

## MULTI-DIMENSIONAL CHROMATOGRAPHY USING DIFFERENT DEVELOPING METHODS

### II. MICROSCOPIC AND COLORIMETRIC INVESTIGATION OF COMPOUNDS SEPARATED BY GAS CHROMATOGRAPHY IN SUBMICRO-QUANTITIES

JAROSLAV JANÁK

*Laboratory of Gas Analysis of the Czechoslovak Academy of Science,  
Brno (Č.S.S.R.)*

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In a previous communication<sup>1</sup> a new two-dimensional method of chromatography was described, in which the gas chromatogram is continuously developed on to the start-line of a thin layer of adsorbent or sheet of chromatographic paper, which is moved under the outlet orifice of the gas chromatographic column in a certain program. In this paper, the case will be discussed where instead of a plate with a thin layer of adsorbent (or a sheet of paper) a simple plate is used, made of glass or other material. In addition it is pointed out that it is possible to use crystallographic or colorimetric methods for identification of nanogram to picogram quantities of matter, using microscopy techniques. Only two earlier papers<sup>2,3</sup> dealing with a similar matter in another way and in the milligram range can be cited.

#### EXPERIMENTAL AND DISCUSSION OF RESULTS

##### *Sublimated chromatograms*

The technique described previously<sup>1</sup> was used. High boiling gas chromatographic fractions were condensed on a thick glass plate, while for lower boiling substances a jacketed metallic plate of the same size which was cooled with an internally circulating liquid (water, brine) was used. The investigation was carried out with tar substances having boiling points above 200°. Samples of 2–10 mg of mixture were injected, at a column temperature of 280°; the distance between the column capillary outlet and the glass plate was 1–2 mm; carrier gas velocity was 1 ml/sec. Gas chromatographic fractions leaving the column were condensed as a sublimate on the glass plate, well developed zones consisting of characteristic microcrystals of the substances being formed. The shape of the crystal zones is the same as is known from the recording of the gas chromatographic peaks. In Fig. 1 a sublimatogram of the mixture of biphenyl, acenaphthene, fluorene and phenanthrene is shown. The width of the sublimated zones was 3–4 mm, the diameter of the GC-column orifice being 1.5 mm. Each zone begins and ends with a crystalline bloom while the center of the zone generally consists of well developed microcrystals. The form and type of the microcrystals is characteristic. In Figs. 2–4 a series of photomicrographs of sharp and typical tran-



Fig. 1. Sublimatogram of a mixture of diphenyl, acenaphthene, fluorene, phenanthrene (photograph taken in the plane of the surface of the supporting glass plate with lateral illumination).

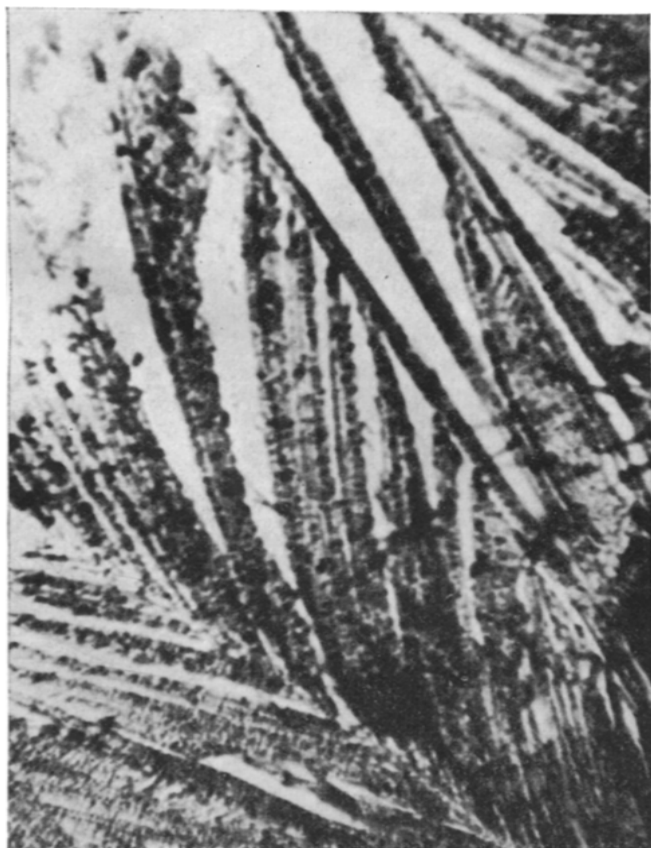


Fig. 2. Start line of the acenaphthene zone (magnified 50  $\times$ ).

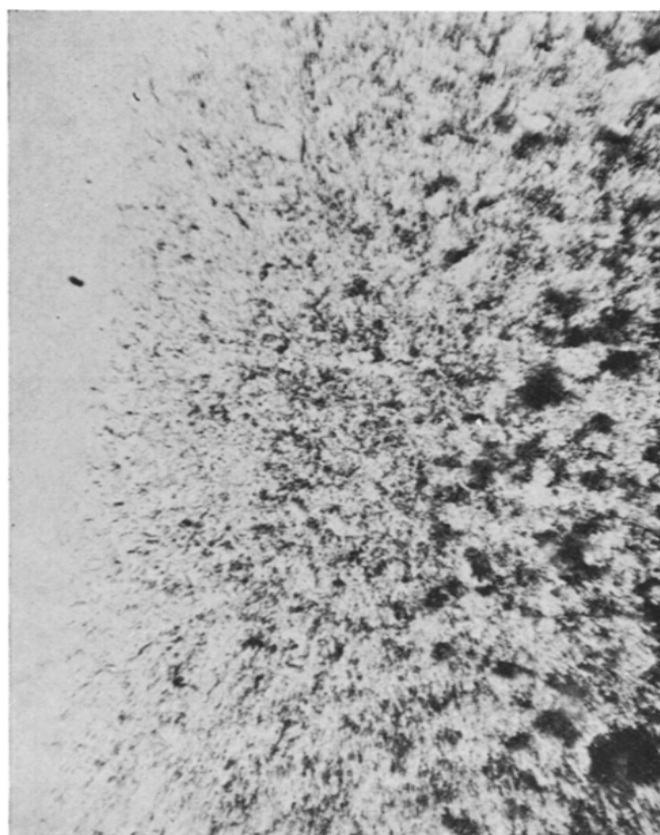


Fig. 3. Start line of the fluorene zone (magnified 50  $\times$ ).

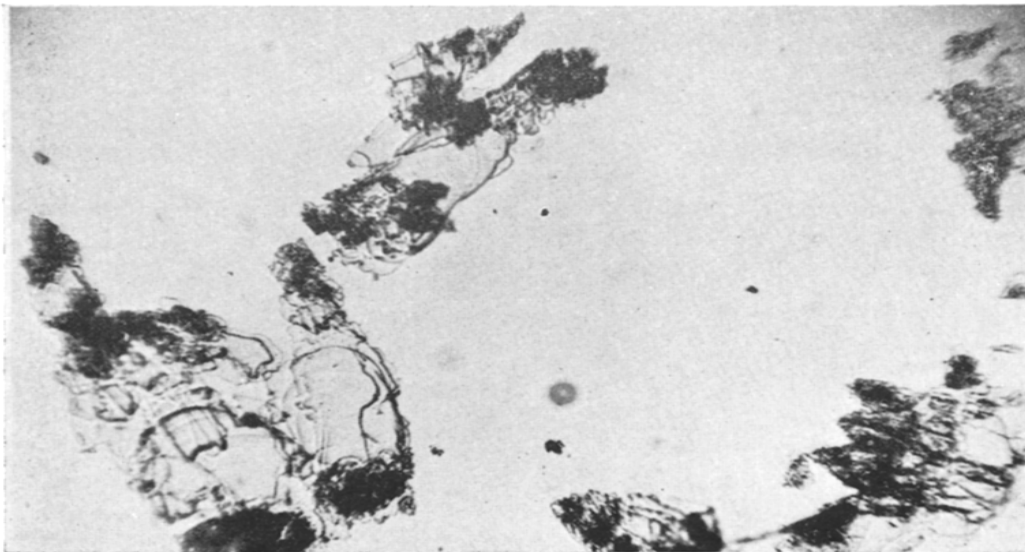


Fig. 6. Microcrystals of fluorene (actual size  $10^{-1}$ - $10^{-2}$  mm).

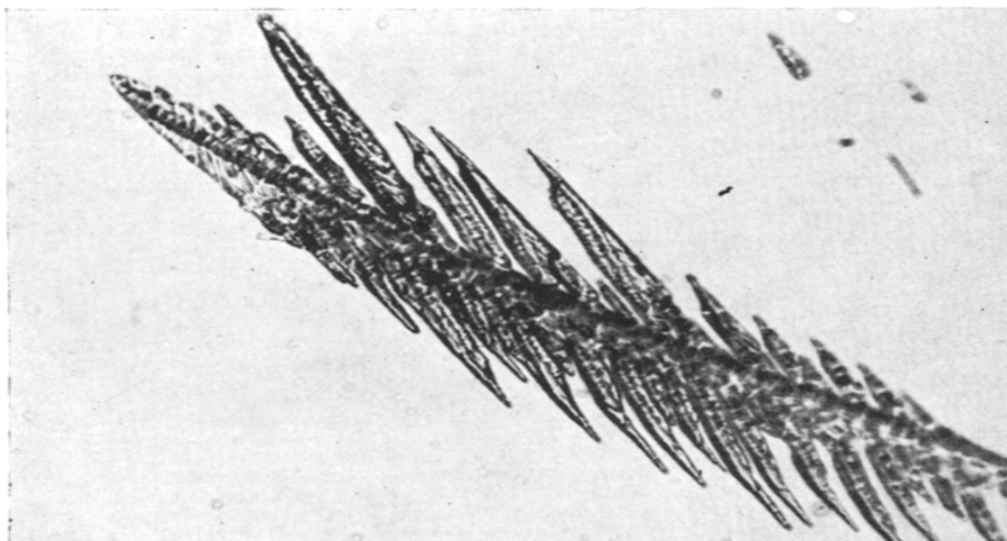


Fig. 5. Microcrystals of acenaphthene (actual size  $10^{-1}$ - $10^{-2}$  mm).

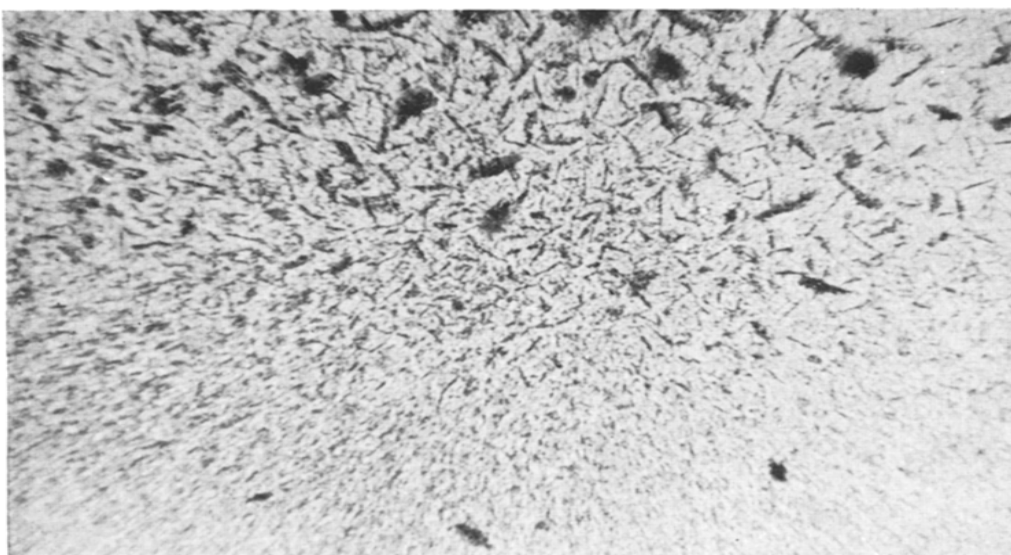


Fig. 4. Start line of the carbazole zone (magnified  $50\times$ ).

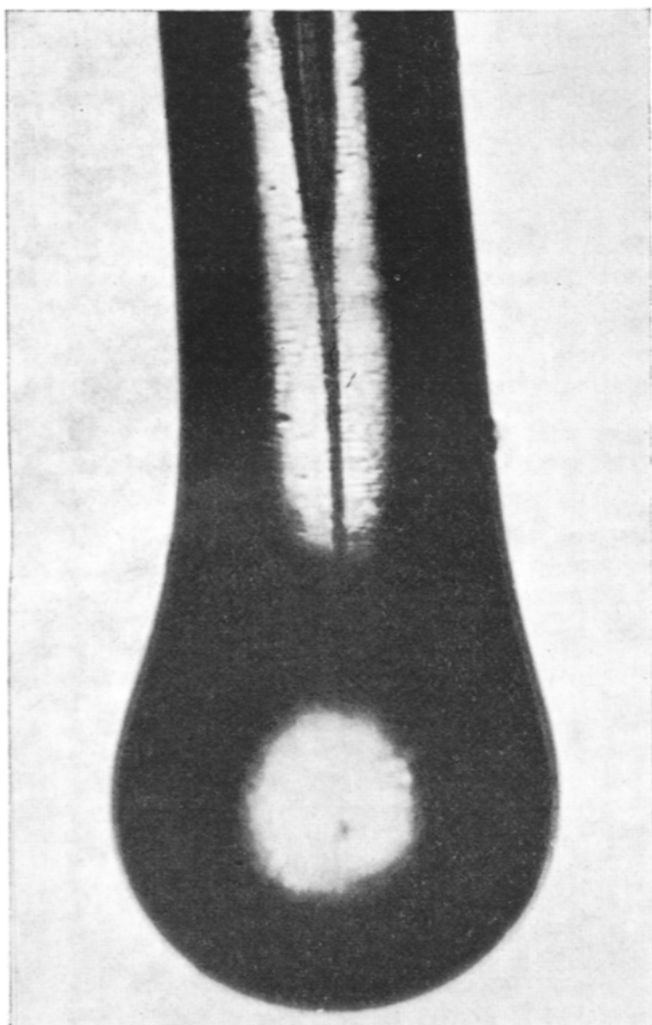


Fig. 7. Glass rod with ball for the transfer of microcrystals (thickness of the rod 0.10 mm, ball diameter 0.15 mm).

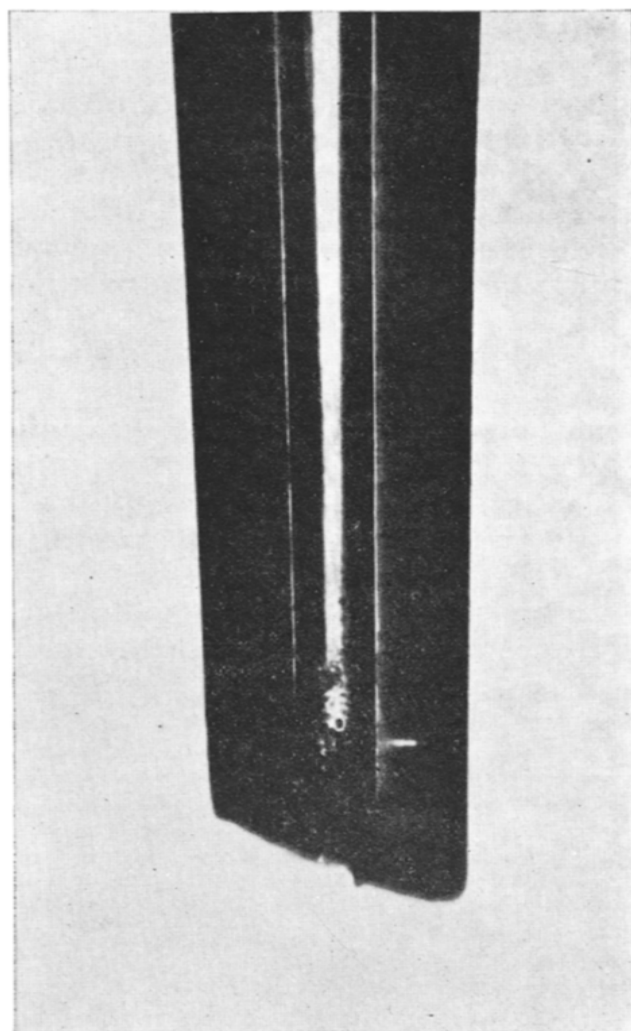


Fig. 8. Glass micropipette (internal diameter 0.07 mm, smooth tip with an angle of 45-60°).

sitions of gas-chromatographic fractions are shown. In Fig. 2 the start of the acenaphthene zone can be seen, beginning with characteristic needles. In Figs. 3 and 4 the edges of fluorene and carbazole zones are illustrated. The growth and increase of the thickness of the crystal layer and their different arrangement are apparent.

Further microscopic examination showed the crystal structure in detail. Fig. 5 shows a characteristic microcrystal of acenaphthene. It is usually a tree-like needle with oblique growth, angle almost exactly  $30^\circ$  and  $210^\circ$ . The actual size is  $10^{-1}$  to  $10^{-2}$  mm; the weight is of the order of  $10^{-2}$  micrograms. It is evident that its character is quite different from that of the fluorene crystals (Fig. 6), whose ill defined leaflets can be described on the basis of planes of symmetry as 001. By comparison carbazole crystals (see Fig. 9) also develop a tree-like structure, whose needles grow at an angle of  $85^\circ$  and  $265^\circ$  on the supporting axis.

In the case of a mixed zone, when overlapping of chromatographic zones occurs, sharply differentiated eutectics or mixtures of well developed crystals of pure substances are formed.

*Handling of crystals and colorimetric investigation*

Figs. 7 and 8 show the main devices for handling small quantities of substances. A glass rod (Fig. 7) 0.05–0.10 mm thick, ending in a small ball, maximum diameter 0.2 mm, serves to the transport of crystals weighing few tens of nanograms. On handling the rod is fastened in two fixed points, the motion of its end being controlled by means of a micrometer screw while being observed under a microscope with 100-fold magnification. For the transport of solvents and solutions a thin glass capillary (Fig. 8) with outer diameter of 0.1–0.2 mm and inner diameter of 0.07–0.15 mm has proved to be useful. It has to have a smooth tip, the best angle being 35° to 60°.

The use of the device and the method are shown in an example. A crystal weighing approximately  $10^{-2}$  micrograms with a volume of *ca.*  $10^{-5}$  mm<sup>3</sup> was placed near a grain of silica gel with a volume of *ca.*  $10^{-3}$  mm<sup>3</sup> by means of the rod (see Fig. 9). The crystal was dissolved by adding *ca.* 0.1 mm<sup>3</sup> benzene (*ca.* 5–10 mm of the benzene column inside the micropipette). The dissolution is carried out in the following way: the tip of the pipette is placed in contact with the silica gel grain near the crystal. A droplet of solvent wets the silica gel, spreads out, reaches the crystal and dissolves it. The benzene, together with the solution, is all spontaneously absorbed, due to capillary forces, by the silica gel grain. Evaporation of the benzene, taking 5–10 sec, leaves the dissolved matter of the crystal within the grain.

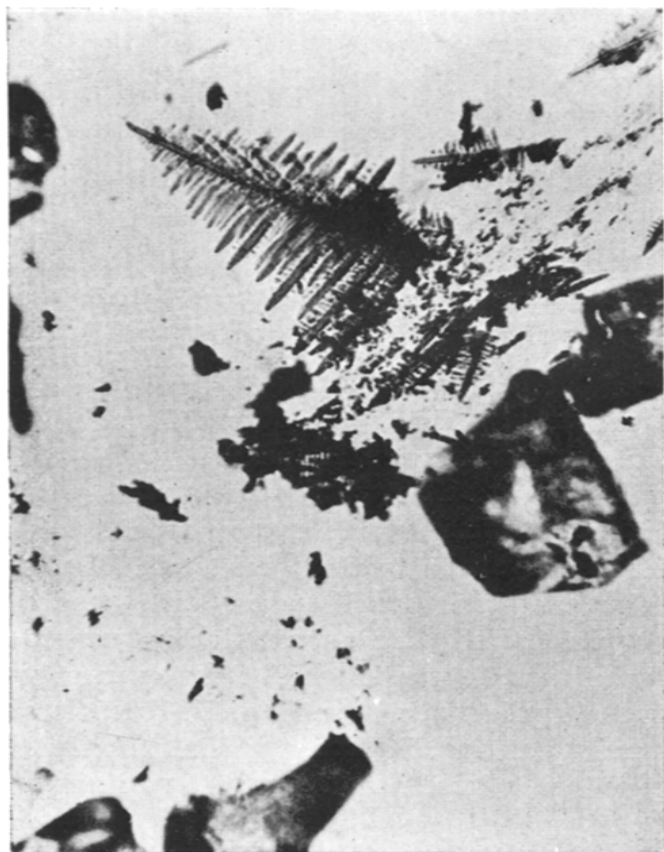


Fig. 9. Carbazole crystal transferred among the silica gel grains (the approximate diameter of a grain is 0.15 mm).



Fig. 10. Colour intensity representation of the carbazole-(TCE) complex in black and white ( $20 \cdot 10^{-9}$  g of carbazole).

TABLE I

COLOURS OF TETRACYANOETHYLENE COMPLEXES WITH SOME AROMATIC AND HETEROCYCLIC COMPOUNDS

<i>Compounds</i>	<i>Colour</i>
<i>Hydrocarbons</i>	
2,3,6-Trimethylnaphthalene	blue
1-Phenylnaphthalene	grey
2-Methylfluorene	blue-grey
3:4-Benzofluorene	green
9,10-Dihydroanthracene	orange
1,2,3,4-Tetrahydroanthracene	blue-violet
1:2-Benzanthracene	blue-green*
3-Methylphenanthrene	violet
3,6-Dimethylphenanthrene	violet
1:2-Benzopyrene	grey-green
3:4-Benzopyrene	grey-blue
1,2,3,4-Tetrahydrofluoranthene	blue-violet
Benzo-[ <i>m,n,o</i> ]-fluoranthene	violet
3:4-Benzofluoranthene	violet
Perylene	yellow
<i>O-compounds</i>	
Phenol	orange-brown
2-Methylphenol	brown
3-Methylphenol	brown
4-Methylphenol	violet
2,3-Dimethylphenol	brown
2,4-Dimethylphenol	violet-brown
2,5-Dimethylphenol	violet-brown
2,6-Dimethylphenol	brown
3,4-Dimethylphenol	blue-violet
3,5-Dimethylphenol	brown
2-Ethylphenol	brown
3-Ethylphenol	brown
4-Ethylphenol	violet
2-Methyl-4-ethylphenol	black-violet
2-Ethyl-4-methylphenol	blue-violet
3,4-Diethylphenol	brown
2-Ethyl-5- <i>n</i> -propylphenol	violet-brown
2-Ethyl-5- <i>n</i> -butylphenol	brown-violet
3- <i>n</i> -Propylphenol	red brown
3- <i>n</i> -Butylphenol	brown
2,2'-Dihydroxybiphenyl	red-brown
2-Methylbiphenylene oxide	brown
1,8-Dimethylbiphenylene oxide	red-violet
Braza	faint grey-violet
2-Hydroxybiphenylene oxide	blue-violet
2-Hydroxyfluorene	blue-green
4-Phenanthrol	red
<i>N-compounds</i>	
2,3-Dimethylcarbazole	sky blue
2-Methylacridine	yellow
4-Azafluorene	yellow
2-Azafluoranthene	yellow
1-Azapyrene	yellow
1-Azacarbazole	yellow
7-Azaindole	yellow
<i>S-compounds</i>	
6:7-Benzothionaphthene	blue-violet

\* Colour disappears within a few seconds.

The process presents the following interesting possibilities. The concentration of material within the grain is approximately  $1:10^2$ . The visibility limit of the most coloured complexes of aromatic hydrocarbons with tetracyanoethylene (TCE)<sup>1,4</sup> on a thin layer of silica gel is  $1-2 \mu\text{g}$  in an area of *ca.*  $120 \text{ mm}^2$  and a thickness of  $0.9 \text{ mm}$ , *i.e.* a volume of *ca.*  $100 \text{ mm}^3$ . The visibility limit in silica gel is therefore of the order of  $1:10^5$ . As the concentration of carbazole in the grain is of the order of  $1:10^2$ , it exceeds the visibility limit of this coloured complex by 3 orders.

If  $0.05-0.1 \text{ mm}^3$  of a benzene solution of tetracyanoethylene is added in the same way, an intense colour due to the formation of the complex within the grain is seen, even if a quantity of less than 20 nanograms is treated. In Fig. 10 a black and white photograph of a silica gel grain, from Fig. 9, containing the coloured complex (ultramarine blue) is shown.

A series of coloured pictures (Figs. 11-14) shows the colours obtained with nano- to picogram quantities of various materials (acridine, carbazole, 2-methylcarbazole, and phenanthrene) in silica gel. In Table I colours of the tetracyanoethylene complexes with a larger number of aromatic and heterocyclic substances which can be used in the investigation of the composition of tar fractions are listed. Table I completes the data given in a previous paper<sup>1</sup> for about 100 compounds.

It will be seen that classical colorimetric methods can be applied to the identification of substances in concentrations that can be only detected by ionization methods at present. It is shown that by means of this technique it is possible to investigate colorimetrically even gas chromatographic fractions obtained from a capillary chromatograph. This has not been considered possible up to now.

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#### SUMMARY

Many classical colorimetric and crystallographic methods can be applied to the identification of nano- to picogram quantities of compounds obtained after separation by gas chromatography, if the compounds are examined under a microscope.

Techniques of handling crystals are described. The method is useful for the investigation of capillary gas chromatographic effluents.

#### REFERENCES

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- <sup>4</sup> P. V. PEURYFOY, S. G. SLAYMAKER AND M. NAGER, *Anal. Chem.*, 31 (1959) 1740.



Fig. 11. Colour of the acridine-TCE complex ( $10^{-7}$  g of acridine).

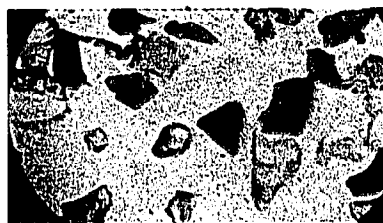


Fig. 12. Colour of the carbazole-TCE complex ( $10^{-9}$  g of carbazole).

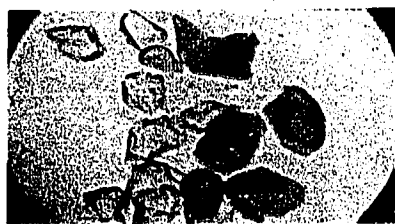


Fig. 13. Colour of the 2-methylcarbazole-TCE complex ( $10^{-9}$  g of methylcarbazole).



Fig. 14. Colour of the phenanthrene-TCE complex ( $50 \cdot 10^{-12}$  g of phenanthrene).

The coloured plates were printed by Grafia, ČSSR.