MULTI-DIMENSIONAL CHROMATOGRAPHY USING DIFFERENT DEVELOPING METHODS

III. THE IDENTIFICATION OF SUBSTANCES BY MEANS OF PROGRAMMED DISTRIBUTION OF FRACTIONS IN TWO-DIMENSIONAL CHROMATOGRAPHY

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In earlier papers[1,2] new possibilities were pointed out of preparation and identification by two-dimensional chromatography, where gas chromatography is used as a method for placing the fractions on the moving start line of a thin layer or paper. The possibility of identification by the programme-controlled shifting of the plate was also mentioned[2]. KAISER[3] tried the method suggested[1] and the start line of a thin layer was intermittently moved along as soon as some components left a gas-chromatographic column featuring programme-controlled temperature. The signal for movement was taken from the recording instrument. Each fraction from the gas chromatograph was separately plotted as a discrete point on the start line of the plate. This method, however, does not permit utilization of the characteristic distribution of spots on the thin-layer chromatogram for the purpose of identification.

In this communication the case formerly suggested[2] is dealt with, where the gas chromatogram remains isothermal, but the shifting of the plate is logarithmically programmed with time. Proof is furnished that by logarithmic shifting of the thin layer or chromatographic paper, the distance between the zones of the individual members of the homologous series is the same and the reading of the two-dimensional chromatogram greatly facilitated. Further, the possibility is pointed out of noting the position of the zones or of identifying them directly in KOVÁTS retention indices[4], and in a suitable group of substances of correlating them with their boiling points[5].

THEORY

The shift of the chromatographic zone of a substance 1 is proportional to the velocity of the carrier gas u so that $u_1 = R_F \cdot u$,

where: $R_F = 1/(1 + K_1)$
$K_1 = a \cdot e^{Q_1/RT}$
K_1 = the separation coefficient
a = a constant
Q = the heat of adsorption or solution
R = the gas constant
T = the absolute temperature.

For a pair of homologous substances 1 and 2

$$Q = RT \cdot \ln \frac{K_2}{K_1} = RT \cdot \ln \frac{t_2}{t_1} \quad (1)$$

where: Q = sorption energy increase for the homologous increment,
t_1, t_2 = elution times of maximum of zones of homologues 1 and 2.

K and likewise t, increase exponentially with an increasing number of increments. If we make a logarithmic recording t, the distance in the chromatogram corresponding to the increment of the $-CH_2-$ group will be the same, and additive, for substances having the same structure.

The position of the individual components in the chromatogram can be established for $y_1 = \log t_i$ and $y_{i-1} = \log t_{i-1}$ from the equations of the straight lines for

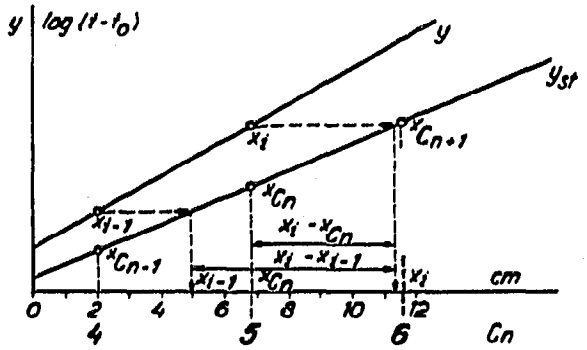

Fig. 1. Graphical illustration of position of substance i (in cm) measured from the edge of the thin layer, in relation to a chosen homologous series.

the standard series of n-paraffins and the homologous series where the component i occurs as follows from the definition of the KOVÁTS' indices[4]. The procedure is graphically illustrated in Fig. 1.

$$x_i - x_{i-1} = (x_{C_n} - x_{C_{n-1}}) \frac{\log t_i - \log t_{i-1}}{\log t_{C_n} - \log t_{C_{n-1}}} \quad (2)$$

$$x_{C_n} - x_i = (x_{C_n} - x_{C_{n-1}}) \frac{\log t_{C_n} - \log t_i}{\log t_{C_n} - \log t_{C_{n-1}}} \quad (3)$$

In the logarithmic shifting of the plate, $\log t$ is directly given by the linear section L on the start line of the plate. If we correlate some of the zones in the chromatogram to the elution indices of n-paraffins, we can read directly from the start of the plate the values for KOVÁTS' retention indices, corresponding to the component or increment of the series:

$$I_{\text{subst.}} = 100 \frac{L_i - L_{C_n}}{L_{C_n} - L_{C_{n-1}}} + 100 \cdot C_n \quad (4)$$

$$I_{CH_2} = 100 \frac{L_i - L_{i-1}}{L_{C_n} - L_{C_{n-1}}} \quad (5)$$

Similarly, proceeding from the assumption that the boiling points of non-polar substances are determined by the dispersion forces, we can, when using the non-polar stationary phases in a sufficiently wide range of boiling points, estimate the boiling point of the component i in the chromatogram:

$$(\text{B.P.})_i = (\text{B.P.})_x + \{(\text{B.P.})_y - (\text{B.P.})_x\} \frac{L_x - L_i}{L_x - L_y} \qquad (6)$$

EXPERIMENTAL AND DISCUSSION

Gas chromatography

The experiments were performed on a chromatograph CHROM III (Laboratorní přístroje, N.E., Praha) equipped with exchangeable detectors (thermal conductivity, flame and argon ionization), columns for preparation and analysis in packed and capillary columns. The katharometer and the packed column with isothermal and programme-controlled heating system were chosen for use. The column measured 3 m in length and 0.6 cm in diameter. The packing was SE-30 silicon elastomer (General Electric Inc., U.S.A.), 20% weight on Chromosorb W (Johns-Manville, Ltd., London, Great Britain). The carrier gas was hydrogen. An adapter for the trapping device was used and under the outlet were placed the stage[2] and the carrier plate with the thin layer. The fractions ahead of the outlet to the thin layer were registered by means of a recording instrument.

Logarithmic shifting device

The apparatus illustrated in Fig. 2 was constructed for the logarithmic shifting of the plate. This apparatus consists of a Wheatstone bridge, the logarithmic potentiometer P_1 (Aripot, manufactured by ARITMA, N.E., Praha) with an electron-tube impedance transformer, the amplifier Z and the motor M moving the stage (for carrying the plate), coupled with the potentiometer slide P_6. The synchronous motor moves the slide of the logarithmic potentiometer P_1, thus producing on the cathodes of the EF-80 electron-tube a voltage which is deducted from the voltage of the bridge. The voltage difference is conveyed into the amplifier Z feeding the two-phase in-

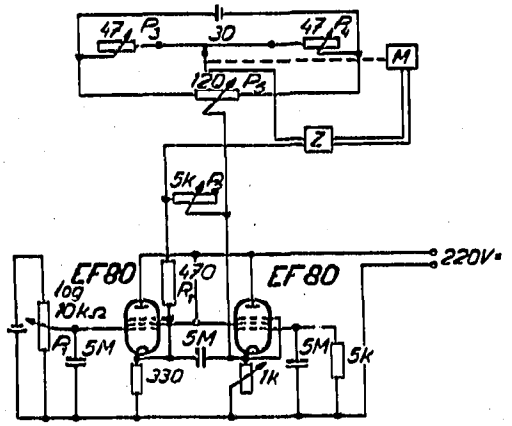

Fig. 2. Scheme of device for logarithmic shifting.

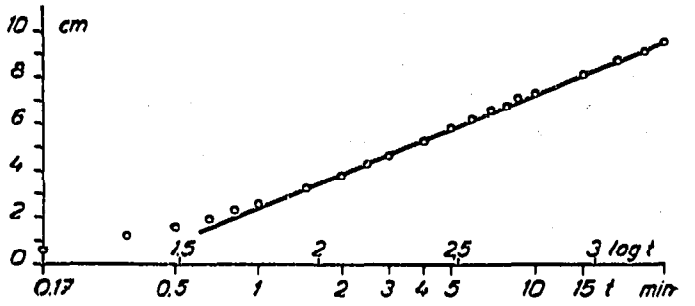

Fig. 3. Time behaviour of shifting of plate with thin layer.

duction motor (type RD 09, manufactured by VEB Magdeburger Armaturen Werke, D.D.R.). The slide of the Wheatstone bridge moves so that the voltage difference on the input side of the amplifier approaches zero. In the position of the stage corresponding to the displacement of the logarithmic potentiometer P_1, the amplifier input shows zero voltage and the stage stops. With the aid of the potentiometers P_2, P_3 and P_4 the modulus of the logarithmic shifting can be changed. The normal position of the stage can be adjusted by means of the potentiometer P_5. The actual moving of the stage depending on time is graphically illustrated in Fig. 3. The shift corresponds, with the exception of the first minute, satisfactorily to the logarithmic course.

Thin-layer chromatography

The technique of the fraction trapping on a thin layer was described in a previous paper[2]. The chromatograms were developed in a chamber corresponding to the S-chambers[6] for dried slurry plates. The type used was adapted for work with layers made with dry powder. This is shown in Fig. 4. The quantity of solvent used

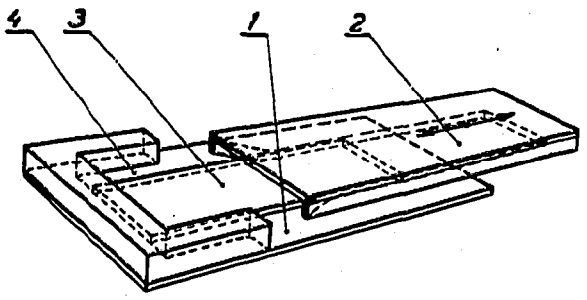

Fig. 4. Glass chamber for developing chromatograms on a thin powder layer. (1) Bottom part of chamber; (2) lid of chamber; (3) plate with thin layer; (4) space for solvent.

was approximately 25 ml. Examination of Merck thin-layer silica gels showed that only used silica gel PHH (see ref. 2) gave no colouring after treatment with tetracyanoethylene.

Chemicals

n-Propylbenzene and *n*-butylbenzene were synthetized by Friedel-Crafts' reaction and purified by means of rectification. The other substances used were commercial preparations: benzene, toluene (Lachema, Brno), ethylbenzene (Fluka, Buchs, Switzerland). Silica gel, tetracyanoethylene, aromatic hydrocarbons, phenols, indoles, and quinoline were the same as those used in our previous experiments[2].

The reading of the zone position in Kováts' indices and the correlation to the boiling points of aromatic hydrocarbons

In order to verify the theoretical assumptions, the homologous series of aromatic hydrocarbons benzene to *n*-butylbenzene was chosen. This mixture was complemented by C_6, C_7, C_8 and C_{10} *n*-paraffins. The individual components of the mixture were adsorbed on a logarithmically moving thin layer of silica gel, placed under the outlet of the column. The logarithmic shifting was begun always at the time interval $t - t_0 = 1$ minute. The position of the aromatics was detected with tetracyanoethylene, the silica gel having been moistened with a solution of tetracyanoethylene and

dried prior to the experiment. The positions of the aromatic hydrocarbons quoted are recognized by the yellow to orange-yellow spots of the complexes formed. Only the detection of benzene, which evaporates quickly and thus reduces the possibility of exact localization of the spot, presents certain difficulties. The position of *n*-paraffins, which do not yield a colour complex with tetracyanoethylene, was registered by reference to the linear recording on the gas chromatograph, taking place at the same time. Fig. 5 presents the chromatogram of the mixture with linear movement of the chromatographic chart paper.

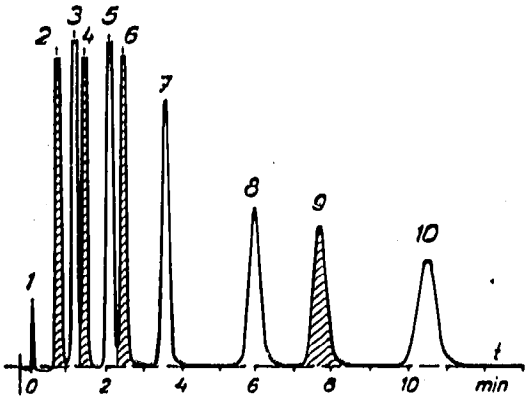

Fig. 5. Isothermal chromatogram of aromatic hydrocarbons and *n*-paraffins (t = 110°, F = 100 ml H_2/min). (1) Air; (2) *n*-hexane; (3) benzene; (4) *n*-heptane; (5) toluene; (6) *n*-octane; (7) ethylbenzene; (8) *n*-propylbenzene; (9) *n*-decane; (10) *n*-butylbenzene.

Table I gives the values $L_{max} - L_0$ for linear shifting (measured on the chart paper of the chromatograph) and L_{max}' (measured from the plate edge) for the bulk of spots for logarithmic displacement. KOVÁTS' retention indices were calculated for linear shifting from the known relations[4] and for logarithmic shifting from the values read on the start line of the thin layer according to eqn. (4).

The comparison between the positions of the individual aromatics on the plate

TABLE I

COMPARISON OF KOVÁTS' INDICES, CALCULATED FROM A CHROMATOGRAM WITH LINEAR MOVEMENT OF THE CHART (GAS CHROMATOGRAPH RECORDING) AND WITH LOGARITHMIC SHIFTING (THIN LAYER)

Substance	*Linear shifting*		*Logarithmic shifting*	
	$L_{max} - L_0$ (*mm*)	$I^{SE-30}_{170°}$	L_{max}' (*mm*)	$I^{SE-30}_{170°}$
n-Hexane	6.5	600	—	—
Benzene	11.5	676	5.5	675*
n-Heptane	13.0	700	14.0	700
Toluene	20.5	774	39.0	772
n-Octane	24.0	800	48.0	800
Ethylbenzene	35.5	867	69.0	865
n-Propylbenzene	59.5	956	97.0	951
n-Decane	77.0	1000	113.0	1000
n-Butylbenzene	105.0	1052**	128.0	1046**

* Computed by extrapolation from C_8 and C_7.
** Computed by extrapolation from C_8 and C_{10}.

(measured from the plate edge) calculated from eqn. (2) and the actual ones is given in Table II. Toluene and ethylbenzene were taken as standards.

Similarly, the position of the zones, referring to any optimal standard, *e.g.*, *n*-paraffin, can be calculated from eqn. (3). Finally, the boiling points could be calculated from eqn. (6) (Table II, right-hand side). As a starting point for the calculations, the boiling points of toluene and ethylbenzene were used.

TABLE II

COMPARISON OF CALCULATED AND MEASURED POSITIONS OF MODEL SUBSTANCES ON A THIN LAYER AND OF THE CALCULATED AND MEASURED BOILING POINTS

Substance	*Position (mm)*		*Boiling points (°C)*	
	Calculated	*Measured*	*Calculated*	*Measured*
Benzene	5.5	8.5	83	80
n-Propylbenzene	97.0	100.0	159	158
n-Butylbenzene	130.5	128.0	184	183

Applications in coal tar chemistry

The applicability of this method to the examination of the composition of wash oils from coal tar was tested on a model mixture consisting of the following fifteen substances: 2-methylnaphthalene, 2,7-dimethylnaphthalene, 2,3-dimethylnaphthalene, 2,3,6-trimethylnaphthalene, acenaphthene, fluorene, indole, 3-methylindole, 2-methylindole, 4-hydroxyhydrindene, 5-hydroxyhydrindene, 2-hydroxydiphenyl, 1-naphthol, 2-naphthol and quinoline. The individual fractions were

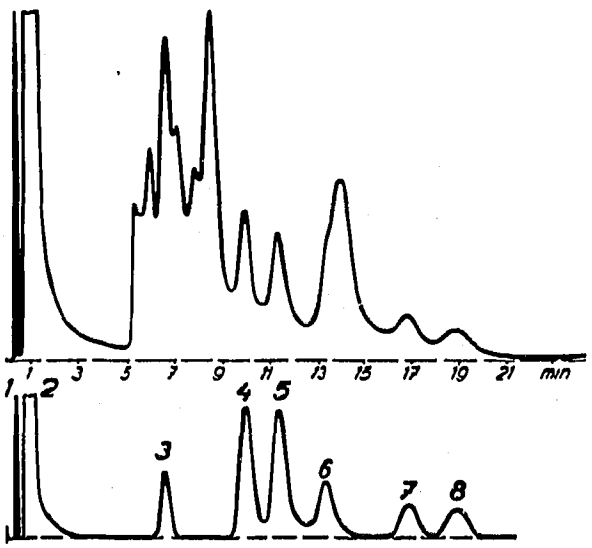

Fig. 6. Isothermal chromatograms. (a) Model mixture (t = 170°, F = 100 ml H_2/min), containing: 2-methylnaphthalene, 2,7-dimethylnaphthalene, 2,3-dimethylnaphthalene, acenaphthene, 2,3,6-trimethylnaphthalene, fluorene, indole, 3-methylindole, 2-methylindole, 4-hydroxyhydrindene, 5-hydroxyhydrindene, 2-hydroxydiphenyl, 1-naphthol, 2-naphthol, quinoline. (b) Fraction of aromatic hydrocarbons (t = 170°, F = 100 ml H_2/min). (1) Air; (2) solvent; (3) 2-methylnaphthalene; (4) 2,7-dimethylnaphthalene; (5) 2,3-dimethylnaphthalene; (6) acenaphthene; (7) 2,3,6-trimethylnaphthalene; (8) fluorene.

deposited by the aforementioned technique on a logarithmically moving thin layer of silica gel. The thin layer was developed with benzene and the individual substances were detected with a solution of tetracyanoethylene. Fig. 6a presents the chromatographic recording of the complete mixture.

In Fig. 7, the completed two-dimensional chromatogram on the plate after elution with benzene is presented.

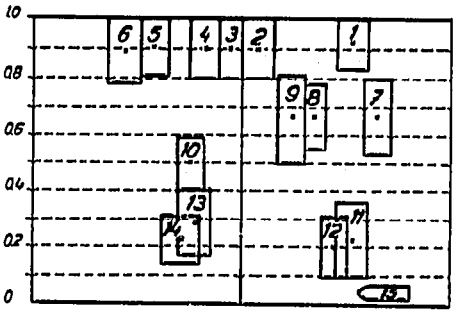

Fig. 7. Two-dimensional chromatogram on plate. Gas chromatogram: $t = 170°$, $F = 100$ ml H_2/min; chromatogram on thin layer: $t = 20°$; adsorbent: silica gel; solvent: benzene. (1) 2-Methylnaphthalene, violet; (2) 2,7-dimethylnaphthalene, black-violet; (3) 2,3-dimethylnaphthalene, blue-violet; (4) acenaphthene, green; (5) 2,3,6-trimethylnaphthalene, blue-violet; (6) fluorene, red-violet; (7) indole, brown; (8) 3-methylindole, violet, later brown; (9) 2-methylindole, black-violet, later brown; (10) 2-hydroxydiphenyl, dark ochre; (11) 4-hydroxyhydrindene, brown; (12) 5-hydroxyhydrindene, violet; (13) 1-naphthol, orange; (14) 2-naphthol, grey-violet; (15) quinoline.

Fig. 6b shows the chromatogram of the hydrocarbon portion. Both chromatograms in Fig. 6 were made under identical conditions.

The individual components were identified on the basis of the various colours of their complexes with tetracyanoethylene, the R_F values[2,6] and the quantities quoted above.

In the case of elution with benzene on silica gel, we can assume that the substances at the front of the plate, $R_F = 0.9$, are hydrocarbons (*cf.* Fig. 6b). If we use two known substances, in our case 2-methylnaphthalene and fluorene, we can establish the boiling points of the other fractions with the aid of eqn. (6). From Table III, it can be seen that the calculated and the actual boiling points of substances, whose presence is assumed, agree to a sufficient extent.

By comparing the chromatograms in Figs. 6a and 7 (6b respectively), we see that the zone of acenaphthene lies in the forward part of the common zone with the higher phenols, the zone of 2-methylnaphthalene forms the third peak in the chromatogram, etc.

TABLE III

CALCULATED AND ACTUAL BOILING POINTS OF HYDROCARBONS WITH R_F 0.90

Substance	*Boiling point* (°C)	
	Calculated	*Measured*
2,7-Dimethylnaphthalene	265	262
2,3-Dimethylnaphthalene	271	269
Acenaphthene	277	277
2,3,6-Trimethylnaphthalene	290	288

The importance of eqn. (5) can be verified in the case of the indoles, whose R_F values are around 0.75. The dimethylindoles in the tar fractions would be found on the plate at the same distance from the methylindoles as that formed by the interval between the indole and the methylindoles of a certain structure. The procedure is similar with phenols, whose R_F values generally vary over a range from 0.2–0.55 depending on the steric hindrance of the hydroxyl functional group. Finally, if we know the values of the KOVÁTS' retention indices for substances that are likely we can, with their help, very well determine directly the position of the individual substances on the plate.

SUMMARY

It was shown that by logarithmic shifting of the thin layer (or chromatographic paper), an equivalent distance for each of the individual members of an homologous series can be obtained along the start line, when developing a gas chromatogram. The identification of the substances in the two-dimensional chromatogram is greatly facilitated. The type of the substance can be found through measuring the distance directly in accordance with the values of KOVÁTS' retention indices, or with suitable groups of substances according to the boiling points of appropriate standards. In this manner, the position of a spot in the two-dimensional chromatogram, can be anticipated according to its structure and molecular size.

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