MULTI-DIMENSIONAL CHROMATOGRAPHY USING DIFFERENT DEVELOPING METHODS*

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The efficiency of chromatographic separations of complex mixtures is commonly reduced by the fact that peak or zone overlapping occurs with compounds of different chemical structure. In paper chromatography (PC), thin-layer chromatography (TLC) or electrophoresis the use of two-dimensional chromatograms is known and widely applied[1–4]. In gas chromatography (GC) the application of two or more stationary phases selectively retaining the different components of mixtures is in principle equal to two-dimensional chromatography.

Only few papers[5,6] have been published on the pre-separation of compounds using TLC and the subjection of pre-separated parts of a mixture to gas chromatography. One of these papers used a more detailed combination[6]: pre-separation by means of GLC on one type of stationary phase, followed by TLC separation of the simplified GLC cuts and subsequent gas–liquid chromatographic separation of extracted TLC cuts on another type of stationary phase. In this paper it is shown that the separation on thin layers of adsorbent is a process occurring in the system independent of the vapour pressure of the substances. Under these circumstances the polarity of the adsorbent is much higher than the polarity of all known polar stationary phases used in GLC. Therefore the separation depends in the first place upon the type of functional group and/or their steric hindrances, while the molecular weights play a secondary role. On the other hand, non-polar stationary phases in GLC systems separate to the first approximation according to the increasing C-number in a molecule or according to the increase in molecular weight, as well as the dispersion forces of molecules. The separation is therefore a process in which the vapour pressure of the substances plays a minor role.

If we use GLC as a sampling stage for TLC or PC it is clear that we can open up some new possibilities in the field of isolation and identification of components from complex mixtures of different substances. This type of two-dimensional chromatographic separation exploits to the maximum both separation extremes given by all existing chromatographic methods, *viz.* separation according to the number of C atoms by GLC in the direction of the time axis along the start of the thin layer or the paper and, according to the type of functional group, by TLC or PC in the direction of the capillary flow of the solvent. After this two-dimensional chromatography, it is possible to extract the separated compounds from the thin layer or paper and, if

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necessary, chromatograph microgram quantities by means of GLC on a suitably selected stationary phase.

Other methods of identification, *e.g.* microscopy, crystallography or colorimetry, as described in another paper[7], can be used in connection with the above-mentioned procedure.

THEORY

When different chromatographic methods are applied in sequence, it is necessary to consider the effect of different diffusion processes on zone broadening. If the products of a gas chromatogram are developed on the start of the thin layer or the paper, the condition that neighbouring components are accurately and quantitatively resolved[8] is given by

$$t'_{max} - t_{max} = \Delta t_{max} = 4\sigma \tag{1}$$

As a result of diffusion on the plate, overlapping of both zones may appear, as shown in Fig. 1, when a thin-layer chromatogram is developed. This overlap may be so great that under unsuitable conditions the separatory effect of the gas chromatography is annulled. In order that two different zones may be clearly detected after the TLC separation, the relation between b and σ must also be limited, $b \leqq 2\sigma$.

The width of the zone, b, on the thin layer can be defined in a common form similar to that used in chromatography for defining the degree of separation[9]:

$$n = 16\left(\frac{V_{max}}{\Delta V}\right)^2 = 16 \cdot \left(\frac{d}{b}\right)^2 \tag{2}$$

where n is the number of theoretical plates, V_{max} is the volume of mobile phase at the maximum of the concentration of the zone, ΔV is the volume corresponding to the zone width (cut out with tangents at the points of inflexion), and d is the distance of the zone from the start on the layer.

$$b = 4\sqrt{\frac{d^2}{n}} \tag{3}$$

After putting the number of theoretical plates n in terms of the height equivalent of a theoretical plate H, where $n = d/H$, $d = R_F \cdot L$, and L is the length of the layer, equation (3) can be written as

$$b = 4\sqrt{H \cdot d} = 4\sqrt{H \cdot R_F \cdot L} \tag{4}$$

b therefore increases linearly[10] with respect to d and $\Delta b = K_1 \Delta d$ (definition (2)). Since $d^2 = K_2 t$, t being the development time of the chromatogram on the plate, the width of the zone b increases in accordance with $\sqrt{t}$. The spreading from the starting point of the spot to a point of definite width is parabolic. This was the conclusion drawn for paper chromatography[11] and chromatography on plates[9] prepared by drying of a slurry of silica gel G.

The position of the chromatographic zones on the start line is given by $\Delta t_{max} \cdot v$, where v is the velocity of the plate (when the sample from the gas chromatography column is deposited on it), and along the start line our condition for non-overlapping may be written as

$$b \leqq 2\sigma \leqq \frac{1}{2}\Delta t_{max} \cdot v \tag{5}$$

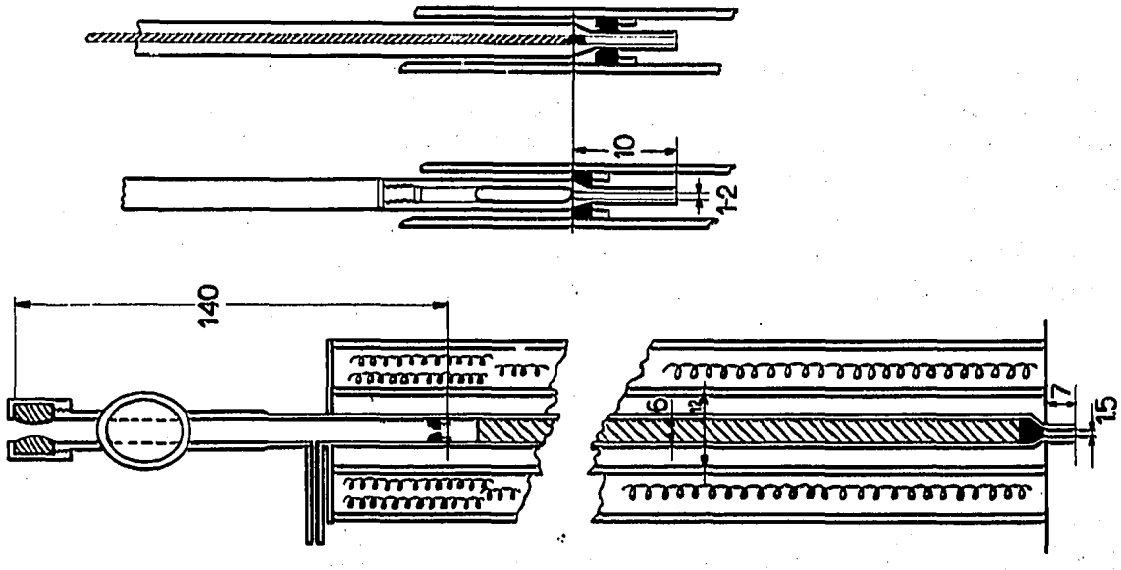

Fig. 2. Scheme of the gas chromatographic column with sampling device in detail (dimensions in mm).

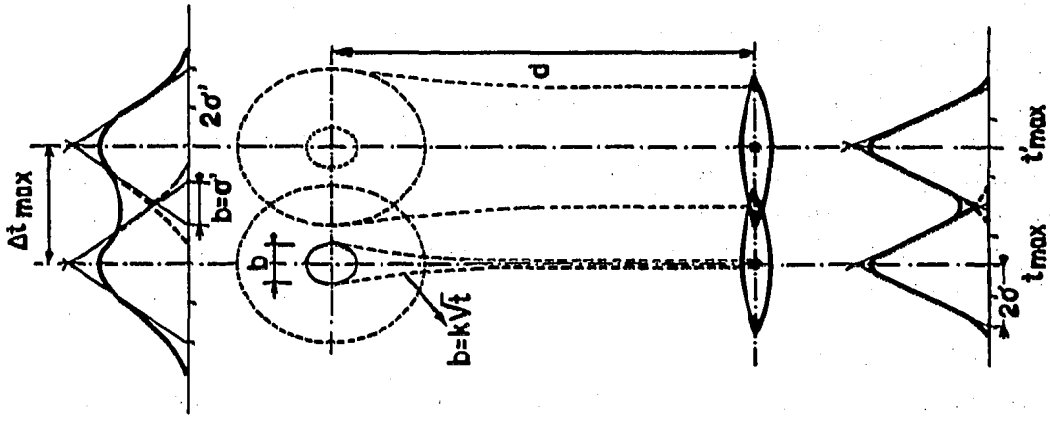

Fig. 1. Band broadening of gas-chromatographic zones on a thin layer.

Combining equations (3) and (5) gives

$$v \geqq \frac{8}{\Delta t_{max}} \sqrt{H \cdot R_F \cdot L} \tag{6}$$

The speed necessary to fulfil this condition will be the lower the larger Δt_{max}, and/or the lower the height equivalent of a theoretical plate, or the ratio of the rate of movement of the solute zone to the solvent front and the length of the layer (that is, the value under the root determining the value of b).

When artificial restriction of the lateral diffusion of the zones has been created by means of dividing the layer into a system of narrow strips of width a, the relation for the minimum speed can be given in the final form:

$$v_{min} = \frac{a}{b} \cdot \frac{8}{\Delta t_{max}} \sqrt{H \cdot R_F \cdot L} \tag{7}$$

METHODS AND MATERIALS

Gas chromatography

The gas chromatography column was a stainless steel tube of 6 mm I.D. and of 100 cm of usual length, as shown diagrammatically in Fig. 2.

The column was placed in a steel jacket of 12 mm I.D. surrounded by 8 semi-circular electric heating elements each of 250 mm length. Current was supplied from the mains supply through an auto-transformer. The injection chamber was heated with a small element to approximately 50° above the temperature of the chromatographic column. The colum outlet was reduced to a capillary of 1.5 mm I.D. and 7 mm long. The sample was introduced into the gas chromatographic column by means of a slightly modified TENNEY–HARRIS pipette[12] or a plunger-operated injection needle[13]. In the first case the sampling pipette was pressed through a silicone gasket which was held tightly in place above the column filling by a metallic O-ring, and the liquid or melted sample was transferred by the carrier gas stream into the heated sampling chamber and thence in vapour form into the column filling. In the second case the sample was forced out from a robust injection tube by a steel wire acting as a plunger. The second method gives better results for low-viscosity liquids and for solids with melting points higher than 100°.

Phenyl methyl silicone elastomer of molecular weight approximately 370,000 (East Bohemian Chemical Works, N.E., Pardubice, ČSSR) was applied as stationary phase as a 10% w/w coating of celite (Johns–Manville Ltd., London, England) of grain size 0.2–0.3 mm. The stationary phase was stable up to 300°. Nitrogen or argon was used as carrier gas.

Thin-layer chromatography

Plates of mirror glass, 100 × 200 mm and 200 × 200 mm, were used and coated with loose powdered silica gel PHH (Spolana N.E., Neratovice, ČSSR; this material is more polar than Merck silica gel G for thin-layer chromatography). An applicator of a common type was used[14] for the silica gel plates of 80 × 190 mm and 180 × 180 mm, grain size 0.05–0.15 mm, thickness of the layer 0.6–0.9 mm. In the case where lateral diffusion is restricted, metal plates made from nickel-coated brass were used. These

were notched at 3-mm intervals with a groove 1 mm in width to provide a thin-layer strip of 1 mm width between the grooves (see Fig. 3).

A glass chromatographic chamber of 150 × 300 × 150 mm or 300 × 300 × 150 mm was used. The slope of the coated plates was 20–30°. The following analytically

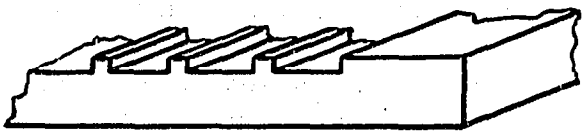

Fig. 3. Schematic illustration of the type of notches.

pure solvents, *n*-hexane, cyclohexane, benzene, chloroform, acetone, ether (Lachema N. E., Brno, ČSSR), were used for measuring R_F values. For some analytical separations, benzene, cyclohexane and a mixture of cyclohexane–benzene (1:1 v/v) were chiefly employed. Developing times of the TLC were usually 8–15 min.

Gas chromatographic sampling technique for TLC

The silica gel plates were placed on desk D on a small flat carriage as shown in Fig. 4.

The movement of the carriage was effected by a continuous screw, S_3, driven by an electric motor, M, which can be operated at different speeds by means of gears S_1 and S_2. The driving velocity was regulated in the range of 2–75 mm/sec. The outlet from the chromatographic column was placed 2 mm above the surface of the silica gel layer and 10–15 mm from the edge. No disturbance of the silica gel was caused by velocities of carrier gas up to 1 ml/sec and under these conditions the starting zone was usually less than 3 mm wide.

Detection and recovery of substances from a TLC plate for further GLC analysis

The spots were detected by forming coloured complexes of the substances under examination with tetracyano-ethylene (TCE)[15]. A saturated solution of TCE in benzene (3.6 g/100 ml) was dropped from a capillary pipette or sprayed on by means of a wetted tooth-brush rubbed on a metal sieve. After evaporation of the benzene in a drying oven at 100°, the yellow-coloured silica gel becomes white and coloured spots of

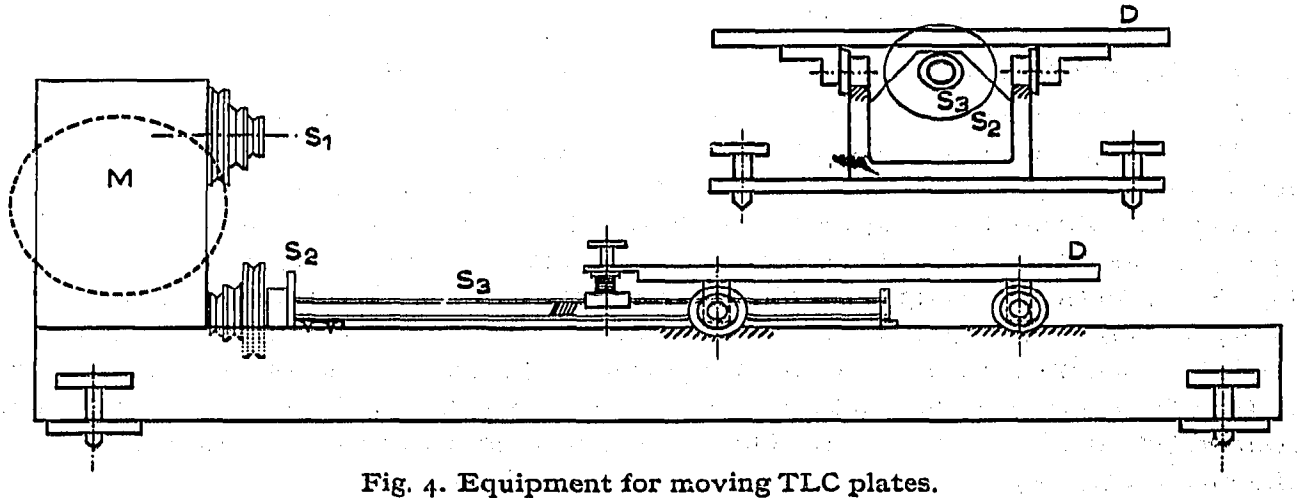

Fig. 4. Equipment for moving TLC plates.

complexes appear. The intensity of the various colours is constant for 1–2 h, but substances of phenolic character deepen in colour during this time. After this time the colours fade according to the type of substance[16], though the effect of the moisture content of the silica gel on the reaction becomes important too. Similar results are observed at higher drying temperatures. In the case of analytical treatment of the chromatograms, TCE solution was dropped on places where spots were expected.

For further gas-chromatographic examination the silica gel corresponding to a zone characterized by a t_{max} value (gas chromatography) and R_F value (thin-layer chromatography) is transferred into a glass capillary shown in Fig. 5.

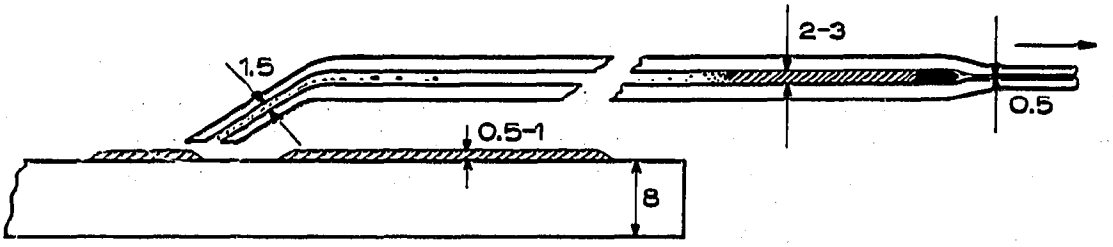

Fig. 5. Transfer capillary for extracting TLC material for GLC analysis (dimensions in mm).

The silica gel is removed by suction into the capillary starting at the centre of the expected zone on the plate and working spirally to the edges. The small silica gel column in the capillary is eluted with methanol or pyridine (0.1 ml at a time). Usually the first two drops from the column contain all the adsorbed material and serve as a storage solution for injection into a sensitive ionisation gas chromatograph. The apparatus used was a commercial high-temperature flame ionisation model (Chrom I, Laboratory Equipments N.E., Prague, ČSSR).

Reference substances

These substances were compounds specially prepared for research purposes (Gesellschaft für Teerverwertung, Duisburg, W.-Germany; The Benzole Producers Ltd., Watford, Herts., England; Coal Tar Research Association, Gomersal, Leeds, England) and purified by GC-sublimation[7], so that they were gas-chromatographically pure.

EXPERIMENTAL AND DISCUSSION OF RESULTS

R_F values of standard compounds and colours of their TCE complexes

The R_F values obtained are summarized in Tables I and II.

The group character of the separation of compounds with different functional groups on silica gel, as described earlier[6], is evident. Aromatic hydrocarbons and heterocyclics without strong electron affinity to silica gel have R_F values of about 0.4–0.5 in non-polar solvents (*n*-hexane, cyclohexane), and heterocyclics with –NH– and –N= functional groups and phenols have R_F values of less than 0.05. In benzene the R_F values of aromatic hydrocarbons increase to 0.90–0.95, those of indoles and carbazoles to 0.60–0.75. Phenols have R_F values of 0.15–0.50, according to the degree of steric hindrance of the hydroxyl group. Heterocyclics with tertiary nitrogen are retained practically on the start. The molecular weight of most substances has little influence on the R_F value.

TABLE I

R_F VALUES OF AROMATIC HYDROCARBONS AND COLOURS OF THEIR COMPLEXES WITH TCE

Compound	*Colour of complex with TCE*	R_F		
		n-Hexane	*Cyclohexane*	*Benzene*
Naphthalene	rose-violet	0.50	0.40	0.94
1-Methylnaphthalene	grey-violet	0.47	0.46	0.94
2-Methylnaphthalene	red-violet	0.46	0.47	0.93
1,2-Dimethylnaphthalene	grey-blue	0.48	0.56	0.94
1,3-Dimethylnaphthalene	grey-violet	0.42	0.53	0.95
1,4-Dimethylnaphthalene	grey-blue	0.55	0.54	0.95
1,5-Dimethylnaphthalene	green-blue	0.44	0.47	0.93
1,6-Dimethylnaphthalene	green-blue	0.47	0.47	0.94
1,7-Dimethylnaphthalene	dark grey	0.50	0.52	0.95
2,3-Dimethylnaphthalene	blue-violet	0.36	0.40	0.95
2,6-Dimethylnaphthalene	blue-violet	0.46	0.44	0.93
2,7-Dimethylnaphthalene	violet	0.45	0.44	0.94
2-Ethylnaphthalene	brown	0.55	0.52	0.93
2,3,5-Trimethylnaphthalene	blue-grey	0.50	0.47	0.93
2-Phenylnaphthalene	red-violet	0.34	0.38	0.92
Biphenyl	orange	0.45	0.45	0.95
3,5-Dimethylbiphenyl	orange	0 40	0 42	0.93
4,4'-Dimethylbiphenyl	light violet	0.39	0.44	0.94
4,5-Benzindane	grey-green	0.50	0.50	0.96
Acenaphthene	coffee green	0.52	0.53	0.95
Fluorene	red-violet	0.40	0.40	0.95
Phenanthrene	violet	0.36	0.40	0.93
1-Methylphenanthrene	violet	0.29	0.32	0.94
Anthracene	blue-green*	0.40	0.42	0.95
2-Methylanthracene	blue-green*	0.40	0.40	0.94
Pyrene	grey-green	0.35	0.38	0.93
Fluoranthene	brown-violet	0.30	0.25	0.92
Chrysene	grey	0.18	0.18	0.94

* Colour disappears within a few seconds.

The colours of TCE complexes are also given in Tables I and II. It is known[16] that the colour formation is connected with the donor–acceptor charge transfer between π electron pairs of aromatic conjugated systems and cyano groups on the unsaturated C skeleton[17]. The colour of the complexes in the visible part of the spectrum has some relation to the structure of the substances under investigation. Hydrocarbons with simple benzene nucleic or substituted nuclei have colours from yellow to red, *e.g.* benzene – yellow, tetramethyl benzene – red, diphenyl – orange, diphenylmethane – orange, 3,5-dimethyldiphenyl – light violet. Tri- or polycyclic aromatic hydrocarbons are grey to violet. A special position is occupied by phenols and some heterocyclics, where, as previously mentioned, the colour change with time indicated that the complexing proceeds by stages. There are also characteristic group colours, *e.g.* red to brown for indoles and blue for carbazoles. Heterocyclic compounds with tertiary nitrogen in the nucleus yield weak yellow to yellow-brown colours.

TABLE II

R_F VALUES OF AROMATIC S, O AND N COMPOUNDS AND COLOURS OF THEIR COMPLEXES WITH TCE

Compound	*Colour of complex*	R_F					
		n-Hexane	*Cyclohexane*	*Benzene*	*Chloroform*	*Ether*	*Acetone*
S compounds							
Thionaphthene	brown	0.47	0.53	0.93			
Diphenylene sulphide	violet	0.46	0.44	0.90			
O compounds							
Cumarone	yellow-orange	0.41	0.41	0.90			
Diphenylene oxide	brown-red	0.37	0.40	0.92			
4-Hydroxyhydrindene	red-brown	0.02	0.01	0.29	0.21	0.81	0.94
5-Hydroxyhydrindene	grey-violet	0.00	0.00	0.18	0.16	0.88	0.95
2,3,5-Trimethylphenol	dark violet	0.00	0.00	0.28	0.33	0.82	0.96
2,4,5-Trimethylphenol	grey	0.00	0.00	0.24	0.26	0.91	0.92
3,4,5-Trimethylphenol	brown-violet	0.00	0.00	0.15	0.17	0.90	0.96
3-Methyl-5-ethylphenol	brown	0.00	0.00	0.19	0.18	0.90	0.94
1-Naphthol	orange	0.01	0.01	0.32	0.26	0.85	0.94
2-Naphthol	brown	0.00	0 00	0.20	0.17	0.86	0.95
2-Hydroxybiphenyl	yellow-brown	0.03	0.02	0.55	0.50	0.90	0.94
4-Hydroxybiphenyl	blue-violet	0.00	0.00	0.23	0.20	0.92	0.95
N compounds							
Indole	brown (green edge)	0.03	0.02	0.65	0.63	0.95	
2-Methylindole	orange	0.03	0.02	0.65	0.60	0.95	
3-Methylindole	brown (violet edge)	0.02	0.03	0.65	0.70	0.92	
5-Methylindole	brown	0.03	0.03	0 65	0.65	0.93	
7-Methylindole	brown	0.04	0.04	0.64	0.65	0.95	
Carbazole	ultramarine blue	0.03	0.02	0.74	0.75	0.92	
2-Methylcarbazole	sky blue	0.03	0.02	0.77	0.70	0.92	
Quinoline	red-orange	0.00	0.00	0.02	—	0.45*	
2-Methylquinoline	red-orange	0.00	0.00	0.04	0.40*	—	
4-Methylquinoline	brown-orange	0.00	0.00	0.04	0.40*	—	
7-Methylquinoline	brown-orange	0.00	0.00	0.03	—	—	
8-Methylquinoline	brown-orange	0.00	0.00	0.03	0.38*	0.70*	
2,6-Dimethylquinoline	brown-yellow	0.00	0.00	0.04	—	—	
7,8-Benzoquinoline	brown-orange	0.00	0.00	0.01	—	—	
Isoquinoline	brown-orange	0.00	0.00	0.03	—	0.44*	
1-Methylisoquinoline	brown	0.00	0.00	0.02	0.29*	—	
3-Methylisoquinoline	grey-brown	0.00	0.00	0.02	—	—	
2-Phenylpyridine	brown-orange	0.00	0.00	0.02	—	—	
Acridine	yellow-orange	0.00	0.00	0.00	—	0.01	

* Tailing from the start to the given R_F value; in the case of small samples remains at the start.

Estimation of minimum plate driving velocity

Zone broadening during the deposition of the substances from the GC column on the TL plate is a diffusion process[18] which can be described by an effective diffusion coefficient:

$$\sigma = \sqrt{2Dt} = \frac{b}{4}$$

$$D = \frac{b^2}{32\,t}$$

$$b = \sqrt{32Dt} = k\sqrt{t}$$

Fig. 6 shows that the relation d and t only approaches the parabolic one described for PC[11] and TLC[9] (from slurry-prepared thin-layer plates). Deviation from the linearity of the relation between d^2 and t is caused by irregular distribution of the solvent. The condition that the driving velocity of the chromatographic front is proportional to the mass flow of solvent[11] is not completely fulfilled.

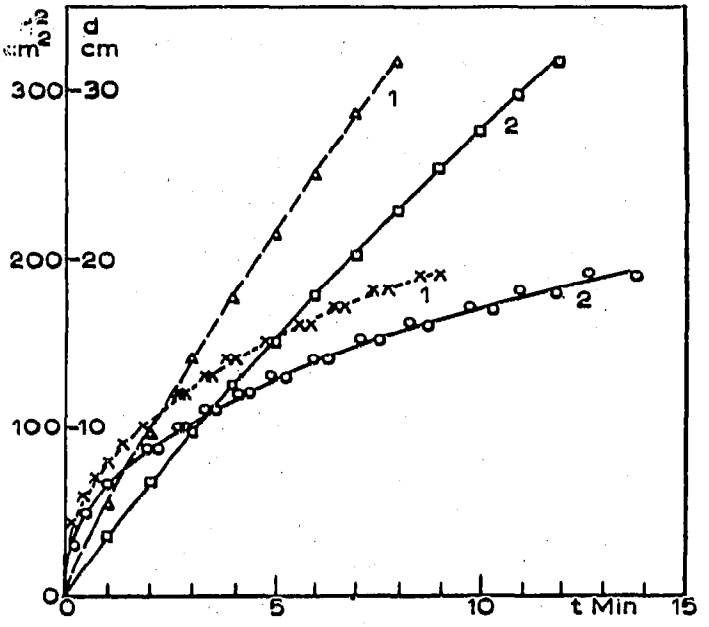

Fig. 6. Relation between distance of the zone from the start and time.
1 = cyclohexane; 2 = benzene.

Data were found for some aromatic hydrocarbons in the system silica gel/benzene, as shown in Table III ($L = 19.0$; $t = 525$ sec; $R_F = 0.94$; $\Delta t_{max} = 1.3$ min).

Values for the minimum driving velocity of the plate estimated from average data:

$$v_{min} = \frac{8}{1.3} \cdot \sqrt{0.00259 \times 18} = 6.15 \times 0.216 = 1.33 \text{ cm/min.}$$

Using notches of $a = 0.30$ cm, the minimum driving velocity is

$$v_{min}\,(\text{diphenyl/acenaphthene}) = 1.33 \cdot \frac{0.30}{0.84} = 0.475 \text{ cm/min.}$$

This is in accordance with experimental results, as may be seen from Fig. 7.

With a driving velocity of 0.22 cm/min (Fig. 7a) the pairs diphenyl and acenaphthene and acenaphthene and fluorene overlap ($b > 2\sigma$), but with a velocity of 0.63 cm/min (Fig. 7b) the separation is better than required by $b = 2\sigma$ and no overlapping can be recorded with a driving velocity of 1.49 cm/min (Fig. 7c) ($b \ll 2\sigma$).

On the other hand, the zones of fluorene and phenanthrene (Fig. 7a) are well separated, even when the driving velocity of the plate is 0.22 cm/min, because Δt_{max} is 3.7 min and

$$v_{min}\ (\text{fluorene/phenanthrene}) = \frac{8}{3.7} \cdot \frac{0.30}{0.84} \cdot 0.216 = 0.163.$$

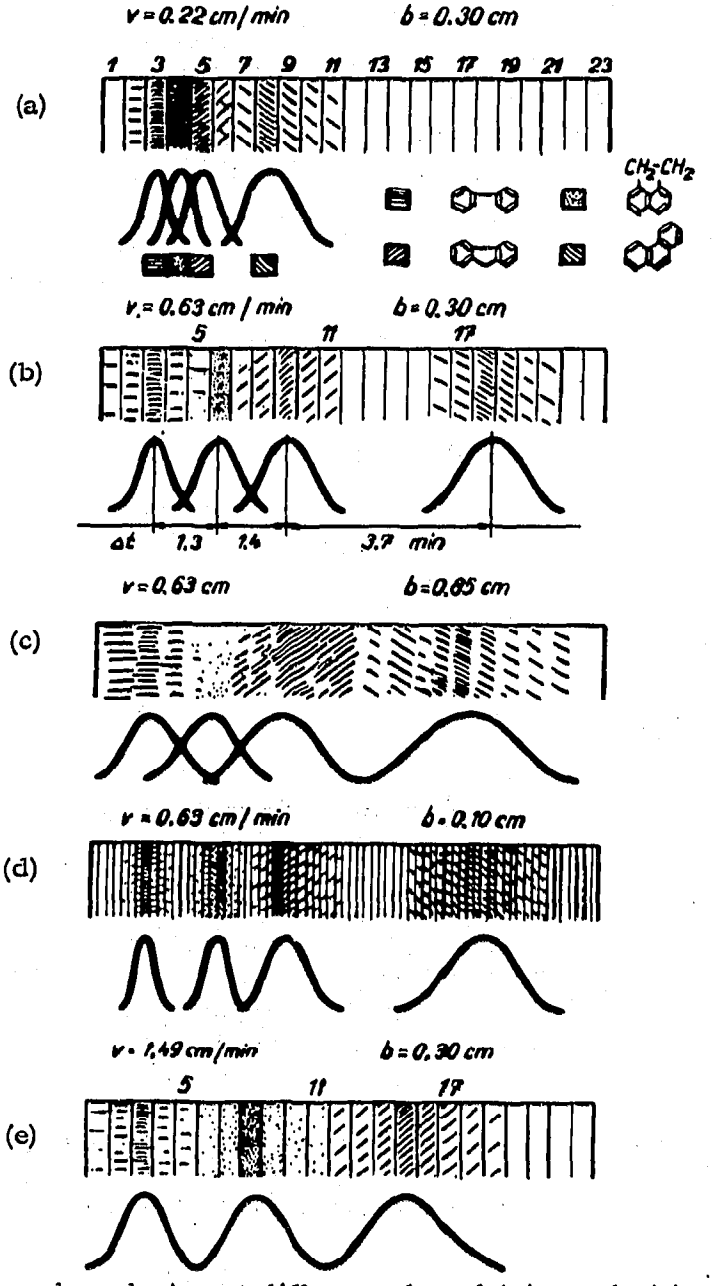

Fig. 7. Examples of zone broadening at different plate driving velocities and different side diffusion hindrances.

TABLE III

MEASURED VALUES OF EFFECTIVE DIFFUSION COEFFICIENT D, PROPORTIONALITY FACTOR k BETWEEN TIME AND ZONE WIDTH, AND HEIGHT OF A THEORETICAL PLATE H FOR SOME AROMATIC HYDROCARBONS

Compound	*b* (cm)	*d* (cm)	$D \times 10^4$ (cm²/sec)	$k \times 10^2$ (cm/sec)	$H \times 10^2$ (cm)
Biphenyl	0.75	18.0	0.335	6.55	2.06
Acenaphthene	0.85	18.0	0.43	7.42	2.65
Fluorene	0.95	18.0	0.56	8.49	3.30
Phenanthrene	0.80	18.0	0.38	7.00	2.35
Mean	0.84	18.0	0.43	7.37	2.59

Finally the example in Fig. 7c shows that the zone broadening on the plate without prevention of lateral diffusion gives the same results with a driving velocity of 0.63 cm/min only as was found in the case of a driving velocity of 0.22 cm/min (Fig. 7a) and notches 0.30 cm wide, because $b > 2\sigma$. A thin-layer chromatogram developed with the same parameters, but with artificial prevention of lateral diffusion at 0.10-cm intervals is practically a copy of the corresponding gas chromatogram, because $b \ll 2\sigma$.

Application to coal tar research

The complexity of coal tar mixtures is extremely great. Gas chromatography alone is not able to yield a complete separation of compounds. It may be shown that the amount of GC-information about the composition of coal tar and similar materials decreases with increasing boiling points of the compounds[19–21]. The new possibilities

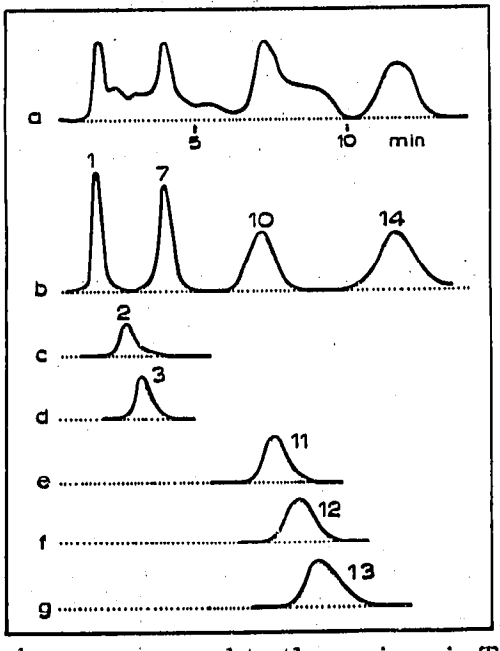

Fig. 8. Gas chromatograms (numbers correspond to those given in Table IV and Fig. 9). (a) original mixture; (b) hydrocarbons extracted from thin layer; (c) extracted zone of quinoline; (d) extracted zone of 4-hydroxyhydrindene; (e) extracted zone of 2-hydroxybiphenyl; (f) extracted zone of 1-naphthol; (g) extracted zone of 2-naphthol.

TABLE IV

PHYSICAL AND CHROMATOGRAPHIC VALUES FOR COMPONENTS OF THE STANDARD MIXTURE I

No.	*Compound*	*Boiling point (°C)*	*Gas–liquid chromatography silicone elastomer (220°) $r_{1,2}$*	*Thin-layer chromatography silica gel/benzene R_F*	*Colour of complex with tetracyano-ethylene*
1	Naphthalene	238	0.46	0.95	rose-violet
2	Quinoline	237	0.67	0.02	yellow-brown
3	4-Hydroxyhydrindene	247	0.77	0.29	brown
4	3-Methylisoquinoline	251	1.0	0.02	yellow-brown
5	Indole	255	0.60	0.60	brown (rose*)
6	5-Hydroxyhydrindene	255	0.94	0.18	violet (light blue-violet*)
7	Biphenyl	255	1.0	0.95	orange
8	3-Methylindole	266	1.1	0.65	purple (rose*)
9	2,4-Dimethylquinoline	273	1.4	0.03	brown-yellow
10	Acenaphthene	277	1.8	0.95	green
11	2-Hydroxybiphenyl	286	1.9	0.50	brown
12	1-Naphthol	288	2.1	0.32	orange
13	2-Naphthol	296	2.2	0.20	brown
14	Fluorene	298	2.9	0.95	violet

* Self-colouring in the presence of air oxygen at 100°.

of the method described appear to be clear from the information in Table IV on the composition of standard mixtures of compounds with boiling points ranging from 200 to 300°.

The chromatographic data are summarised in Fig. 9, which shows the separation on the plate and this is followed by gas chromatograms of the original mixture and separated materials extracted from a group of zones or from individual zones (Fig. 8).

The mixture of compounds with boiling points up to 400° was separated in the same way and is described in Table V and illustrated in Fig. 10.

This method was successfully exploited in research on the composition of coal tars, but it must be mentioned that it may be used without serious changes for the separation of other complex mixtures of industrial nature.

TABLE V

PHYSICAL AND CHROMATOGRAPHIC VALUES FOR COMPONENTS OF THE STANDARD MIXTURE II

No.	*Compound*	*Boiling point (°C)*	*GLC, silicone elastomer (300°) $r_{1,2}$*	*TLC, silica gel/ benzene + n-hexane (1:1 v/v) R_F*	*Colour of complex with tetracyano-ethylene*
1	Acenaphthene	277	0.35	0.93	green
2	1-Naphthol	288	0.40	0.12	orange
3	2-Naphthol	296	0.40	0.07	brown
4	Fluorene	298	0.59	0.93	rose-violet
5	Phenanthrene	340	1.0	0.92	violet
6	Acridine	344	1.1	0.01	yellow-green
7	Carbazole	353	1.2	0.55	ultramarine-blue
8	1-Methylphenanthrene	359	1.4	0.92	violet
9	2-Methylcarbazole	364	1.6	0.58	sky blue
10	Fluoranthene	384	2.0	0.92	violet
11	Pyrene	394	2.60	0.90	grey-green

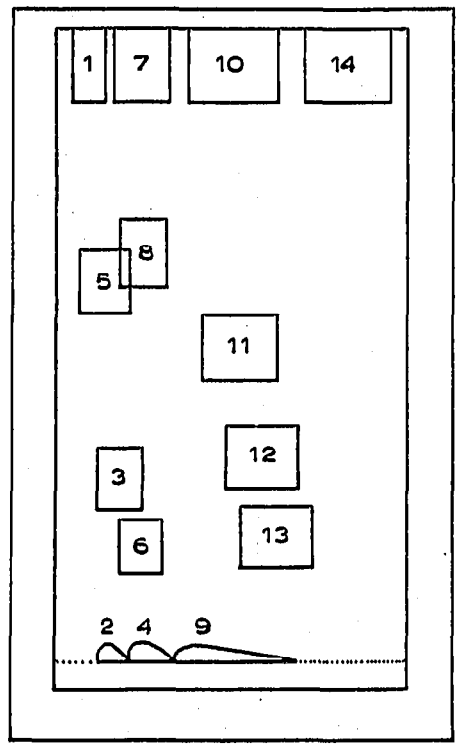

Fig. 9. Position of chromatographic zones after the combined developing (the numbers correspond to those given in Table IV). GLC–220°; TLC–silica gel/benzene.

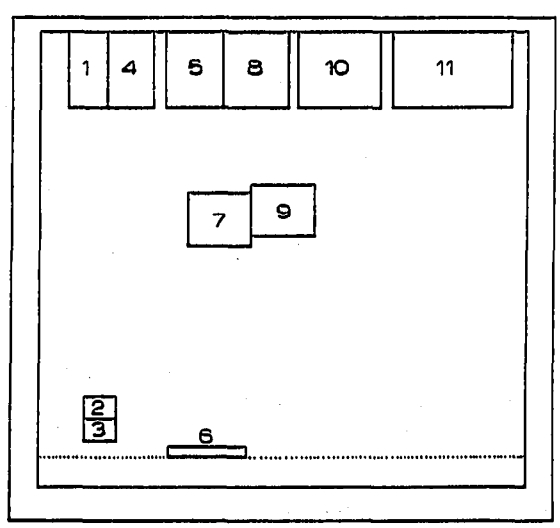

Fig. 10. Situation of chromatographic zones after the combined developing (numbers correspond to those given in Table V). GLC–300°; TLC–silica gel/benzene + *n*-hexane (1 : 1 v/v).

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SUMMARY

A new multi-dimensional chromatographic technique is described. μg to mg quantities from the gas chromatogram are placed on the start-line of a thin layer of adsorbent or of a sheet of chromatographic paper, which is moved past the orifice of the gas chromatograph and then developed in the usual manner. Thus, separation of the materials is obtained by exploiting the two most extreme possibilities given by the existing chromatographic methods, *viz.* gas–liquid chromatography, which separates according to the relative volatility of compounds (in the direction of the time-axis along the start), and thin-layer chromatography or paper chromatography, which separate according to the type of the functional group (in the vertical direction). After the extraction of the separated materials from the thin-layer or paper, GLC, on a suitably selected stationary phase, may be repeated if necessary. The theory for separation and experimental details are given. Further possibilities are obtainable by programming the driving velocity of the plate (paper) which acts in the same manner as temperature programming of a GC column.

The method described was used successfully in the research on the composition of coal tar, but can be applied to other complex mixtures of compounds of different chemical character.

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