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COMBINATION OF FLAT-BED CHROMATOGRAPHIC TECHNIQUES WITH MODERN COLUMN TECHNIQUES

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SUMMARY

A critical comparison of existing chromatographic techniques is given for the purpose of evaluating promising combinations for both qualitative and quantitative treatments of chromatograms. Developments in the main chromatographic techniques are considered in terms of the frequency of published papers, based on about 60,000 items.

"Flat-bed chromatographic techniques" is a collective term for separation methods that are characterized by developing the chromatogram in a planar mode. Typical examples are paper chromatography, thin-layer chromatography (TLC) and planar electrophoresis.

In this introduction, we discuss the combination of these techniques with column chromatographic techniques. Typical column techniques are gas chromatography (GC), liquid chromatography, gel permeation chromatography, counter-current distribution and column electrophoresis. In the context of a discussion of the present-day perspectives of column chromatography I understand the term "liquid chromatography" to mean recent rapidly developing, high-pressure, high-efficiency liquid chromatography[1] rather than the classical Tswett column chromatography. Similarly "counter-current distribution" implies its capillary mode[2] and "column electrophoresis" implies the procedure realized in capillaries, the so-called "displacement electrophoresis" or "isotachophoresis"[3]. Ion-exchange chromatography is not specifically mentioned, because from the point of view of its instrumentation it is covered very well by liquid chromatographic and gel permeation chromatographic techniques.

The potentiality of any combination of chromatographic techniques is a combination of the analytical characteristics of the individual chromatographic techniques (Table I).

The aim of this meeting is to discuss the problems of the quantitative aspects of flat-bed techniques. From the general data in Table I, it can be seen that all flat-bed techniques suffer from certain imperfections in this respect. The evaluation in Table I is meant in its analytical sense: three plus signs indicates a mean of better than 0.1%, two plus signs 1 % and one plus sign about 5 % relative, respectively. As a result of recent evaluations[4], the precision of flat-bed separations only exceptionally reaches 3 % relative; in most instances it is 5–7 % and sometimes greater than 10 % relative.

TABLE I

PARAMETRICAL EVALUATION OF PLANAR AND COLUMN CHROMATOGRAPHIC TECHNIQUES

Chromatography	*Time of separation*	*Resolving power*	*Quantification*	*Separation ability*		
				Volatile substances	*Non-volatile substances*	*High molecular weight substances*
Flat-bed techniques						
Paper	Long	Medium	+	No	Yes	Poor
Thin-layer	Short	Medium	+	No	Yes	Poor
Electrophoresis	Long	Medium	+	No	Yes[a]	Yes[a]
Column techniques						
Gas	Short	Extremely high	+++	Yes	No	No
Liquid	Short	High	++	Yes	Yes	Poor
Gel	Medium	Medium	++	No	Yes	Yes
Counter-current	Medium	Medium	++	Poor	Yes	Poor
Ion-exchange	Medium	Good	++	No	Yes[a]	No
Electrophoresis (capillary mode)	Medium	High	+++	No	Yes[a]	Yes[a](?)

[a] Ions or charged molecules.

Quantification is limited by the fact that all types of chromatographic papers as well as layers of adsorbents have a certain thickness of material, which is not completely transparent and homogeneous. Therefore, the precision of the measurement of light transmission and reflection cannot exceed certain limits, which unfortunately lie beyond the analytical precision of at least 1 % relative. Only the scanning of radioactive substances gives slightly better results.

There is a tendency to determine a substance by physical or chemical treatment of the plate while determining the content of substances outside the flat bed. An example of physical treatment is simple extraction, a procedure that is suitable for all flat-bed materials[5]. An example of chemical treatment is the pyrolysis of the substance on the layer followed by the determination of the pyrolytic products[6], *e.g.*, CO_2 and CH_4. This technique, because of the thermal treatment involved, is applicable only to layers of materials that are sufficiently stable to heat. Only inorganic sorbents can be used. In all instances, the precision achieved was not greater than 1–2 % relative because of a background noise signal.

The combination sequences must be selected according to the proposed application. There are several simple and also more complicated possibilities (Table II). In general, sequences in which the column technique is the last procedure are to be

TABLE II

COMBINATIONS OF CHROMATOGRAPHIC TECHNIQUES

Flat-bed → column
Column → flat-bed
Flat-bed → column → flat-bed
Column → flat-bed → column

preferred for quantitative analysis whereas any combination with a flat-bed technique as the second (end) step can be used for qualitative analysis[7]. The method of MANGOLD AND KAMMERECK[8] is recommended as one of the simplest examples of the first alternative. In their work, fatty acids were pre-separated by TLC and analyzed quantitatively by GC. Incidentally, it is necessary to emphasize that there is no problem in applying high-efficiency liquid chromatography in order to obtain very similar results. This arrangement has not been applied up to now with modern liquid chromatographs. The reverse sequence of the techniques is also possible and has already been applied[9]. Certain new possibilities can be achieved when depositing[10] the effluent of a column chromatogram on to the driven starting line of a flat bed (Fig. 1). This mode of sampling can be effected in consecutive steps (one spot beside another) for each fraction or for a particular fraction of the effluent, or continuously along the starting line of the layer or paper. In an uninterrupted sampling procedure, the flat bed can be moved at a linear or programmed, *e.g.* logarithmic, rate. The method is most commonly used in the direct combination of GC and TLC[11]. The method can be further modified by combining high-efficiency liquid chromatography with TLC.

Another potentiality of the flat-bed–column technique has been developed by VAN DIJK[12]. The sampling line is a circle around a disc covered with a chromatographic layer. The solvent does not flow from the centre to the edge, as is the case in circular-paper chromatography or TLC. The solvent flow is directed from the edge to the center of the disc so that the separated substance is collected, at a characteristic time, in the central outlet. Liquid chromatographic and GC detectors can be used for monitoring and quantification.

The flat-bed–column technique can increase the precision of analytical quantification, but the limit of about 0.1 % relative cannot be obtained, which will influence the practical application of flat-bed techniques. Three years ago[7], I was a little pessimistic about their use in laboratory work.

It is of interest to evaluate the chromatographic literature of about the last 10 years. In Fig. 2, the frequency of published papers is given from a set of about

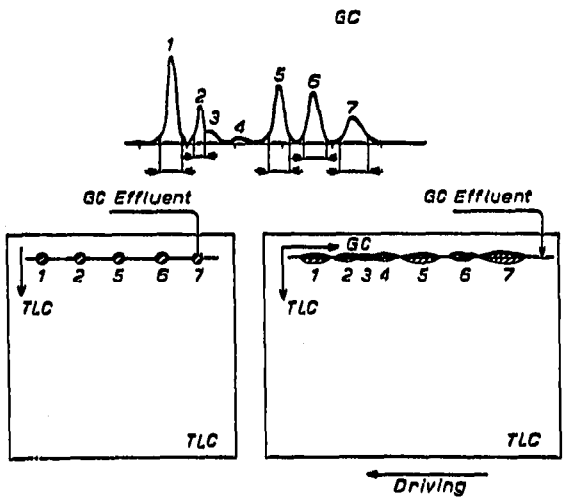

Fig. 1. Combination of column (GC) and flat-bed (TLC) techniques. Left: stepwise sampling; right: continuous sampling.

60,000 items. Although Fig. 2 reflects much more the situation in research activity rather than the actual situation in routine laboratory work, the statistics give an indication of the trends. The frequency of the papers published on paper chromatography is decreasing so rapidly that very little research activity is to be expected in 1975. This fact is caused by the replacement of this flat-bed technique by TLC, a technique that has for the same instrumental and parametrical values, an advantageous speed of analysis. However, in TLC also the maximum research activity has passed, having reached its maximum in about 1968/69. This fact does not mean that TLC is losing its practical value at present. Similarly the decrease in the number of published papers on GC does not imply a decrease in practical value. Both techniques are increasingly gaining in importance in practical routine work all over the world.

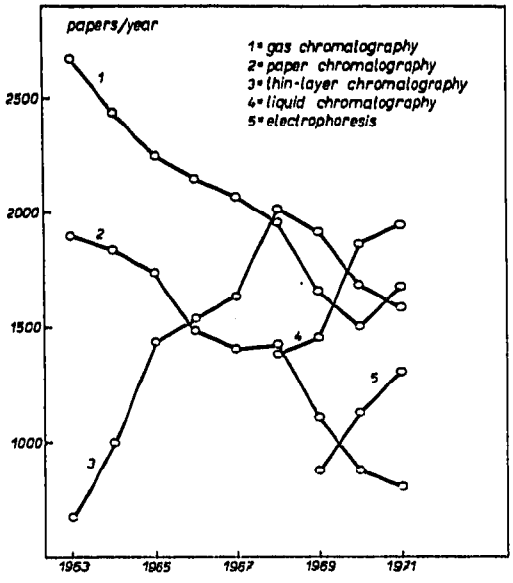

Fig. 2. Frequency of publication of papers in different branches of chromatography.

A growing interest in liquid chromatography and electrophoresis can be noticed. The replacement of TLC by liquid chromatography can be expected with certainty, as shown by the substantial differences in the parameters given in Table II. Nevertheless, the situation will not be the same as with the replacement of paper chromatography by TLC. A balance will be reached, given by the higher precision of the liquid chromatographic instruments that will be available in laboratory practice within several years on the one hand, and their high costs on the other hand, and by the lower precision of TLC and the cheapness of its instrumentation. It is true that the precision of TLC can be increased to a certain extent. However, it should be noted that the disadvantage of this improvement is that TLC will perhaps lose its simplicity and cheapness.

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DISCUSSION

GOODALL: I should like to comment on some of Dr. JANÁK's tables in so far as experience in the pharmaceutical industry applies. Here chromatography is extensively used for quality control and for assessing stability (shelf-life, etc., of formulated products). In the analytical laboratories at Macclesfield, flat-bed chromatography is used extensively for qualitative work, *e.g.*, identifications and limit tests; partition chromatography on columns is used for the assay of formulated products by UV monitoring of column effluent: when possible the partition column was being superseded by the methods of high pressure liquid chromatography, which are much faster and more precise. Quantitative flat-bed chromatography followed by optical or radioactive determinations is used when other methods are not satisfactory. Vapour-phase chromatography is used so extensively for volatile compounds or those that are convertible to volatile derivatives that the array of VPC's have been organised under the control of a "Datachrom" computer system, which also prints out the results.

HAIS: In addition to the factors tabulated in evaluating various chromatographic procedures, the following may also be considered.

(1) *Ease of automation*. Here the column methods are certainly more promising than the flat-bed techniques.

(2) *Efficiency, defined as number of analyses divided by either total or active working time*. Here the possibility of carrying out many mutually comparable parallel analyses is the advantage of flat-bed techniques.

(3) *Economy*. The number of analyses divided by expenses on equipment and wages.

The increase in the use of electrophoresis during recent years surprises me. Let us consider the succession of advances, beginning with the classical moving-boundary "free" electrophoresis methods, which were later supplemented with zonal methods involving the use of paper, gels, various powders or density gradients as anti-convection media as well as immunoelectrophoresis and the two-dimensional combination of chromatography with electrophoresis, all of which took place prior to recent years. One wonders whether there has not been a systematic error in the collection of data on which your statistics have been based. Has the biological and medical litera-

ture not been neglected, so that a breakthrough of electrophoresis in non-biochemical analysis would be overemphasized?

DEYL: Dr. JANÁK's conclusions are correct. There has been a real increase in the number of papers dealing with electrophoresis in recent years, accounting for an increase of almost 100 % every year. However, the increase is due only to the separations of hydrophilic substances, mainly proteins and polynucleotides. Another source of increasing frequency of papers dealing with electrophoresis is the fact that SDS/polyacrylamide gel electrophoresis replaces more complex methods of molecular weight determination. If a new series of substances of known molecular weight is used for calibration, these papers cannot be disregarded as simply routine applications.