CHROM. 4439

COMBINATION OF PAPER OR THIN-LAYER CHROMATOGRAPHY WITH OTHER CHROMATOGRAPHIC PRINCIPLES

INTRODUCTION

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SUMMARY

Combination of planar (thin-layer and paper) and column (gas and liquid) chromatographic techniques is discussed in detail. Use of gas chromatography as a sampling technique for thin-layer and paper chromatography is shown in the results as a common principle of a new two-dimensional chromatographic development. Liquid (column) chromatography, on its new level, is discussed in this connection, too.

A critical search of adsorbents for thin-layer chromatography is given, and new sorbents are introduced. From some examples of R_F values of several model substances measured on thin layers of organic porous polymers like Porapak Q, R, S, and T as well as on thin layers of porous glass beads with chemically modified surface like Durapaks, the prospect of applying such materials in thin-layer chromatography is shown and discussed.

INTRODUCTION

Paper (PC) and thin-layer (TLC) chromatography are very suitable methods for the separation of complex mixtures of substances with different chemical structure. Their application is limited, however, since zone overlapping often takes place and the chromatograms can be only semiquantitatively evaluated. For this reason new approaches have been sought in the combination of PC and TLC with other methods.

In the case of PC and TLC we will speak about "planar" chromatographic techniques to differentiate those techniques which are effectuated by percolating a column packed with a suitable sorbent.

In planar chromatographic techniques, two-dimensional development is used in order to improve separation. After the development of a mixture of substances with one solvent in one direction of the plane and after evaporation of the solvent used, a second development is accomplished using another solvent in a perpendicular direction. During the experiment, one type of layer and two kinds of solvent of suitable chemical composition are used to obtain the best separation.

In "column" chromatographic techniques such as gas chromatography, liquid

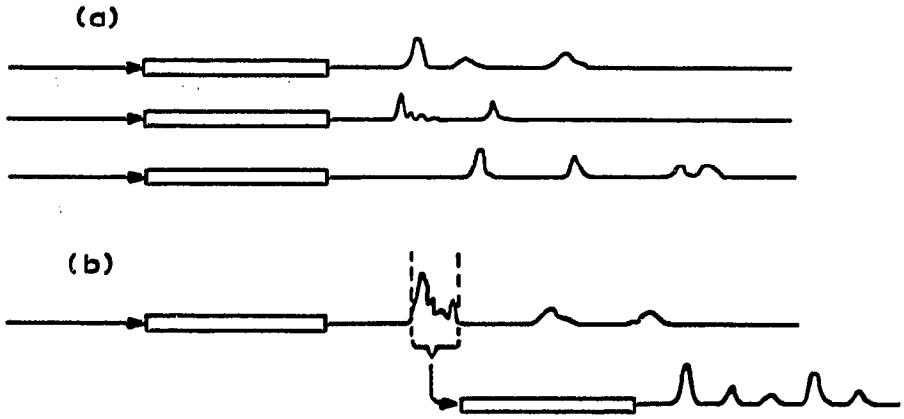

Fig. 1. Combination of GC columns, (a) in parallel; (b) in a series.

(column) chromatography, but also ion-exchange (column) chromatography or gel filtration, use is made of two or more stationary phases selectively retaining the different components of a mixture. Development is performed in one of the following ways (Fig. 1): (a) the whole mixture is chromatographed in two or more parallel columns packed with different sorbents and percolated with suitable solvents or gas; (b) the mixture is separated into simpler fractions which are subsequently introduced onto another column for further separation. The results obtained by the latter procedure are close to those obtained by two-dimensional chromatography.

The only chromatographic method which can be defined at the present stage as a quantitative analytical method is GC. This means that its precision is better than 1%. However, it is restricted to compounds with a volatility higher, for example, than 1 mm Hg at the temperature used. Present research in liquid (column) chromatography attempts to make this method applicable for quantitative analysis. Both principles will be discussed in some detail.

GAS CHROMATOGRAPHY

Recently, many combinations of planar and column techniques have been used in order to obtain a more complete separation of all components of a complex mixture (for a recent review see ref. 1). In most of the cases the mixture is subjected to preliminary separation by TLC followed by a complete separation of the pre-separated compounds of the mixture by GC[2] (Fig. 2). A more intricate method[3] has been described in which GC on one stationary phase is used with a subsequent separation of the simplified gas chromatographic cuts on paper or a thin layer, followed by gas chromatographic separation of the extracted thin layer cuts on a second stationary phase (Fig. 3).

The main advantage of the combination of TLC or PC with GC is based on quite different distribution factors which control the separation process. In gas chromatography, the separation is principally determined by the volatility of the substances. The use of a non-polar stationary phase makes this phenomenon most remarkable. The separation is realized in accordance with the dispersion forces so that the number of C atoms in the molecule of the substance under consideration or

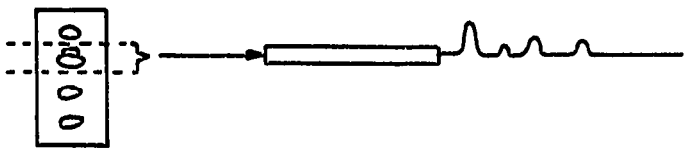

Fig. 2. Combination of TLC with subsequent GC.

the molecular weight plays the main role. When different polar stationary phases are used, certain changes in the distribution coefficient may take place due to deviations from Raoult's law. However, the temperature dependence of the orientation forces decreases the effectiveness of the selective retention considerably so that the volatility factor remains, with few exceptions, the most decisive contributor to the chromatographic retention of substances in gas chromatography.

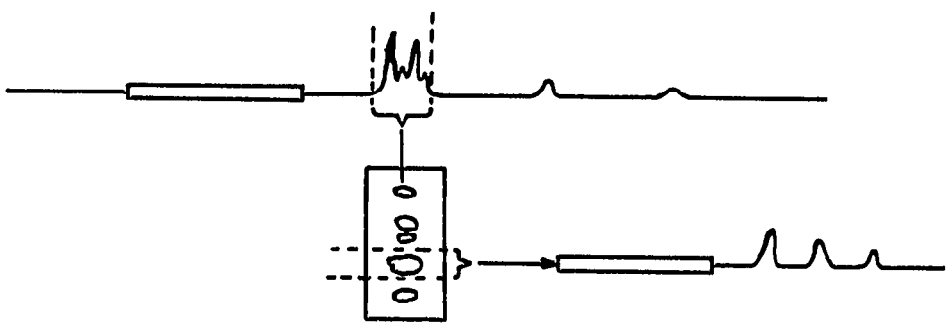

Fig. 3. Combination of GC with subsequent TLC of a GC-cut and followed by a second GC of a TLC-cut.

Using planar chromatographic techniques, however, polar adsorbents used at room temperature show a much higher effective polarity than do any of the known polar stationary phases in GC. Due to this fact the distribution coefficient is principally determined by the type of the functional groups and/or their steric shielding, while their volatility plays a less important role than in GC.

How to utilize those differences? There are two ways: TLC or PC before GC, and GC before TLC or PC. Since the first combination[2] has already been mentioned briefly, we shall direct our further attention to the second one[1], in which the gas chromatographic effluent is sampled directly on the starting line of a dry thin-layer or chromatographic paper (Fig. 4). The sampling can be effectuated in consecutive steps[4], *i.e.* one point for each gas chromatographic cut, or continuously[5]. In the case

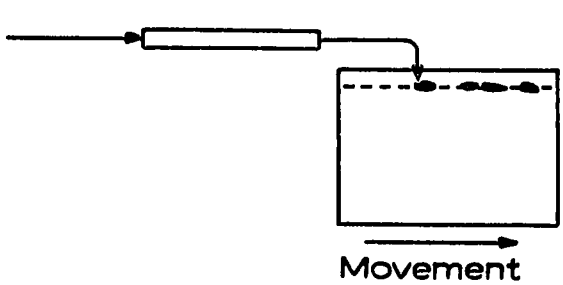

Fig. 4. Development of a gas chromatogram on the starting line of a thin layer.

of an uninterrupted sampling procedure, the thin layer or paper can be moved under the gas chromatographic column exit at a linear[5] or programmed, *e.g.* logarithmic[6], rate. After trapping of the gas chromatographically separated substances on the starting line of the layer or paper a second development can be carried out along the plane.

This new type of two-dimensional chromatography exploits to the maximum both separation extremes offered by the existing chromatographic methods, *viz.* separation according to dispersion forces (as mainly determined by the number of C atoms and/or molecular weight) by GC along the time axis coinciding with the start of the thin layer (paper) and separation according to orientation forces (determined mainly by the type of functional group) by thin-layer (paper) chromatography in the direction of the capillary flow of the solvent.

The potentiality of the above method for the identification of compounds can be seen on inspecting the two-dimensional chromatograms of some terpene compounds[4] (Fig. 5), steroids[7] (Fig. 6), fatty acids[8] (Fig. 7) and constituents of coffee flavors[9] (Fig. 8) phenols[10] or alkaloids[10a]. In Fig. 5 we can see the thin-layer separation of a single gas, chromatographic peak which contains many overlapping terpene compounds trapped

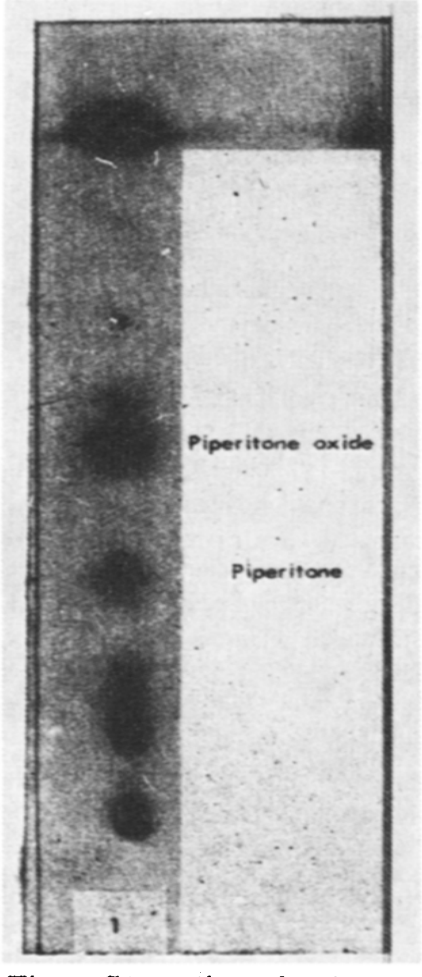

Fig. 5. Separation of a single GC-peak of a terpene mixture trapped at one point at the starting line of a thin layer. (For details see ref. 4.)

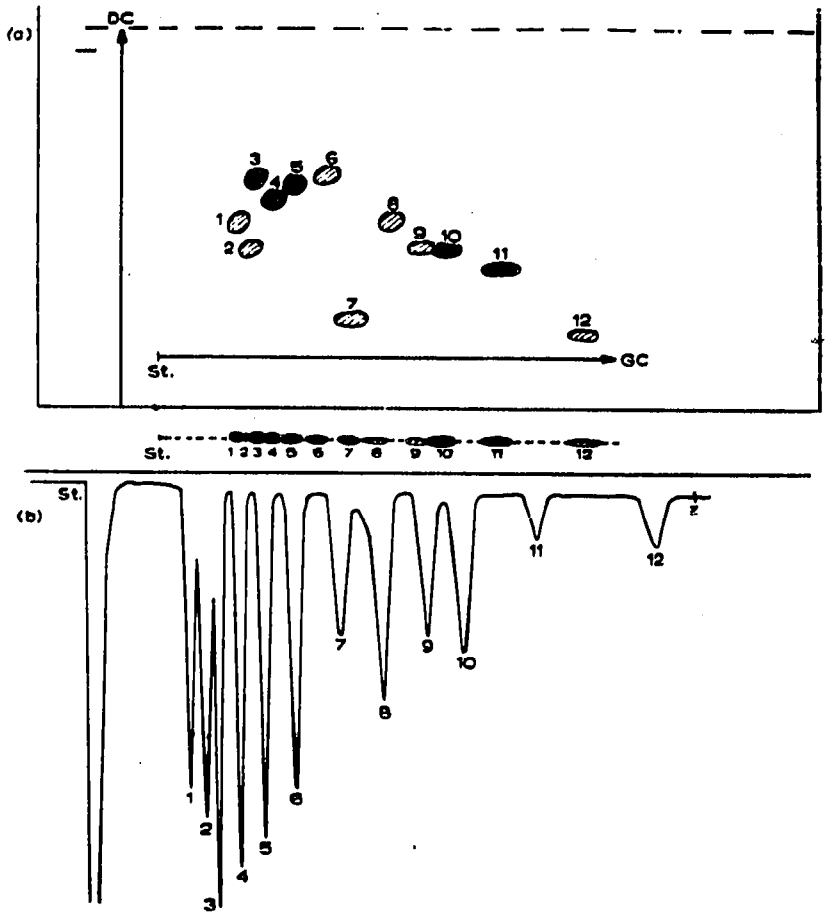

Fig. 6. GC–TLC two-dimensional chromatography of steroids with continuous linear movement of the starting line of the thin layer. (For details see ref. 8.)

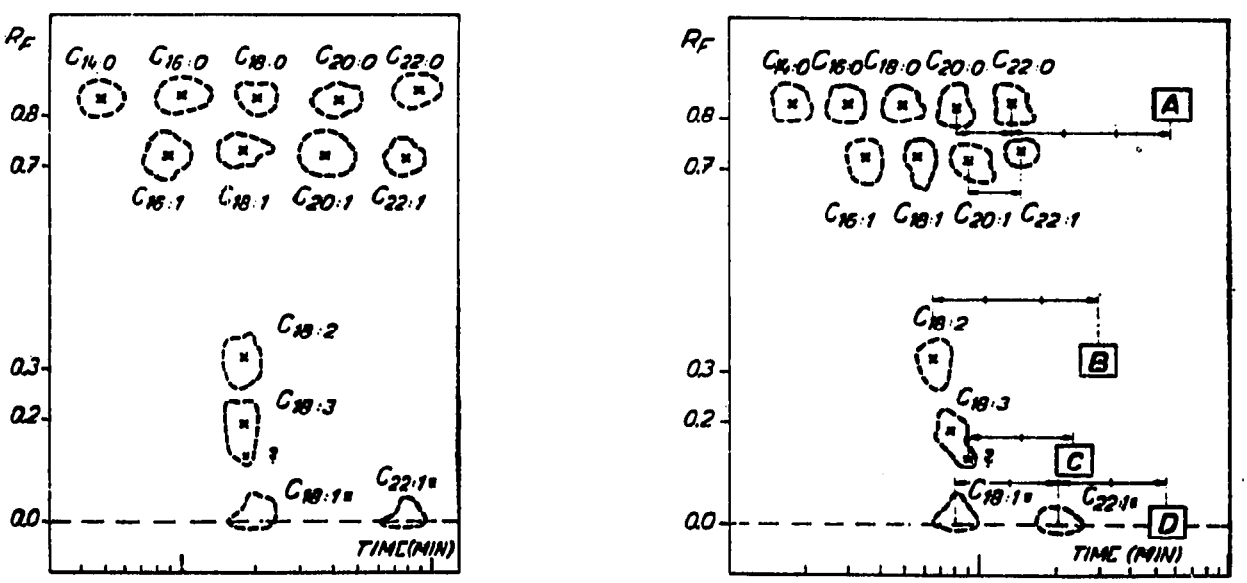

Fig. 7. GC–TLC two-dimensional chromatography of a complex mixture of fatty acid esters with logarithmic movement of the thin layer. (For details see ref. 7.)

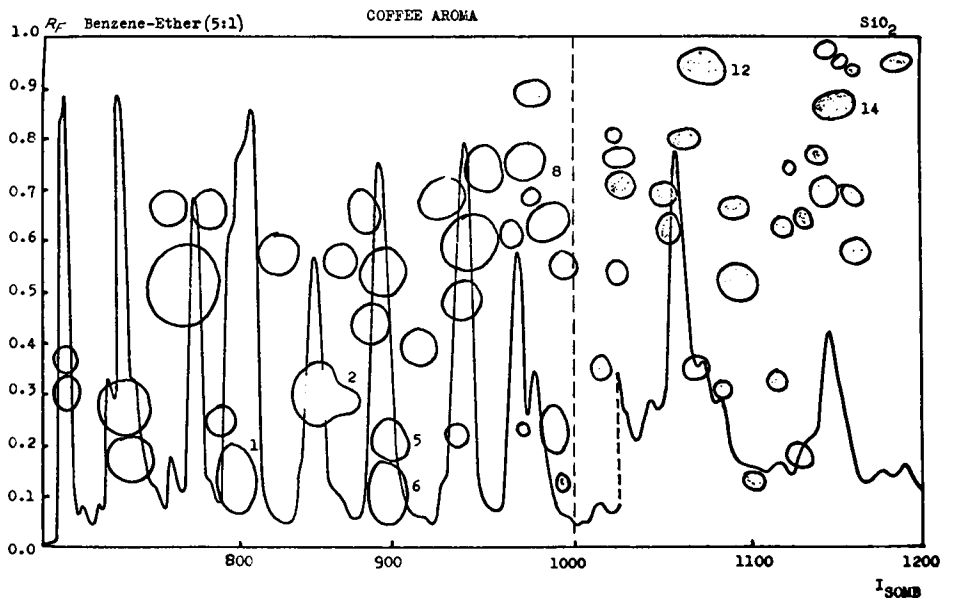

Fig. 8. GC–TLC two-dimensional chromatography of a complex mixture of coffee aroma constituents with logarithmic movement of the thin layer. The retention time is expressed in Kovats' indices. (For details see ref. 9.)

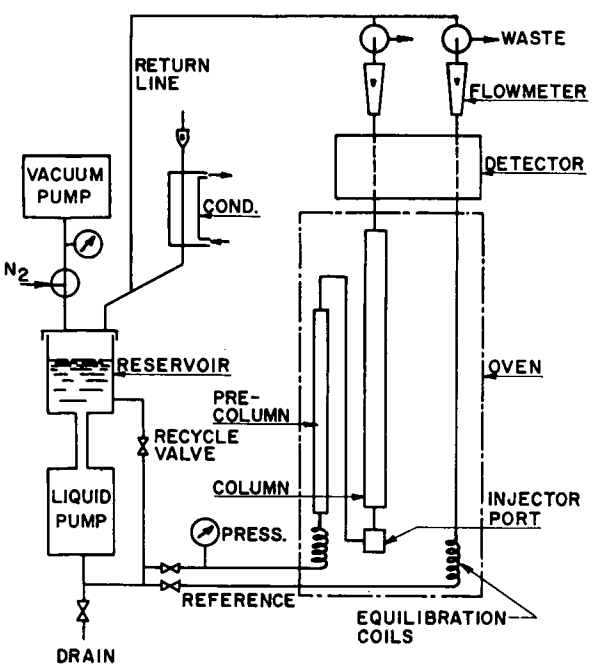

Fig. 9. Diagram of a new liquid (column) chromatograph.

at one point at the start of the layer. In the case of steroids (Fig. 6) exposition of the gas chromatogram is made by linear movement of the starting line of the thin layer. In the case of fatty acid methyl esters (Fig. 7), the gas chromatograms (the first from an Apiezon L column, the second from a Reoplex 400 column) were developed on the logarithmically moving thin layer covered with silica gel coated with silver nitrate. It must be emphasized that under these conditions the distances between the homologous members of the fatty acids are linear so that the place in which a fatty acid ester of expected structure should be placed can easily be found (Fig. 7). The last picture (Fig. 8) shows the case in which the gas chromatographic eluent is trapped on the logarithmically moving starting line of the layer, and the time axis is expressed directly in Kovats' indices.

LIQUID (COLUMN) CHROMATOGRAPHY

Although it is the oldest variant of chromatography, liquid chromatography was utilized for many years without great development, and we know now that it was used under experimental conditions which are far from optimum. It will be interesting to elucidate which key points were limiting the development: (i) The diffusion coefficients of most substances in the liquid phase are two to four orders of

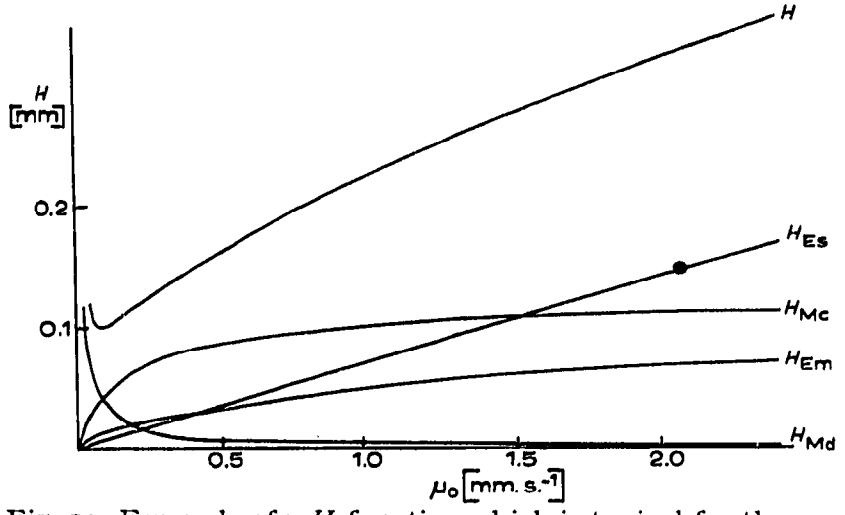

Fig. 10. Example of a H-function which is typical for the present state of liquid chromatography in the column.

magnitude lower than in the gaseous phase so that the size of a classical liquid column, the percolating velocity of the solvent and the particle diameter used up to date in liquid chromatography manifest themselves in a substantially great broadening of peaks, which means a low separation power of the column; (ii) the detection limits of common analytical reactions are much lower in liquids than in gases; due to this fact, greater samples must be applied on a classical column, which manifests itself in column overloading and again in loss of resolving power of the column.

However, the improvement in the theoretical treatment of GC is beneficial for variants of liquid chromatography too. Theoretical consideration and comparison of experiences in gas–liquid systems with those in liquid–liquid or liquid–solid systems[11–15]

give very positive results now. A new instrument for liquid column chromatography[16] (Fig. 9) is characterized by narrow columns packed with very fine particles of sorbent and by a detector with an extremely small dead volume[17]. The solvent is pressed through the column by a pressure of often over hundreds of atmospheres. The contributions to the height of a theoretical plate (as a measure of column performance) are expressed[11] (Fig. 10) in a way similar to the Van Deemter equation used in GC.

However, four independent transport mechanisms contribute to the value of the plate. The terms H_{Md} and H_{Mc} reflect the contributions of molecular diffusion, and mixing by convection, respectively. H_{Em} and H_{Es} are contributions of mass transfer phenomena in the mobile and stationary phases, resp. It is clear that the resolving power of a liquid column at a low percolating velocity is comparable with that in gas chromatography so that it is possible to reach a much better effectiveness than in the case of the "classical" liquid columns.

Practical chromatography in columns has been improved considerably with respect to time and precision. An example of the separation of some herbicides[18] (Fig. 11) shows how much the present strong position of TLC and PC in research as well as in control laboratories is threatened.

NEW ADSORBENTS

One of the greatest limitations of the separation power in TLC is the random character of the porosity of frequently used adsorbents, *e.g.* silica gel, alumina,

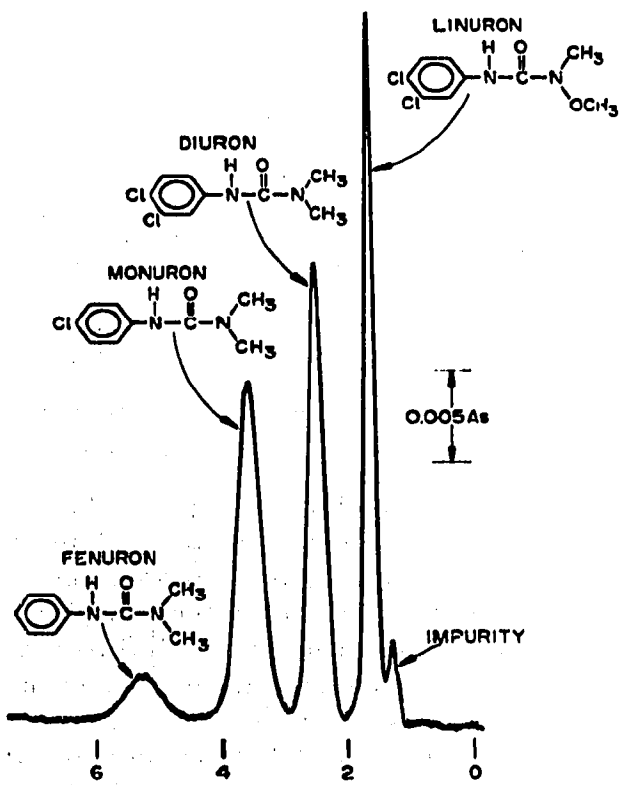

Fig. 11. Example of a rapid liquid chromatographic separation of some herbicides. (For details see ref. 18.)

TABLE I

CLASSIFICATION OF ADSORBENTS FREQUENTLY USED IN GC AND THEIR POSSIBLE USE IN TLC

Stationary phase	*Characterization of action in GC*	*Utilization in TLC*	*Note*
Liquid solvents	These solvents make possible the unrestricted three-dimensional sorption of solute molecules.	Frequently used	Solubility of stationary phase in solvents limits the utilization to mutually non-soluble liquids only.
Liquid crystal solvents[19]	The smectic region of such liquids has a two-dimensional parallelly layered lattice structure; discrimination of molecules on the basis of shape[20].	Probably not applicable	
Gel solvents	Intermolecular bonding forms temporarily existing pores of molecular size; discrimination of molecules on the basis of size.	Possible in certain regions between coagulation and swelling.	
Solids with random porosity	Pores of small diameter form a large surface area for adsorption.	Frequently used.	Since pores are homogeneous, the sorption isotherms tend to linearity in the region of concentration that is of interest for chromatography (see ref. 21).
Inorganic solids with constructed porosity	*Crystals*: lattice with geometrical homogeneous pores of molecular size (molecular sieves); discrimination of molecules on the basis of shape.	Possible, but only briefly mentioned[3].	Filling of pores with gases soluble in solvent used necessary.
	Solid melts: part of the solid is chemically removed, the rest of the lattice has regular pores of rather homogeneous diameter (porous glass).	Possible, but not used.	Commercially available as Porasils (Water Associates, Framingham, Mass.).
Inorganic solids with constructed organic surface	The inorganic crystal lattice is chemically modified. *Montmorillonites*[22]: OH groups of planar developed silicate lattice are substituted by alkylammonium salts; discrimination of molecules with different spacel oriented groups.	Used[23]	Commercially available as Bentones; swelling occurs.
	Substituted porous glass[24]: OH groups of silicate surface hydrolysed under the control (Porasil) are substituted by alkyl-, polyalkoxy-, and cyanoethoxy- groups; separation is based on sorption on a unimolecular layer.	Possible, now used[25].	Commercially available as Durapaks (Water Associates).
Organic polymers with constructed porosity	Styrene-ethylvinylbenzene copolymers cross-linked by divinylbenzene; by copolymerization of polar constituents (acrylonitrile, polyglycol etc.) the chemical structure can be varied.	Very promising; now used[25,26]	Commercially available as Porapaks (Water Associates).

TABLE II

R_F VALUES FOR SOME MODEL SUBSTANCES OF DIFFERENT CHEMICAL STRUCTURE ON PORAPAK Q, R, S, AND T LAYERS

Solvents: ethanol (Et), benzene (Bz) and cyclohexane ($\triangle C_6$).

		Porapak			
		Q	*R*	*S*	*T*
Fluorene	Et	0.31	0.52	0.47	0.48
	Bz	0.97	0.83	0.94	0.90
	$\triangle C_6$	0.48	0.60	0.79	0.37
Carbazole	Et	0.54	0.56	0.55	0.49
	Bz	0.90	0.41	0.79	0.51
	$\triangle C_6$	0.05	0.03	0.03	0.03
3,4,5-Trimethylphenol	Et	0.73	0.79	0.78	0.76
	Bz	0.88	0.17	0.45	0.29
	$\triangle C_6$	0.14	0.02	0.02	0.02

charcoal, etc. Improvements in separation power were made by using adsorbents of more uniform graining and pore distribution. One of the most fruitful effects resulting from the development of GC is the better knowledge of column support and packing problems, so that it is possible to construct columns with predicted properties. Let us compare the probable usefulness in TLC or PC of adsorbents classified according to the knowledge derived from GC (Table I).

Two examples (Tables II and III) show the possible usefulness in thin-layer, but also in column chromatography, of new adsorbents like Porapaks or Durapaks. Particularly organic porous polymers are of great interest[25]. The distribution coefficient seems to be linear for a very wide range of concentrations; the overloading effect begins with higher amounts of solute as compared with common adsorbents, and there

TABLE III

R_F VALUES FOR SOME MODEL SUBSTANCES OF DIFFERENT CHEMICAL STRUCTURE ON DURAPAK LAYERS

Solvents, same as in Table II.

		Porous glass with chemically bonded:		
		n-Decane	*Polyethylene glycol 400*	*Oxypropionitrile*
Fluorene	Et	0.88	0.77	0.83
	Bz	0.98	1.00	0.97
	ΔC_6	0.90	0.90	1.00
Carbazole	Et	0.92	0.83	0.84
	Bz	0.93	0.83	0.65
	ΔC_6	0.00	0.19	0.05
3,4,5-Trimethylphenol	Et	0.98	0.86	0.93
	Bz	0.95	0.77	0.86
	ΔC_6	0.30	0.21	0.10

is so little adsorption of polar solvents that the composition of the mobile phase undergoes only extremely small changes during capillary flow along the layer. Therefore such types of adsorbent are very suitable for a better theoretical approach to the chromatographic processes during TLC.

Porapak Q (Table II) represents a non-polar stationary phase which is insoluble in hydrocarbons or other non-polar solvents, *e.g.* tetrachloromethane. Therefore, a reversed retention sequence for a triad (3,4,5-trimethylphenol–carbazole–fluorene) has been found when applying ethanol as the percolating solvent (R_F values: 0.73, 0.54 and 0.31, resp.).

In the case of styrene copolymers (Porapak R, S, and T) we can see remarkable separation selectivities, which can be controlled very well by the choice of the correct Porapak and a solvent of suitable polarity (or functionality, see ref. 27). As a result of those preliminary experiments I wish to express the opinion that this kind of artificial solid, which can be constructed for any type of chromatographic separation, is very promising.

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DISCUSSION

PROCHÁZKA: As gas chromatographers are used to employing the expression "carrier gas", they are also beginning to call the mobile phase in liquid chromato-

graphy "carrier". However, paper and thin-layer chromatographers are used to the word carrier for the material holding the stationary phase or the impregnation medium, etc. Since the introduction of partition chromatography (in silica gel columns) by MARTIN AND SYNGE, this word has been used in this way. I propose to use the expression "eluant" for the mobile phase in liquid chromatography and the word "carrier" only for the support of the stationary phase.

JANÁK: I subscribe to your view that it would be unfortunate to call the mobile phase of liquid column chromatography a "carrier liquid". Incidentally, the sorbent to which the stationary liquid phase is attached is called support in gas chromatography.

HAIS: This discussion shows that it would be worthwhile unifying the nomenclature. Thus "carrier gas" means mobile phase in GC and the sorbent holding stationary liquid is called "carrier" in liquid column and mostly in flat bed partition chromatography but "support" in GC (sometimes in TLC as well), while the word "support" is also generally used for glass plates or plastic sheets on which thin layers, gels or papers are held in chromatography or electrophoresis.

TURINA: Which detector was used in this work and how much did the data obtained oscillate from the mean values?

JANÁK: The detector used for scanning of the chromatogram on Fig. 11 was a DuPont Model 410 precision photometer. This chromatogram was published by J. J. KIRKLAND, *J. Chromatog. Sci.*, 7 (1969) 12; for details it is necessary to consult the original source. As far as I know, the oscillation of the base line was negligible in comparison with the response.