Journal of Chromatography, 78 (1973) 1–3
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 6459

QUANTITATIVE FLAT-BED CHROMATOGRAPHY

K. MACEK
3rd Medical Department, Charles University, Prague (Czechoslovakia)

Despite the fact that the major importance of both paper and thin-layer chromatography lies in their qualitative aspects, since the first discovery of these methods, virtually every evaluation of chromatograms has been concerned with the semi-quantitative determination of the compounds present in the mixture being analyzed. The exploitation of the relationship between the concentration of a compound and the size of the spot or the intensity of the colour of spots or zones appeared to be very attractive mainly because of the simplicity of the procedure. The first publications in the field of paper chromatography appeared in 1948. These papers dealt with procedures involving the use of retention analysis (WIELAND[1]), the comparison of the size of the spot or the intensity of the colour of an unknown spot with a series of standards (FISHER and co-workers[2,3]), or methods involving the objective measurement of the sizes of spots and the intensity of colours, *e.g.*, densitometry (BLOCK[4]). Significant progress in the latter technique followed closely the increase of applications of paper electrophoresis in laboratories of clinical chemistry. Along with these *in situ* methods another direction was followed, namely quantitative analysis after the elution of the components of spots from paper chromatograms. In this case, paper chromatography served as the separation procedure and the determination was carried out by routine techniques of quantitative analysis.

At a very early stage of development, the biological effects of some substances were also used for quantitation and the methods of assaying radioactively labelled compounds became more popular. The techniques of quantitative analysis in thin-layer chromatography were analogous and used the experience gained earlier in paper chromatographic studies. At first, methods to be applied after elution were more popular, as they were more simple and faster than the analogous techniques in paper chromatography. The commercial availability of ready-made layers and foils has catalysed the rapid development of *in situ* procedures during recent years.

Much attention has been paid to quantitative analysis in flat-bed techniques during the last few years, because the procedures that had been used previously were less accurate than those used in column chromatographic techniques. Many studies were devoted to the theoretical basis of quantitative *in situ* analysis. In studies of techniques, most effort was directed towards obtaining more precise results, towards better reproducibility and towards increased sensitivity of these procedures. The accuracy of these methods was considerably improved by new commercially available apparatus for the measurement of colour or fluorescence intensity. Of similar importance was the development of devices for applying very small amounts of sample at the origin of the chromatogram. The accuracy of analysis was increased in parallel with the increase in knowledge of bed quality and with the possibility of using numerous commercially available products. One of the most delicate steps in flat-bed techniques is the application of detection reagents. With

some reagents (mainly with charring agents), this problem is avoided by adding the particular substance directly into the stationary phase. Only limited attention has been paid to automation and to the computerization of results, which reflects the poor applicability of these techniques in flat-bed chromatography.

In order to achieve increased sensitivity, several different approaches were investigated: the chromatography of fluorescent derivatives, the application of detectors used in gas chromatography, and combinations of flat-bed techniques with radioactive labelling or biological methods. More and more frequently it has been possible to combine flat-bed techniques with column chromatographic techniques. In this combination, flat-bed chromatography serves mainly as the separation step while gas chromatography, for example, is the final step that also involves quantitation. The appearance of these procedures is the answer to the general needs of biochemistry and especially clinical biochemistry, which are the fields that require the analysis of compounds in the nanogram and sometimes in the picogram range. Very good examples of this requirement are the procedures that are used in steroid hormone analysis.

I have to disagree with the optimism of some investigators as I believe that when making the choice of the most appropriate analytical procedure to use, it is necessary to accept the fact that flat-bed techniques are less accurate than column chromatographic techniques. The relative standard deviation in techniques that have been carried out perfectly is in the range 2–4 %. On the other hand, however, this accuracy is quite sufficient for many problems and the choice of an appropriate method may be considerably influenced by the fact that the cost of apparatus for paper or thin-layer chromatography is two or three orders lower than that for column chromatographic procedures.

During our third meeting in 1967 in Liblice, there was only one section that was devoted to quantitative analysis. At that meeting, the special problem of reproducibility was discussed. During the present meeting we would like to discuss all possible aspects of quantitative analysis in paper and thin-layer chromatography. Our experience of the previous symposia made us limit the total number of lectures, so that we expect to have enough time for both plenary and private discussions.

In conclusion, I wish to express my thanks to those scientists who accepted our invitation to present the introductory lectures of the individual sections: Drs. GOLDMAN, GOODALL, JANÁK, JORK, MENDOZA and SNYDER. I also would like to express our gratitude to the various Companies and Institutions for their financial aid in organizing this meeting: to the Kavalier, Macherey-Nagel, Merck and Serva companies. And, of course, I would like to thank all of my collaborators who participated in the actual organization of this Symposium.

DISCUSSION

HARA: One wonders why the term "flat-bed chromatography" is coming to the fore in Europe. In Japanese, a term is used which I would translate as plate, planar or flat chromatography.

HAIS: We have adopted the term already introduced (I could not say by whom or when) for chromatographic techniques in which the mobile phase flows through porous material arranged in such a way that two dimensions prevail, one

of which is in the direction of flow. This excludes columns, capillaries and threads. We leave it to our English-speaking colleagues to decide whether the term flat-bed chromatography should be retained or changed. It is obvious, however, that *some* term covering both paper and (thin)-layer chromatography is needed and that its use is likely to grow steadily, because paper and thin-layer chromatography have much in common and sometimes even cannot be properly distinguished, such as in the case of materials containing both fibres (cellulose, glass...) and powders, or in the case of foils. Of course, by "bed" we do not mean a piece of furniture used to sleep on but rather something like a river-bed.

REFERENCES

1 T. Wieland, *Angew. Chem.*, 60 (1948) 313.
2 F. Fisher, D. S. Parsons and G. A. Morrison, *Nature (London)*, 161 (1948) 764.
3 R. B. Fisher and R. Holmes, *Biochem. J.*, 44 (1949) LIV.
4 R. J. Block, *Proc. Soc. Exp. Biol. Med.*, 72 (1949) 337.