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Short Communication

Capability of a carbon support to improve the gas chromatographic performance of a liquid crystal phase in a packed column for some volatile oil constituents

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ABSTRACT

"Graphpac" was investigated to see if carbon was a better gas chromatographic support than silica for columns of 3% of the liquid crystal bismethoxybenzilidinebitoluidine or $(MBT)_2$. Six volatile oil component solutes exhibited the same elution sequence as from silica, but with lower relative retention times with respect to linalol —about 50% for some aromatics, reflecting the much lower polarity of the carbon-supported packing indicated by cuminal/caryophyllene ratio. Caryophyllene had greatly increased relative retention, and this was further raised if a low-loading of 0.3% $(MBT)_2$ was used on "Graphpac", indicating even lower polarity. This column was not reliable below 200°C, but an initial period at 203°C, followed by rapid heating to 230°C, gave reasonable results for some significant constituents of teatree oil. Nevertheless, 3% $(MBT)_2$ on "Graphpac" was preferable for assaying this and sweet fennel oil by providing a more reliable melted liquid crystal stationary phase, with low temperature versatility.

INTRODUCTION

We have previously [1–3] used packed columns of liquid crystal phases for the gas chromatographic study of constituents of volatile oils. Some authors claim that the normal silica support we have used is not the optimum for liquid crystal performance. In 1984 Rayss and Waksmundzki [4] studied the influence of graphite support for the liquid crystal *p*-butyl-*p*-hexanoylazobenzene (PHPB). The logarithm of retention volumes for *n*-octanol against the reciprocal of temperature showed breaks in the plot indicating the two transition temperatures (solid to nematic liquid crystal; liquid crystal to normal isotropic liquid) of PHPB only if at least 3% of it was present. However, the lower loading of 1.3% still revealed the "influence of the graphite support", in contrast to silica support where at least 7.7% PHPB was needed to show the transitions. Without such amounts of PHPB "the occurrence of a solid/nematic phase transition becomes impossible (due to) the influence of the silica gel surface on the liquid crystal layer directly adjacent" to it. They concluded that "in the surface film of PHPB on graphite, about ten times more molecules are present than on silica (in) a polymolecular film" so that carbon, as graphite, was a more 'empathetic' support for their liquid crystal than silica.

Economic low loadings of expensive liquid

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crystals should thus be possible, and recently Nazarova *et al.* [5] studied a presumed monolayer of the liquid crystal bis(hexyloxybenzylidine)phenylenediamine [$(HB)_2PD$] on carbon. Polyaromatic isomers were better resolved than with a 5% loading on normal support "Chromaton", although the latter did indicate the transitions. In contrast, the 0.33% $(HB)_2PD$ on graphitized carbon they used gave only straight line plots like a normal liquid of log retention volume against temperature reciprocal, indicating that "no phase transition ... takes place". This was because they considered $(HB)_2$ PD was "in a (continuous) structured state similar to the mesomorphic" liquid crystal at temperatures above and below its transition points. Rayss and Waksmundzki [4] had agreed in observing practically a straight line plot with 0.5% PHPB on graphite. Their explanation was that "at the solid surface a film of PHPB molecules may be formed oriented differently than in the layer more distant" when thicker loadings were present.

It was therefore of interest to see if a carbon support would improve the performance of a liquid crystal that we previously used on silica [1,3], bismethoxybenzilidinebitoluidine $(MBT)_2$, for the gas chromatography of volatile oil constituents, applied in both normal (3%) and very low (0.3%) loadings. It is a dianil aromatic diether, like $(HB)_2$ PD, but with four aromatic rings instead of three.

EXPERIMENTAL

Apparatus

A Pye GCD gas chromatograph was used fitted with a flame ionisation detector. This had no temperature programming facility, but altering the temperature-setting dials caused rapid heating at about 40°C min^{-1}. Cooling from high temperature was allowed without opening the oven to minimise disturbance of the liquid crystal phase. The oven temperatures were observed with a Technoterm 7300 probe. Chromatographs were plotted with a Hewlett-Packard 3390A integrator/recorder.

The glass columns (1.5 m × 2 mm I.D.) were packed with 3% or 0.3% (w/w) $(MBT)_2$ from T.C.I. (Tokyo, Japan) [1] on uncoated "Graphpac-GC" 80–100 mesh (Alltech) claimed to have a surface area of 10–13 m^2 g^{-1}. The weighed amounts of liquid crystal and support were dispersed in dichlormethane and taken to dryness in a rotary evaporator.

Materials and methods

Sources of most solutes [1–3] and oils [1,6] have been given before. Fenchone was from Aldrich. Injections were 0.1 μl oil, or trace residues of solutes from an "emptied" microsyringe. Nitrogen was the mobile phase flowing at 15 ml min^{-1} at the outlet of the 3% $(MBT)_2$ column, but twice as fast from the low-loaded column. Holdup times were deducted from observed retention times, obtained by extrapolating to methane the retention times of *n*-heptane and *n*-hexane plotted on semi-logarithmic graph paper.

RESULTS AND DISCUSSION

Average results are given in Table I where some significant solutes are numbered at the left. The present values are related to those previously obtained on silica supports.

The 3% $(MBT)_2$ on Graphpac column showed the same sequence for four aromatic solutes (Table I, solutes **2**, **3**, **4** and **5**) as on silica [1,3], namely estragole (quickest)–safrole–cuminal–thymol (slowest). However, the 0.3% on Graphpac phase showed thymol ahead of cuminal, almost level with safrole. Typically for liquid crystals [3], safrole was always quicker than anethole. In the present work, anethole (the *trans*-isomer) was constantly slower than thymol (solutes **6** and **5**), indicating that the Graphpac columns always differed from the "conventional" sequence, even when first used. Once again, there was a distinction between unmelted (heated from cold) behaviour of 3% loading at 160°C and the supercooled response at the same temperature after heating above the 180°C melting point of $(MBT)_2$. On the unmelted phase, thymol was only just ahead of anethole, and never well behind it as on silica support [1]. There was no anethole-thymol sequence "shift"

TABLE I

AVERAGE RELATIVE RETENTION TIMES (LINALOL = 1.00) ON GRAPHPAC AND SILICA SUPPORTS (ITALIC RESULTS ON SILICA) COATED WITH $(MBT)_2$

3% $(MBT)_2$ loading used except where indicated otherwise. Values in italics obtained previously [1–3].

Solute	230°C			203°C		200°C	181°C	175°C	160°C *supercooled*		160°C *unmelted*	
	3%	3%	0.3%	0.3%	3%							
6 Anethole	2.77	*4.81*	2.62	2.8–3.1	3.07	*5.87*	3.19	*7.06*	3.41	*7.88*	2.65	*3.72*[b]
Caryophyllene	2.68	*2.00*[b]	3.68[b]	4.04[b]	2.75	*2.22*[b]	2.81	*2.41*[b]	2.98[b]	*2.33*[b]	3.00[b]	*2.80*[b]
5 Thymol	2.35	*3.96*	1.86[b]	2.00[b]	2.59	*4.90*	2.75	*5.19*	3.00	*5.88*	2.62	*4.53*
4 Cuminal	2.26	*3.71*	2.08	2.1–2.4	2.34	*4.38*	2.30	*4.62*	2.36	*4.82*	2.06	*3.35*
3 Safrole	2.11	*3.55*	1.85	1.97	2.17	*4.10*	2.15	*4.24*	2.12	*4.46*	1.79	*2.93*
Geraniol	1.77		1.70	1.90			1.97	*3.29*				
2 Estragole	1.58	*2.38*	1.44	1.42	1.59	*2.58*	1.57	*2.75*	1.57	*2.80*	1.35	*1.79*
1 α-Terpineol	1.53	*1.96*	1.44	1.47[b]	1.52	*2.20*	1.51	*2.34*	1.51	*2.39*	1.43[b]	*1.94*[b]
4-Terpineol	1.35		1.25	1.25			1.33					
Fenchone	0.87		0.72	0.67			0.75					
Limonene	0.62		0.62	0.58			0.54			*0.49*		*0.39*[b]
Cineole (1,8-)	0.59		0.51	0.50			0.50			*0.43*		*0.43*
α-Pinene	0.36		0.34	0.32			0.30					
c ratio[a] and polarity of column	*0.63 Low*	*1.39 High*	*0.42 Very low*	*0.43 Very low*	*0.64 Low*	*1.48 High*	*0.61 Low*	*1.44 High*	*0.59 Low*	*1.55 Very high*	*0.51 Low*	*0.90 Intermediate*

[a] *c* ratio is 3 × (cuminal value)/4 × (caryophyllene value) [8].
[b] Value out of sequence in table.

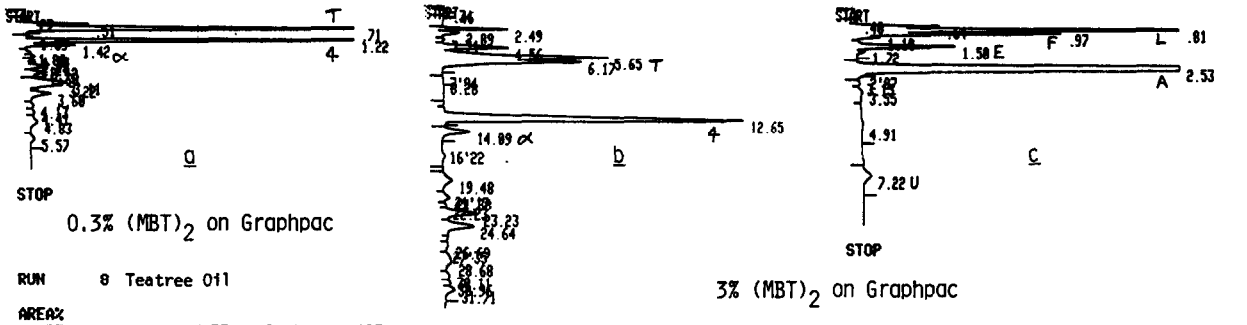
on melting the liquid crystal; although the terpineol-estragole one was still evident (Table I), α-terpineol being ahead unless the $(MBT)_2$ was not melted, or the low loaded (0.3%) Graphpac was used (solutes **1** and **2**).

There was no sign of "naive" column behaviour with the 3% $(MBT)_2$ on Graphpac, even when new, which would have been shown by anethole emerging ahead of cuminal (solutes **6** and **4**) and safrole ahead of α-terpineol (solutes **3** and **1**) [1]. After melting the liquid crystal (results at 181°C and above, and supercooled to 160°C) the sequence of six original test solutes (**1–6** in Table I) was the same as on silica support [1]. However, relative retention values were usually lower, about 50% of the values on silica for the three chemically distinct aromatics **3**, **4** and **5**, although over 60% for α-terpineol (solute **1**). This and aromatics **2**, **3** and **4**, exhibited almost constant values. Although $(MBT)_2$ is a tetraaromatic molecule, the carbon support does not promote its relative retention of different aromatic substances. Surprisingly, this packing did increase the relative retention of the sesquiterpene caryophyllene (Table I) and it may be useful for chromatographing this type of hydrocarbon, especially at 0.3% loading. Other solutes with long relative retention times (**5** and **6**) gave the usual decline in values with increasing temperature [7] whilst those with short times showed the usual increase on the $(MBT)_2$ on Graphpac.

The low-loaded (0.3% $(MBT)_2$) on Graphpac could not be used below 200°C, at which temperature some aromatics gave variable results —larger amounts injected producing shorter retention times, unlike conventional phases. Most values were lower than with the 3% loading on Graphpac, but caryophyllene relative retention times increased considerably (well after anethole) so indicating a considerable reduction in polarity [8] with the low loading. Reduced values for thymol were distinctive here, bringing this polar solute close to safrole. Nevertheless,

by using an initial isothermal period at 203°C, followed by rapid heating to about 230°C, quite good volatile oil assays resulted, although early monoterpene hydrocarbons were not resolved. A teatree oil previously evaluated on a silica column of 3% $(MBT)_2$ gave values (Fig. 1a) a little lower than those before [1] of 41.1% 4-terpineol and 3.8% α-terpineol. These two monoterpenols probably provide the desired antiseptic character of this oil, and are better resolved and more reliably assayed on the 3% $(MBT)_2$ on Graphpac which can be heated up in stages from initial lower temperatures (unmelted) like 110°C (Fig. 1b) to give the terpineol separation at 130°C. It also gives some resolution of the earlier terpinenes. Fig. 1c shows that a sample of sweet fennel oil recently assayed [6] on four different capillaries (including a liquid crystal polysiloxane) gave results close to their averages of 6.3% fenchone, 4.1% estragole, 66.4% *trans*-anethole and 0.8% feniculin.

On silica, the 3% melted liquid crystal allowed caryophyllene to pass through as quickly as α-terpineol, indicating a high polarity packing [8] in relation to cuminal (Table I). The same loading on Graphpac saw caryophyllene emerging after, or with, thymol; the packing now being of low polarity. The low-load of 0.3% $(MBT)_2$ on Graphpac rates as "very low" polarity, 0.4, although not as low as the about 0.3 *c* ratio of methylpolysiloxane. With its high operational temperature (above 200°C) this should make it useful for sesquiterpene hydrocarbons like the later peaks from tea tree oil. Increasing the load to 3% (melted) on Graphpac raises the *c* ratio to 0.6 —still low polarity. Intermediate polarities are given by values between 0.8 and 1.2, and this is exhibited by unmelted 3% $(MBT)_2$ on silica. On this support with melted liquid crystal, the *c*



RUN 8 Teatree Oil

AREA%

RT	AREA	TYPE	AR/HT		AREA%
0.27	6252	D BP	0.075		0.103
0.51	231440	D PV	0.076		3.820
0.71	2331100	D VB	0.102	T	38.474
1.09	26086	BV	0.078		0.431
1.22	2202900	D VV	0.093	4	36.358
1.42	226250	D VP	0.097	α	3.734
1.80	27549	PV	0.123		0.455
1.93	44029	D VV	0.126		0.727
2.13	32127	VV	0.109		0.530
2.24	23202	VV	0.090		0.383
2.42	89945	VP	0.149		1.485
2.68	70657	PB	0.123		1.166
3.11	244820	BV	0.155		4.041
3.22	168850	D VV	0.123		2.774
3.60	143650	VP	0.163		2.371
4.13	18859	PV	0.168		0.311
4.41	33298	VV	0.209		0.550
4.83	95752	VP	0.322		1.580
5.57	42885	PB	0.243		0.708

RUN 11 Teatree Oil

AREA%

RT	AREA	TYPE	AR/HT		AREA%
0.34	4653	D BV	0.069		0.023
0.46	12259	D VB	0.082		0.061
2.49	547940	BV	0.177		2.714
2.89	163250	VV	0.200		0.809
3.71	26968	PP	0.245		0.134
4.56	1124700	PV	0.366		5.570
5.65	3950600	VV	0.507	T	19.567
6.17	2947800	VB	0.452	T	14.600
8.28	8358	PB	0.250		0.041
12.65	6974000	PB	0.494	4	34.541
14.89	660900	BB	0.540	α	3.273

RUN 2 Sweet Fennel Oil

AREA%

RT	AREA	TYPE	AR/HT		AREA%
0.40	6403	D PV	0.088		0.047
0.64	369080	VV	0.092		2.697
0.81	2454500	D VV	0.099	L	17.935
0.97	897690	D VV	0.091	F	6.559
1.10	115000	D VV	0.097		0.846
1.50	625020	VV	0.132	E	4.567
1.72	55437	D VB	0.133		0.405
2.53	8852100	PB	0.195	A	64.682
3.15	12596	PV	0.164		0.092
3.55	57960	VV	0.332		0.424
4.91	95083	VB	1.104		0.695
7.22	143830	BB	0.390	U	1.051

Fig. 1. Chromatograms of oils on packed columns of liquid crystal phase on Graphpac carbon. (a) Teatree oil on 0.3% $(MBT)_2$ at 203°C for 1.5 min, then rapidly heated to 232°C. α = α-Terpineol; 4 = 4-terpineol; T = α- and γ-terpinenes, plus other monoterpenes. (b) Teatree oil on 3% $(MBT)_2$ at 110°C for 3.8 min, then heated to 131°C till 16.2 min, then to 145°C at 21.1 min, then to 160°C at 27.3 min. All unmelted conditions. Incomplete printout shown. Abbreviations as for (a). (c) Sweet fennel oil on 3% $(MBT)_2$ at 203°C for 2.9 min, then rapidly heated to 230°C. A = *trans*-Anethole; E = estragole; F = fenchone; L = limonene plus other monoterpenes; U = feniculin. Printout peak types: B = peak starts/ends on baseline; D = distorted peak; P = penetration of baseline (reset); V = valley between peaks. RT = Retention time in min. AR/HT = peak width at half height.

ratios rise to a high polarity 1.4, or even over 1.5 under supercooled conditions, a value like Carbowax 20M. This range of apparent polarities agrees with the concept of a molecular-orienting effect by the carbon support on the liquid crystal molecules, changing in relation to their concentration. Carbon here gives packed columns of quite different polarities to silica.

The low-loaded melted Graphpac column gave very similar results to the unmelted 3% $(MBT)_2$ on Graphpac for α-terpineol, cuminal and anethole (solutes **1**, **4**, **6**) and close resemblance for estragole and safrole. This may be due to adsorbing uncoated carbon support dominating the $(MBT)_2$ present. So to reliably see the liquid crystal effect on solutes, it is best to use 3% loading and ensure that it is melted. Graphpac support gave packed columns with closer analysis results than silica for the sweet fennel and mace oils previously assayed on capillaries [6], and the 3% liquid crystal packing was more versatile than the low load. Capillaries provide better resolution of the trace constituents of oils, and so many more peaks. However, well-chosen packed columns should yield good results for the main constituents, which may be all that is needed.

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