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THE EFFECT OF THE COLUMN SUPPORT MATERIAL ON THE GAS CHROMATOGRAPHIC RESOLUTION OF METHYL PARATHION, ETHYL PARATHION, ETHYL PARAOXON, MALATHION, AND MALAOXON*

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SUMMARY

The gas chromatographic capabilities of commercially available solid supports produced from diatomaceous earths were compared, using QF-1 silicone (FS 1265, 10 000 centistokes) as the stationary liquid phase. The relative efficiencies of the columns were evaluated from the chromatographic data obtained with the organophosphate compounds methyl parathion, ethyl parathion, ethyl paraoxon, malathion, and malaoxon.

The data indicated that commercial methods for the preparation of solid supports have improved, but not to a degree sufficient to assure reproducible results with different batches of the same type of support. Each new batch of support should be examined for density and adsorptive characteristics before it is considered as a replacement for the column previously used.

It has been previously reported[1] that the type of solid support used in a gas chromatography column would govern the capability of the column to resolve efficiently the pesticides methyl parathion, ethyl parathion, and ethyl paraoxon. Solid supports are now available which are reported to be produced under more rigidly controlled conditions and capable of being more consistent in performance characteristics. This, in part, has been made possible by the preparation of finer mesh-size grades free from fines, by improved methods for the treatment of the support with silanes, and perhaps other innovations. Ideally, the support should be inert and should not be a factor in the chromatographic separation of a compound. This should be a function of the stationary liquid phase only. However, the availability of an "inert" support is questionable. In partial support of this premise, it has been observed that solid supports do not possess the same performance characteristics from one purchased lot to the next. This is illustrated in the results of a study reported herein on five of the improved types of gas chromatographic supports with a fixed set of gas chromato-

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graphy conditions for the analysis of methyl parathion, ethyl parathion, ethyl paraoxon, malathion, and malaoxon.

MATERIALS AND METHODS

The effects of mesh size, batch, and source of the solid supports on the resolving capability of the stationary liquid phase were studied in an effort to find a correlation between one or more of these factors, or some characteristics of the support not easily definable, that may cause anomalous results. Characteristics such as surface area, active sites, or other physical properties, may be responsible for the variations between

TABLE I

OBSERVED DENSITY VARIATION IN DIFFERENT BATCHES OF CHROMOSORB W[a]

Mesh size	*Weight of 10 c.c. volume[b] (g) A*	*Grams/c.c.*	*Grams in 4 ft. × 4 mm I.D. column[c] B*	*Ratio B/A*	*Liquid phase required for equivalent columns[d] (%)*
45–60	2.25	0.23	3.54	1.57	5.1
50–60	2.11	0.21	3.56	1.69	5.1
60–70	2.24	0.22	3.74	1.67	4.8
60–80	2.00	0.20	3.37	1.68	5.3
70–80	2.01	0.20	3.50	1.74	5.2
80–100[e]	2.53	0.25	4.50	1.77	4.0
80–100[f]	2.09	0.21	3.69	1.77	4.9
80–100 HP[g]	2.19	0.22	3.78	1.73	4.8
100–120	2.30	0.23	4.05	1.76	4.5
Minimum	2.00	0.20	3.37		4.0
Maximum	2.53	0.25	4.50		5.3
Maximum difference, %	26.5		33.5		
Density data on other supports[a]					
Chromosorb G					
100–120	5.20	0.52	9.12	1.75	2.0
Chromosorb P					
60–80	3.80	0.38	6.56	1.73	2.7
80–100	3.90	0.39	6.62	1.72	2.7
Gas Chrom Q					
100–120	2.86	0.29	4.84	1.76	3.7
Aeropak 30					
100–120	2.08	0.21	3.80	1.82	4.7

[a] Acid washed, dimethyl dichlorosilane treated.
[b] Free fall density measurement.
[c] Column packed with aid of vibrator.
[d] 0.18 g QF-1 (FS-1265) fluorosilicone for column weight B.
[e] Purchased from Company A.
[f] Purchased from Company B.
[g] Purchased from Company C, HP = "high performance" grade.

different batches of the same support even though they are considered to be similar according to present methods of standardization. Under controlled conditions, permitting only the type of solid support to be the variable, several pesticides were chromatographed, and their response characteristics were compared to determine any differences the support might exert on the chromatographic capabilities of the pesticide.

The solid supports, listed in Table I, were purchased as acid-washed (AW), dimethyl dichlorosilane (DMCS) treated materials. The Chromosorbs G, P, and W were produced from diatomaceous earth deposits in California by the Johns-Manville Company; the different adsorptive and relative hardness characteristics of the three supports have been created by calcination processes. Chromosorb W, "High Performance" grade, is described as a material "developed to provide a support with the highest inertness"[2]. Aeropak 30, prepared from Chromosorb W, has been sized and processed through a series of controlled steps for silanization of the support[3]. Gas Chrom Q, produced from diatomaceous earth deposits in Nevada by the Eagle-Picher Company, was also sized and silanized under rigidly controlled conditions[4]. The mesh sizes 45–60 through 100–120 Chromosorb W were obtained from the same source at the same time, with the exception of two of the three listed 80–100 mesh grades (see Table I). Each of the two additional 80–100 mesh supports were purchased from different companies.

The stationary liquid phase QF-1 fluoro silicone (10 000 centistokes) was used in all columns. The per cent of liquid phase added to each solid support varied (on a weight/weight basis) because of the different densities of the solid supports; the amount (g) of liquid phase added to the solid support for each column was the same (see Table I). The support material was added to an ethyl acetate solution of the QF-1 silicone with constant swirling. The mixture was placed on a steam bath to evaporate the ethyl acetate, and gently stirred occasionally during this period. The columns were firmly packed with the dried material using suction and a vibrating tool (Vibro Graver) against the column for 30 sec. Undue handling of the support might fracture portions of the support particles, thereby producing fines, which may restrict the gas flow, and expose so-called "active sites". The support was retained in the column with plugs of silanized glass wool. Prior to use, each column was conditioned for 18 h at 200° with a constant flow of gas.

The gas chromatograph system was an F & M Model 810, electron capture detector (200 mC), pulse interval 50 microsec, range 10, attenuation 32; carrier gas argon–methane (95:5), flow rate 65–75 ml/min (200°). The gas chromatography column was a 4 ft. × 4 mm I.D. borosilicate glass spiral; column temperature 200° (except as noted in text); injector temperature 215°; detector temperature 200°. A Leeds and Northrup Speedomax H recorder, 1 mV full scale, chart speed 1/2 in. per min was used. The design of the instrument permitted injection of the samples directly onto the column and no metallic parts occurred in the column area.

The pesticides, graciously supplied by the American Cyanamid Company, Princeton, N.J., were analytical grade quality (99.0–99.8% purity) and were:

Methyl parathion: O,O-dimethyl O-*p*-nitrophenyl phosphorothioate
Ethyl parathion: O,O-diethyl O-*p*-nitrophenyl phosphorothioate
Ethyl paraoxon: O,O-diethyl O-*p*-nitrophenyl phosphate
Malathion: O,O-dimethyl S-(1,2-dicarbethoxyethyl) phosphorodithioate
Malaoxon: O,O-dimethyl S-(1,2-dicarbethoxyethyl) phosphorothioate

RESULTS AND DISCUSSION

Some variation in the densities of the Chromosorb W supports was presupposed and was in agreement with published data[5]. However, the tangible variation in the density measurements of different batches of the same mesh size was unexpected, and this suggested that if the density of a given support was not predetermined, an appreciable error could be established in the preparation of a column if the column was to be a replacement for one of similar composition. For example, three batches of Chromosorb W, each purchased from a different company, each of the same mesh size, 80–100, were sufficiently different in density that a variation of 20 to 25% in the amount of liquid phase would be required to produce columns of equivalent composition (see Table I), if a constant weight of support was used. It is noted that the ratio of the densities of the packed weight of the solid support to the free fall weight was remarkably constant (Table I), indicating that a uniform packing technique was possible. The importance of establishing a rationale for the selection of the liquid phase for a specific pesticide problem in gas chromatography has been recently discussed[6]; it is obviously equally important that the solid support should be classified in terms of density and adsorptive characteristics prior to its use[7].

Retention time data on the organophosphate compounds using four different types of column support materials are given in Tables II and III. It was necessary to

TABLE II

COMPARATIVE DATA ON COLUMNS CONTAINING AN EQUIVALENT AMOUNT OF STATIONARY LIQUID PHASE

Column support[a]		*Liquid phase QF-1 (%)*	*Retention time of pesticide (min)*				
Type	*Mesh size*		*Methyl parathion*	*Ethyl parathion*	*Ethyl paraoxon*	*Malathion*	*Malaoxon*
Chromosorb G	100–120	2.0	3.3	4.3	6.6	3.3	NC[e]
Chromosorb W	45–60	5.1	2.4	3.2	NC	2.2	NC
Chromosorb W	50–60	5.1	2.4	3.2	NC	2.4	NC
Chromosorb W	60–70	4.8	3.0	4.1	5.9	3.0	NC
Chromosorb W	60–80	5.3	2.7	3.5	5.4	2.6	NC
Chromosorb W	70–80	5.2	2.7	3.5	5.0	2.5	NC
Chromosorb W[b]	80–100	4.0	3.1	4.1	NC	3.0	NC
Chromosorb W[c]	80–100	4.9	3.4	4.3	6.3	3.4	NC
Chromosorb W (HP)[d]	80–100	4.8	3.1	4.0	5.6	3.1	4.2
Chromosorb W	100–120	4.5	3.4	4.4	NC	3.4	NC
Aeropak 30	100–120	4.7	3.9	5.0	NC	7.4	NC
Gas Chrom Q	100–120	3.7	2.6	3.4	5.4	2.6	NC

[a] All supports were purchased as acid washed, dimethyl dichlorosilane treated materials. The amount of QF-1 liquid phase was 0.18 g for a 4 ft. × 4 mm I.D. column. See Table I for solid support weights for columns.

[b] Company A.

[c] Company B.

[d] Company C, HP = "high performance" grade.

[e] NC indicates that either the compound did not chromatograph or the recorded curve was too poor to be considered.

TABLE III

RELATIVE RETENTION TIMES, ASSIGNING METHYL PARATHION A VALUE OF 1.0

Column support		*Liquid phase QF-1 (%)*	*Ethyl parathion*	*Ethyl paraoxon*	*Malathion*
Type	*Mesh size*				
Chromosorb G	100–120	2.0	1.30	2.00	1.00
Chromosorb W	45–60	5.1	1.33	NC[d]	0.92
Chromosorb W	50–60	5.1	1.33	NC	1.00
Chromosorb W	60–70	4.8	1.37	1.97	1.00
Chromosorb W	60–80	5.3	1.30	2.00	0.96
Chromosorb W	70–80	5.2	1.30	1.85	0.93
Chromosorb W[a]	80–100	4.0	1.32	NC	0.97
Chromosorb W[b]	80–100	4.9	1.26	1.85	1.00
Chromosorb W (HP)[c]	80–100	4.8	1.29	1.81	1.00
Chromosorb W	100–120	4.5	1.29	NC	1.00
Aeropak 30	100–120	4.7	1.28	NC	1.92
Gas Chrom Q	100–120	3.7	1.31	2.08	1.00

[a] Company A.
[b] Company B.
[c] Company C; HP = "high performance" grade.
[d] NC indicates that either the compound did not chromatograph or the recorded curve was too poor to be considered.

vary the amount of each compound applied to the gas chromatograph, in order to obtain a reasonable and acceptable response on the recorder chart. Recorder response ranged from one-fifth to one-half of full scale for two nanograms of methyl parathion, dependent upon the particular support. To obtain equivalent responses with ethyl parathion, the ratio was 1.5:1 (ethyl parathion: methyl parathion); for malathion it was 4:1 (with the exception of the Aeropak 30 support) and, where applicable, paraoxon varied from 3:1 to 10:1. In the one instance where the compound could be chromatographed, malaoxon required about 10 times the amount of malathion for an equivalent recorder response.

Earlier studies[1] indicated that Chromosorb W would not satisfactorily resolve ethyl paraoxon on the column. The present study showed that this compound could be resolved on Chromosorb W in some instances, and was probably due to the improved methods of producing this support. However, as indicated in Table II, each new purchase of this type of support would require preliminary examination to determine if ethyl paraoxon would be resolved. Chromosorb W, "High Performance" grade, gave excellent results with ethyl paraoxon, both in terms of curve symmetry and lower detectable limits; three nanograms of paraoxon was equivalent to one nanogram of methyl parathion in terms of recorder response. This was also the only support of those studied that produced a curve for malaoxon under the same operating conditions as employed for the other compounds. Malathion and malaoxon chromatographed, with excellent separation, on this column in an approximate ratio of one nanogram of malathion to ten nanograms of malaoxon. A sharp needle-like peak immediately following the origin of the curve, plus a gradual sloping curve back to the base line for the next two minutes suggested a decomposition product of malaoxon; nevertheless,

the recorded curve at about four minutes on the chart was symmetric and could be consistently reproduced on this column.

A fifth type of support, Chromosorb P, proved to be nonusable with the pesticides studied, *i.e.*, only ethyl parathion gave a poor response on this column; the other compounds failed to chromatograph, perhaps because of the greater adsorptive properties of this support attributable to the greater surface area per unit volume (ratio of about 3:1) of Chromosorb P over Chromosorb W[7]. KAWAHARA *et al.*[8] were unable to chromatograph methyl and ethyl parathions at a column temperature of 195°. Their problem may have been caused by the fact that they used Chromosorb P (acid-washed only) mixed with Chromosorb W (not acid washed) in their column packing.

Methyl parathion and malathion were not differentiated on any of the columns except Aeropak 30 (see Tables II and III). Aeropak 30 separated methyl parathion and malathion if it was used within 24 h from the time it was exposed to a temperature 200°. However, the results obtained with malathion on this support were somewhat erratic, which suggested that the support was either reacting with the compound and/or it required more than 18 h of conditioning at 200°. Probably, the characteristics of the other supports would change with increased conditioning time, but the data in Table II suggests that any changes with these columns would be more uniform. There was no change in the weight of any of the columns after 24 h at 200°, indicating that any losses due to column bleeding were below the weighable limits of the analytical balance.

It is emphasized that the primary purpose of this study was to compare column supports available from different commercial sources. Obviously, the methyl parathion–malathion resolution problem is primarily one pertaining to the selection of the stationary liquid phase, and it is known that liquid phases other than QF-1 are available for this purpose.

Experiments with the Aeropak 30 column, conditioned at 200° for 64 h, showed improved sensitivity levels for malathion but at the expense of poorer resolution from the adjacent recorded curve obtained for ethyl parathion. After 96 h conditioning, the malathion and ethyl parathion curves superimposed on the recorder chart, a characteristic which remains unchanged after 117 h of column conditioning. The data obtained on Aeropak 30 were of interest, because this support was processed from Chromosorb W, yet its stability and performance characteristics were tangibly different than those of Chromosorb W (see Tables II and III).

The relative thermal stability of malathion and malaoxon at 200° may be critical[9] and it may become acute if the column support is a contributory factor. At a column temperature of 150°, only Chromosorb G, Aeropak 30, and Chromosorb W, "high performance" grade produced reasonably defined curves with malaoxon, and the pattern of the curves suggested a decomposition product and not the parent compound. A cooler injection temperature of 140° did not improve the performance characteristics of malaoxon. The adsorptive properties of the support with some compounds may be as critical as temperature; the performance of malaoxon on Chromosorb W, "High Performance" grade, when compared to the other supports is a case in point. The retention time of ethyl parathion relative to methyl parathion was practically a constant with all of the columns studied. This would suggest that the column supports could be used interchangeably but for the fact that this charac-

teristic was not consistent with the compound ethyl paraoxon and, in one instance, with malathion. Furthermore, the variation in the recorder response of the compounds with the different supports was additional evidence of some form of interaction of the compounds with the supports. As noted by EVANS AND SMITH[10], the role of the support phase is more complicated than it was previously considered, having been widely regarded as an inert phase. The word "inert" as applied to column supports has been ill-used in gas chromatography literature, and a more precise expression should probably refer to the degree of "adsorptivity" or "reactivity" of the material.

In conclusion, the Chromosorb G and Gas Chrom Q supports were satisfactory for the analysis of mixtures of methyl parathion, ethyl parathion, and ethyl paraoxon when they were used with the stationary liquid phase QF-1 (FS 1265) fluorosilicone. However, subsequent purchase of these supports, as well as the other types mentioned, would require pre-examination to determine if the materials would give consistent results. SIMMONS AND TATTON[11] used Chromosorb G (AW, DMCS) coated with a mixture of 1.3% cyanosilicone (GE-XE 60) and 0.13% Epikote Resin 1001 in a 6 ft. × 1/8 in. I.D. U-tube glass column at a temperature of 200°, and their data indicated only a slight separation of malathion and parathion (probably the ethyl compound). RUZICKA *et al.*[12] noted that Chromosorb G (AW, DMCS) coated with a 10% SE-30 silicone gum rubber was not suitable for the analysis of organophosphorus compounds. This is considered to be a very high loading on this solid support, and is reason enough for the column not to be suitable.

The data in Table II suggests that commercial methods of preparing Chromosorb W have improved, *i.e.*, the apparent decrease in adsorptive characteristics as evidenced by the improved results obtained with ethyl paraoxon. This is especially evident in the new "High Performance" grade. PALFRAMAN AND WALKER[13] are of the opinion that technological development on the diatomaceous earths may have approached a limit. However, some degree of erraticism still exists with supports as indicated by the data obtained with ethyl paraoxon and this suggests that the state of the art of producing chromatographic supports has not advanced completely from the area of empiricism to that of precise reproducibility.

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