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Note

Separation of xylenol isomers on nitrofluorenes and nitro-9-fluorenones by gas–liquid chromatography

I. Mixed columns and effect of temperature on retention behaviour

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Numerous papers[1–13] have been published on the separation of alkylphenols, cresols and dimethylphenol isomers by gas–liquid chromatography (GLC). Brooks[2] separated all the isomers on 2,4-xylenyl phosphate, Kolsek and Maticic[3] separated them on di-(3,3,5-trimethylcyclohexyl)phthalate, Janák and Komers[9] separated them on salicylideneaminoguanidine, Bhattacharjee and Basu[11] separated them on rubidium benzenesulphonate (a clathrate-forming stationary phase) and Mortimer and Gent[8] separated *m*- and *p*-cresols on Bentone-34 and tolyl phosphate.

In spite of these studies, systematic investigations of the separations of the isomers have not been reported. 2,4,7-Trinitro-9-fluorenone[14] has been shown to be an effective stationary liquid phase. Because dimethylphenol isomers are important in the chemical industry, their separation behaviour on nitrofluorenes and nitro-9-fluorenones using GLC was investigated.

EXPERIMENTAL

A Shimadzu GC-5A gas chromatograph with a flame-ionization detector was used. The chromatographic column (2.25 m × 3 mm I.D., stainless-steel U-tube) was packed with C_{22} firebrick (60–80 mesh) coated with liquid stationary phase (see Tables I–III).

The temperature of the column was 140 °C and that of the injector was 270 °C. The flow-rate of the carrier gas (nitrogen) was 25 ml/min. The flow-rate of hydrogen was 50 ml/min and that of air was 1 l/min for the flame-ionization detector. The sample size was 0.5–1.0 μl.

The support was Sil-O-Cel C_{22} firebrick (60–80 mesh) (Johns-Manville, Denver, CO, U.S.A., purchased from Gaskuro-Kogyo, Tokyo, Japan) and was agitated in hot 3 *N* hydrochloric acid for 1 h, then washed with water until neutral and dried prior to use. The C_{22} firebrick was coated with 20 % (w/w) of stationary phase and heated in an electric oven for 16 h in order to remove the solvent at the temperature of the column. The column packed with C_{22} firebrick coated with stationary phase was

kept under a stream of nitrogen for 6 h at a temperature *ca.* 20 °C higher than that of the column.

Stationary phases

The stationary phases, 2-nitrofluorene (2-NF)[15,16], 2-nitro-9-fluorene (2-NFO)[15–17], 2,7-dinitrofluorene (2,7-DNF)[17–19], 2,7-dinitro-9-fluorene (2,7-DNFO)[17–19] 2,5-dinitro-9-fluorenone (2,5-DNFO)[17–19], 2,4,7-trinitro-9-fluorenone (2,4,7-TNFO)[20–23] and 2,4,5,7-tetranitro-9-fluorenone (2,4,5,7-TNFO)[21,24], were synthesized in the laboratory and their purities were confirmed by thin-layer chromatography and IR spectroscopy.

Liquid paraffin (LP), dulcitol (DUL), dioctyl phthalate (DOP), sorbitol (SOR) and inositol (INO) of guaranteed grade were purchased from Nakarai Chemical Co. (Kyoto, Japan) and 1,2,3-tris(2-cyanoethoxy)propane (TCEP) from Gaskuro-Kogyo, and were used without purification.

Samples

2,3-Dimethylphenol (2,3-DMP)[25], 2,6-dimethylphenol (2,6-DMP)[26], 3,4-dimethylphenol (3,4-DMP)[27], 2,4-dimethylphenol (2,4-DMP)[28], 2,5-dimethylphenol (2,5-DMP)[29] and 3,5-dimethylphenol (3,5-DMP)[30] were also synthesized in the laboratory and their purities were confirmed by GLC and infrared and nuclear magnetic resonance spectroscopy.

RESULTS AND DISCUSSION

Separation behaviour at different column temperatures

2-NF, 2-NFO, 2,7-DNF, 2,7-DNFO and 2,4,7-TNFO were recently found to be effective stationary phases for separating DMP isomers. In these important separations, changes in retention times with changes in the column temperature and interesting separation behaviour were found, and these were studied in this work.

The retention times at different column temperatures in these separations on 2-NF, 2-NFO, 2,5-DNFO, 2,7-DNF, 2,7-DNFO, 2,4,7-TNFO and 2,4,5,7-TNFO are shown in Table I, and the relationships between retention time and column temperature (log retention time *versus* $1/T$) on 2-NF, 2,7-DNF, 2,4,7-TNFO and a 2,4,7-TNFO + 2,4,5,7-TNFO mixed column in Figs. 1–4. From Table I, it can be seen that 2-NF, 2-NFO, 2,7-DNF, 2,7-DNFO, 2,5-DNFO, 2,4,5,7-TNFO and 2,4,7-TNFO + 2,4,5,7-TNFO separate 2,4- and 2,5-DMPs from each other but not 2,3-DMP from 2,4-DMP, and 2,4,7-TNFO separates all six isomers from each other. The separation of 2,5- and 2,4-DMPs is the most difficult.

In the separations on 2-NFO, results above 160 °C were not obtained because of its high vapour pressure and tendency to sublime. The separation of 2,4- and 2,5-DMPs is performed effectively between 130 and 170 °C on 2-NFO, 2,5-DNFO, 2,7-DNF, 2,7-DNFO and 2,4,5,7-TNFO, and between 130 and 155 °C on 2,4,7-TNFO, but on the latter no separation can be performed above 155 °C.

Although 2,3-DMP is separated from 3,5-DMP below 170 °C on 2,4,7-TNFO, they cannot be resolved above 170 °C, as shown Fig. 3. In the separations of the isomers on 2,4,7-TNFO, transitions in the relationship between retention time and temperature occurred near 170 °C. With increasing temperature from 130 to

TABLE I

RETENTION TIMES IN SEPARATIONS OF DMP ISOMERS

Melting points of liquid stationary phases: 2-NF, 153-154 °C[15,16]; 2-NFO, 223-225 °C[15-17]; 2,7-DNF, 310 °C[16,17]; 2,7-DNFO, 293 °C[15-17]; 2,5-DNFO, 241 °C[15-17]; 2,4,7-TNFO, 175-176 °C[15-20]; 2,4,5,7-TNFO, 253-254.5 °C[21,24].

| Isomer	Stationary phase	Temperature (°C)				
		130	140	150	160	170
2,6-DMP	2-NF	12.20	7.00	4.50	3.10	1.90
2,5-DMP		19.00	10.60	6.70	4.70	2.70
2,4-DMP		21.40	11.80	7.40	5.20	2.80
2,3-DMP		21.40	11.80	7.40	5.20	2.80
3,5-DMP		34.20	18.20	11.10	7.50	4.30
3,4-DMP		42.20	22.40	13.50	9.10	5.10
2,6-DMP	2,7-DNF	9.00	5.80	3.20	1.80	1.30
2,5-DMP		15.80	9.80	5.40	3.20	2.00
2,4-DMP		18.20	11.20	6.20	3.60	2.30
2,3-DMP		18.20	11.20	6.20	3.60	2.30
3,5-DMP		29.40	18.60	9.80	5.40	3.30
3,4-DMP		35.40	22.20	11.40	6.40	3.90
2,6-DMP	2,7-DNFO	12.40	5.90	4.00	2.60	2.00
2,5-DMP		19.80	10.90	7.40	3.60	3.20
2,4-DMP		21.80	12.30	8.00	5.20	3.60
2,3-DMP		21.80	12.30	8.00	5.20	3.60
3,5-DMP		33.60	18.30	11.60	7.60	5.20
3,4-DMP		39.20	21.30	13.20	8.60	5.80
2,6-DMP	2,5-DNFO	8.30	5.00	3.20	2.20	1.60
2,5-DMP		15.60	9.20	5.60	3.80	2.30
2,4-DMP		17.90	10.60	6.40	4.10	2.70
2,3-DMP		17.90	10.60	6.40	4.10	2.70
3,5-DMP		31.70	16.80	10.20	6.40	3.80
3,4-DMP		37.30	20.00	12.40	7.80	4.60
2,6-DMP	2,4,5,7-TNFO	8.00	4.80	2.70	1.80	1.60
2,5-DMP		13.60	7.90	4.60	2.90	2.40
2,4-DMP		15.60	9.10	5.30	3.40	2.70
2,3-DMP		15.60	9.10	5.30	3.40	2.70
3,5-DMP		25.40	14.40	8.40	4.90	4.20
3,4-DMP		30.40	17.40	10.00	6.15	5.00
2,6-DMP	2,4,7-TNFO	6.60	4.60	3.40	3.80	16.40
2,5-DMP		11.60	8.00	5.60	5.90	23.60
2,4-DMP		12.60	8.60	5.90	5.90	23.60
2,3-DMP		13.80	9.70	7.00	7.40	31.80
3,5-DMP		21.00	13.70	9.00	8.60	31.80
3,4-DMP		26.80	17.60	11.50	11.40	43.00
2,6-DMP	2,4,7-TNFO(16%) ÷ 2,4,5,7-TNFO(4%)	32.30	22.40	15.15	23.80	18.00
2,5-DMP		51.20	34.80	23.20	35.00	26.00
2,4-DMP		51.20	34.80	23.20	35.00	26.00
2,3-DMP		68.00	46.00	31.40	47.80	35.00
3,5-DMP		74.20	49.80	32.90	47.80	35.00
3,4-DMP		101.00	67.80	45.60	65.20	47.00
2,6-DMP	2-NFO	8.20	5.30	2.90	2.40	—
2,5-DMP		15.50	9.30	5.10	3.70	—
2,4-DMP		17.40	10.50	5.90	4.10	—
2,3-DMP		17.40	10.50	5.90	4.10	—
3,5-DMP		28.70	16.30	8.90	5.80	—
3,4-DMP		33.40	18.90	10.70	6.80	—
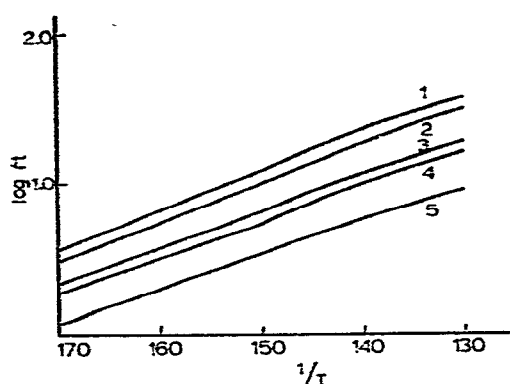
Fig. 1. Separation of DMP isomers on 2,7-DNF. 1 = 3,4-DMP; 2 = 3,5-DMP; 3 = 2,4- + 2,3-DMP; 4 = 2,5-DMP; 5 = 2,6-DMP.
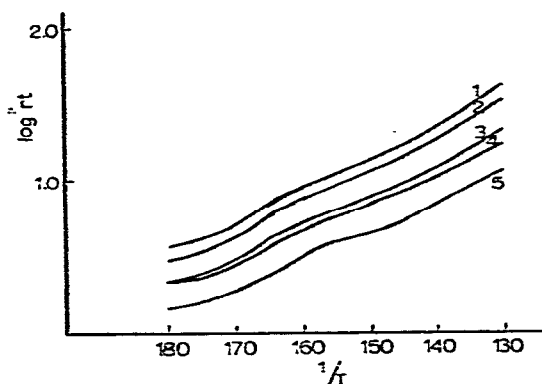
Fig. 2. Separation of DMP isomers on 2-NF. 1 = 3,4-DMP; 2 = 3,5-DMP; 3 = 2,4- + 2,3-DMP; 4 = 2,5-DMP; 5 = 2,6-DMP.

160 °C the logarithm of the retention time of each isomer decreased but from 160 to 170 °C it increased, with maxima at 170 °C. This seems to be due to the phase change of 2,4,7-TNFO from solid to liquid, because the melting point of 2,4,7-TNFO is 175–176 °C, but melting begins at *ca.* 164 °C when measured by differential thermo-analysis. From 130 to 160 °C the separations are due to adsorption and above 170 °C they are due to partition (absorption)[32–34]. Although the separation of 3,5- and 2,3-DMPs is impossible above 170 °C, it will depend on the phase change of 2,4,7-TNFO from solid to liquid.

It is interesting that only 2,4,7-TNFO separates 2,4- and 2,3-DMPs. The reason is not clear but investigations are in progress.

Studies of the variation in retention behaviour with column temperature were performed on 2-NF; small transitions in the graphs of log retention times *versus*
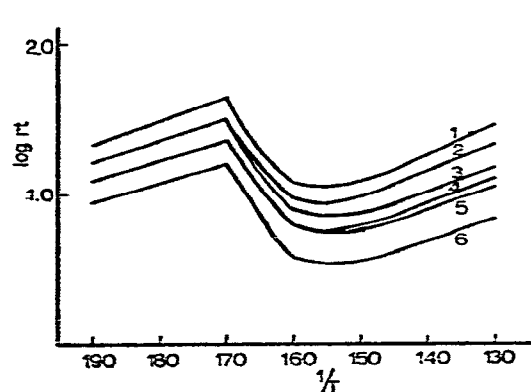

Fig. 3. Separation of DMP isomers on 2,4,7-TNFO. 1 = 3,4-DMP; 2 = 3,5-DMP; 3 = 2,3-DMP; 4 = 2,4-DMP; 5 = 2,5-DMP; 6 = 2,6-DMP.
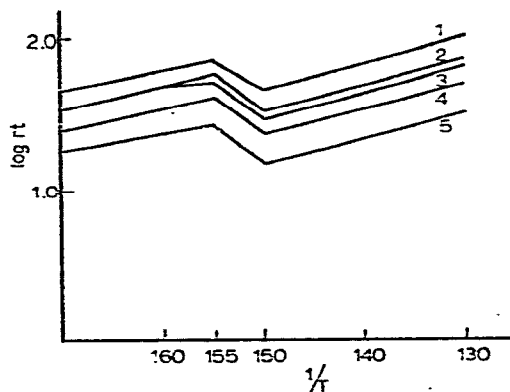
Fig. 4. Separation of DMP isomers on 2,4,7-TNFO (16%) + 2,4,5,7-TNFO (4%). 1 = 3,4-DMP; 2 = 3,5-DMP; 3 = 2,3-DMP; 4 = 2,4- + 2,5-DMP; 5 = 2,6-DMP.

temperature were found, as shown in Fig. 2. The slopes of the lines above and below the range 157–165 °C are almost identical. The reason why the transitions in the retentions are small is not clear, but small transitions on polyethylene glycol 1540 have been reported[34]. The relationship between separation behaviour and column temperature on 2,4,7-TNFO + 2,4,5,7-TNFO is shown in Fig. 4; the transition maxima at 155 °C seem to depend on the melting point of the mixture (146–148 °C).

Comparing Fig. 4 with Fig. 3, the transition range in the former is narrower than that in the latter. If 2,4,7-TNFO liquid stationary phase contains a trace amount of 2,4,5,7-TNFO as an impurity, it cannot separate 2,4- and 2,5-DMPs[23,24,35]. On the other hand, 2,4,7-TNFO may be useful in the analysis of organic compounds.

2,4,7-TNFO and 2,4,5,7-TNFO separate 2,4- and 2,5-DMPs but their mixture cannot effect this separation, and although 2,4,5,7-TNFO alone cannot separate 2,3-DMP from 2,4-DMP its mixture with 2,4,7-TNFO did separate them.

The relationship between retention time and column temperature on 2,7-DNF is shown in Table I and Fig. 1. There is no transition in the retentions, because there is no phase change with 2,7-DNF.

In the temperature range 130–170 °C, the separations on 2,7-DNF are due to adsorption, and 2-NFO, 2,5-DNFO, 2,7-DNFO and 2,4,5,7-TNFO show the same separations as 2,7-DNF. In these systems the separation of the six isomers is performed below the melting points of the liquid stationary phases. In GLC separations, analyses are generally due to partition (absorption), but from these results it was concluded that adsorption of the liquid stationary phases in GLC plays an important role in the analyses of organic compounds. Effective separations of positional isomers of aromatic compounds by adsorption of the liquid stationary phase have not previously been reported.

Separations on mixed liquid stationary phases

2-NF was chosen as the main liquid stationary phase for mixed columns because it separates 2,4- and 2,5-DMPs in both its solid and liquid state. A hydrocarbon (liquid paraffin), an ester (DOP) and polyalcohols (dulcitol, sorbitol and inositol) were chosen as the liquid stationary phases to be mixed with 2-NF because they are also effective in separating DMP isomers, except for 2,4- and 2,5-DMPs.

Tables II and III give the relative retention times and relative peak separations[31] using polyalcohol and mixed stationary phase columns. It was found that a mixture of 2-NF and DUL separates all six isomers (Fig. 5). Although they were also resolved on DUL alone, the retention times were very short and the separations were inadequate for their analysis.

SOR also separates all of the isomers, but the separations were better than with DUL, with symmetrical peaks with good peak separation (Fig. 6). INO separates 2,4- and 2,5-DMPs but cannot separate 2,3-DMP from 2,4-DMP; however, a mixture of 2-NF and INO can separate this pair and also all of the other isomers. A mixture of 2-NF and SOR cannot separate 2,4-DMP from 2,5-DMP, and a mixture of 2-NF and LP cannot separate 2,4- and 2,5-DMPs or 3,5- and 2,3-DMPs. It is characteristic that INO separates all of the isomers when mixed with 2-NF.

TCEP separates all of the isomers except 2,4- and 2,5-DMPs, but its mixture

TABLE II

RELATIVE RETENTION TIMES OF DMP ISOMERS

Stationary phase	2,6-DMP	2,5-DMP	2,4-DMP	2,3-DMP	3,5-DMP	3,4-DMP
2-NF + DUL*	1.00	2.00	2.13	2.74	3.52	4.45
2-NF + DUL	1.00	1.95	2.05	2.64	3.32	4.23
2-NF + LP	1.00	1.44	1.44	1.81	1.81	2.04
2-NF + TCP	1.00	1.70	1.70	2.25	2.50	3.12
2-NF + DOP	1.00	1.52	1.52	1.83	2.03	2.33
2-NF + SOR	1.00	1.95	1.95	2.53	3.11	3.95
2-NF + INO	1.00	1.91	2.14	2.41	3.65	4.28
DUL	1.00	2.13	2.38	2.63	4.13	4.62
INO	1.00	2.00	2.23	2.23	4.03	4.29
SOR	1.00	2.05	2.19	2.74	3.69	4.62
2-NF	1.00	1.51	1.69	1.69	2.60	3.20

* 2-NF + DUL column at 130 °C.

TABLE III

RELATIVE PEAK SEPARATIONS BETWEEN DMP ISOMERS

Relative peak separations: S_{65} = between 2,6- and 2,5-DMP; S_{54} = between 2,5- and 2,4-DMP; S_{34} = between 2,4- and 2,3-DMP; S_{35} = between 2,3- and 3,5-DMP; S_{45} = between 3,5- and 3,4-DMP.

Stationary phase	S_{65}	S_{54}	S_{34}	S_{35}	S_{45}
2-NF + DUL*	1.00	0.07	0.29	0.28	0.26
2-NF + DUL	0.95	0.05	0.29	0.26	0.27
2-NF + LP	0.44	0.00	0.26	0.00	0.13
2-NF + TCP	0.70	0.00	0.32	0.11	0.25
2-NF + DOP	0.52	0.00	0.20	0.11	0.15
2-NF + SOR	0.95	0.00	0.29	0.23	0.27
2-NF + INO	0.91	0.12	0.13	0.52	0.17
DUL	1.13	0.12	0.11	0.57	0.12
INO	1.00	0.12	0.00	0.81	0.06
SOR	1.05	0.07	0.25	0.35	0.25
2-NF	0.51	0.12	0.00	0.54	0.23

* 2-NF + DUL column at 130 °C.

with 2-NF cannot separate 2,4- and 2,5-DMPs, although 2-NF can separate them. In this instance, the separation capabilities of the individual liquid stationary phases are not combined. In the separation of alkylbenzenes, α-naphthylamine separated m- and p-xylenes but not ethylbenzene from p-xylene whereas p-chlorobenzophenone fluorenone separated ethylbenzene from p-xylene. A mixture of α-naphthylamine and fluorenone or chlorobenzophenone separated the three alkylbenzenes[36], indicating that the separation capabilities of the individual liquid stationary phases were combined.

DUL and INO gave effective separations of all of the isomers when mixed with 2-NF. Some liquid stationary phases that individually can separate 2,4- and 2,5-DMPs cannot separate them when mixed with 2-NF, and the principles that govern the effectiveness of mixed columns in the separations of DMP isomers are not clear. In the separations of DMP isomers on mixed columns, effective separations on SOR and DUL alone and on mixed columns of 2-NF with DUL or with INO
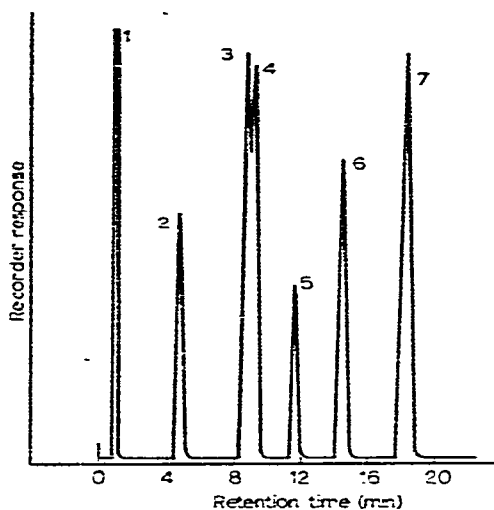
Fig. 5. Separation of DMP isomers on 2-NF (20%) + DUL (20%). 1 = Solvent; 2 = 2,6-DMP; 3 = 2,5-DMP; 4 = 2,4-DMP; 5 = 2,3-DMP; 6 = 3,5-DMP; 7 = 3,4-DMP.
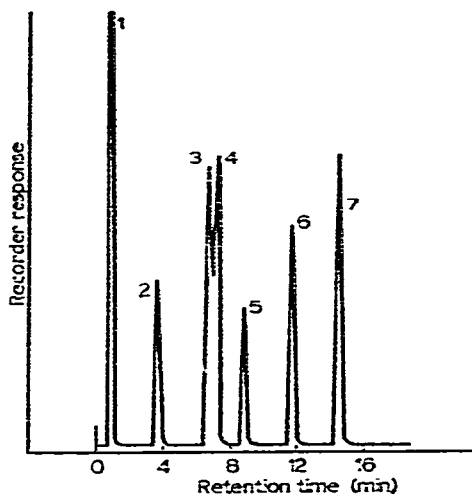
Fig. 6. Separation of DMP isomers on SOR. 1 = Solvent; 2 = 2,6-DMP; 3 = 2,5-DMP; 4 = 2,4-DMP; 5 = 2,3-DMP; 6 = 3,5-DMP; 7 = 3,4-DMP.

were found. These columns are convenient to prepare because DUL and INO are inexpensive and 2-NF[15,16] is easily prepared, but care must be taken in all operations with 2-NF because it is a carcinogen[37–39].

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