPTMS	Y
Carbowax 20M	Pyrex	0.25	–	1.0	Y	Y	N	Y
Carbowax 20M	Pyrex	0.2	10	0.5	Y	Y	N	Y

(Continued on p. 106)

TABLE I *(Continued)*

Phase	Tubing	I.D. (mm)	Length (m)	d_f (μm)	Leaching[a]	Dehydration[b]	Deactivation[c]	Cross-linked
Carbowax 20M	Pyrex	0.19	20	0.1	Y	Y	PEG	Y
Carbowax 20M	Pyrex	0.19	20	0.1	Y	Y	PEG	Y
Carbowax 20M	Pyrex	0.35	20	0.85	Y	N	N	N
Carbowax 20M	Pyrex	0.35	18	0.85	Y	Y	NaCl	N
Carbowax 20M	Pyrex	0.35	18	0.76	Y	Y	NaCl	N
Carbowax 20M	Pyrex	0.35	18	0.85	Y	Y	NaCl	N
Carbowax 20M	Pyrex	0.35	18	0.85	NH_4F	N	N	N
Carbowax 20M	Pyrex	0.2	20	0.1	NH_4F	N	PEG	Y
Carbowax 20M	Pyrex	0.2	11	0.35	NH_4F	N	PEG	Y

[a] Leaching according to Grob [5], NH_4F [12].
[b] Dehydration according to Grob [5].
[c] Treatments with: D4 (octamethylcyclotetrasiloxane) [13], TMSI (trimethylsilylimidazole), HMDS + DPTMDS (hexamethyldisilazane + diphenyltetramethyldisilazane) [5], GPTMS (glycidoxypropyltrimethoxysilane) [5], PEG [14], NaCl [15].

TABLE II

COMPONENTS OF TEST MIXTURES USED FOR POLARITY ASSESSMENT

Component	Code	Non-polar test	Polar test
Undecane	C11	+	–
2,4-Dimethylaniline	A	+	–
Dodecane	C12	+	–
Naphthalene	N	+	+
n-Decanol	ol	+	+
Tridecane	C13	+	–
Carvacrol	Cv	+	–
Methyldecanoate	E10	+	–
Dicyclohexylamine	am	–	+
Nonadecane	C19	–	+
o-Cresol	oCr	–	+

FFAP is distributed by Alltech under the name AT-1000 [20], whereas Supelco only distributes columns under the name SP-1000 [21]. The McReynolds constants for these phases are listed in Table III. While the Carbowax 20M and Carbowax-TPA constants given by McReynolds [6] are almost identical, those of FFAP are clearly different from the latter; other literature values [19,21] are very scattered.

The 31 columns tested with the polar mixture were coated with Carbowax 20M, AT-1000 and FFAP as stationary phases, and were submitted to different preparation treatments as summarized in Table I.

Columns prepared with FFAP and AT-1000 were considered in a first step to belong to the same group (group P1), since the manufacturer of AT-1000 [20] claims that they are equivalent. Columns prepared with Carbowax 20M were included in group P2. When groups P1 and P2 were submitted to stepwise discriminant analysis, the variable with the highest F value was k(N)/k(ol); however, 5 of the 31 columns were incorrectly classified using this single variable (see Fig. 2). The use of more variables somewhat improved the classification; a combination of variables k(ol)/k(am), k(N)/k(am), k(C19)/k(am) and k(N)/k(ol) (canonical variable 1 in Fig. 2) reduced to three the number of incorrect classifications.

In a second step, columns in group P1 were subclassified into groups P1A (FFAP) and P1B (AT-1000). Groups P1A, P1B and P2 were then submitted to discriminant analysis. The results of the best classification, achieved using variables k(ol)/k(am), k(c19)/k(am), k(N)/k(ol) and k(oCr)/k(ol), are shown graphically in Fig. 3; classification was incorrect in 4 of the 31 columns.

There was a clear difference between the results from the non-polar columns (Fig. 1) and

TABLE III

McREYNOLDS CONSTANTS OF THE STUDIED PHASES

Phase	ΔI Benzene	ΔI Butanol	ΔI 2-Pentanone	ΔI Nitropropane	ΔI Pyridine	Ref.
SE-30	15	53	44	64	41	6
OV-1	16	55	44	65	42	6
SE-30	10	53	39	65	58	19
SE-54	33	72	66	99	67	6
FFAP	340	580	397	602	627	6
AT-1000	–	–	–	–	–	20
SP-1000	332	555	393	583	543	21
Carbowax TPA	321	537	367	573	520	6
Carbowax 20M	322	536	368	572	510	6
Carbowax 20M	278	483	322	518	463	19
FFAP	325	541	370	577	511	This work
AT-1000	334	557	384	595	533	This work
Carbowax 20M	335	567	389	594	550	This work
Carbowax 20M	346	580	398	610	561	This work

the polar columns (Figs. 2 and 3). In the first case, the separation into groups was easy: the use of a single variable seems to be enough to classify an unknown column, and even to detect a mixture of phases, in spite of differences in dimensions, materials and preparation methods. However, for columns prepared with Carbowax 20M, FFAP or AT-1000, the separation was less clear. From the plots in Figs. 2 and 3, it can be seen that the dispersion among columns of the same group is close to the separation between groups. The use of discriminant functions including four or more variables was required for classification purposes, but even in this case some classifications were not correct.

A reason for these results could be the high variation in the $r_{x,y}$ values for the Carbowax 20M columns: the mean relative standard deviation was 4.98% for Carbowax 20M, 1.83% for FFAP, 1.29% for AT-1000, 2.35% for SE-30 and 2.54% for SE-54. More important, however, seems to be the difference between the chromatographic

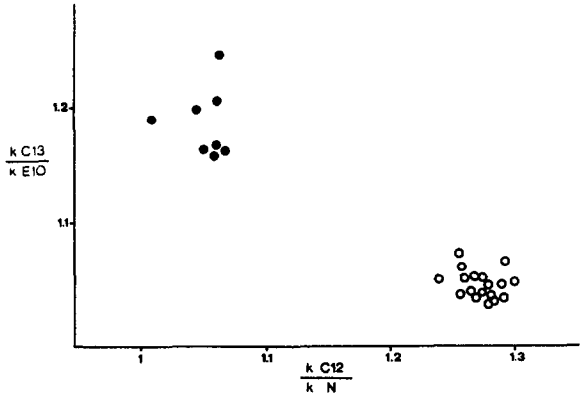

Fig. 1. Plot of non-polar columns using $r_{x,y}$ values. ○ = Group A1 (SE-30, OV-1); ● = group A2 (SE-54).

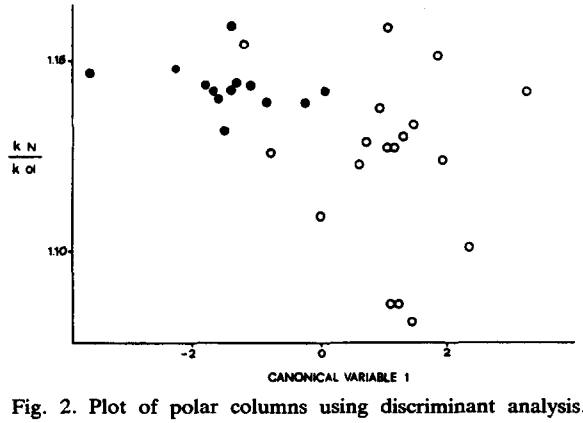

Fig. 2. Plot of polar columns using discriminant analysis. ● = Group P1 (FFAP, AT-1000); ○ = group P2 (Carbowax 20M).

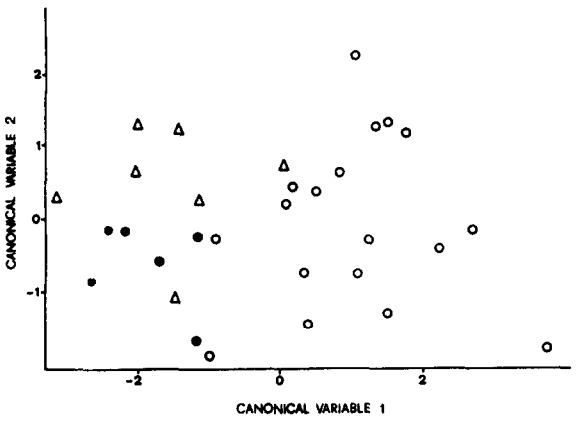

Fig. 3. Plot of polar columns using discriminant analysis. △ = Group P1A (FFAP); ● = group P1B (AT-1000); ○ = group P2 (Carbowax 20M).

properties of these phases. The mean value of the absolute differences between $r_{x,y}$ values for SE-30 and SE-54 columns was 0.214, while for Carbowax 20M and AT-1000 it was only 0.043.

From these results, Carbowax 20M, FFAP and AT-1000 are then difficult to distinguish using this set of variables. Other chromatographic differences, for example the better properties of FFAP in the elution of free acids, also depend on deactivation procedures and are difficult to quantify. However, these phases could probably be correctly classified using chromatographic retention data from other different compounds.

The use of McReynolds constants presents similar problems. Data from refs. 6 and 19–21 in Table III seem to indicate a high dispersion even for the two Carbowax 20M phases. We measured the retention indices of McReynolds probe compounds at 120°C on a FFAP, an AT-1000 and two Carbowax 20M capillary columns (Table III): the values also show a high scatter.

Although our results were calculated using data from capillary columns and could have an important error, they seem to confirm the high degree of chromatographic variation previously found [22] among the columns prepared with PEG-based phases. Since differences of deactivation and dimensions can be discounted (they did not impair the distinction of silicones) the reason for the broad variability of Carbowax 20M should be related to the phase properties. Besides its batch-to-batch variability, this phase seems to be very sensitive to oxidation and ageing [24]. The use of Superox 20M (which is supposed to present better properties than Carbowax) is now in progress in our laboratory; the new data could be used to indicate the extent of degradation of the phases used above.

The method presented here can be applied to other gas chromatographic stationary phases by selecting suitable probe compounds. It can be used in the identification of "unknown" capillary columns and also in the characterization of mixed-phase capillary columns whose phase composition cannot be determined from the coating procedure. The results could also be used to substitute a column for another with a different phase but with an equivalent behaviour: for instance, a column coated with FFAP can be more like a Carbowax one than two different Carbowax columns.

Although the work presented here has been a time-consuming process, it only has to be carried out once. Using the above results as a starting point, it is only necessary to prepare a solution and make a single injection in order to confirm that the chromatographic behaviour of a column corresponds or not to a given stationary phase. The columns are usually correctly identified in a laboratory, but a quality control is always necessary when a column is bought. In some cases, where identification tags are wrong (or lost), the use of a characterization sample affords an easy way to solve the problem.

ACKNOWLEDGEMENT

This work was supported by DGICYT (Projects PB88-034 and PB91-0077-C03-02).

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