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INTRODUCTION

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Originally I prepared a very comprehensive lecture as an introduction to this Symposium, but then I saw that most of the topics would be repeated in the introductions to the individual sections. Therefore I have decided to limit this lecture to a general introduction only and to propose the limits for our discussion.

The identification of compounds is one of the most common tasks connected with paper and thin-layer chromatography apart from the estimation of their purity. Though identification of a compound by means of chromatography in a flat bed seems at first an obvious method, there are still many problems and also many possibilities which up to now have not been fully exploited[1]. That is one of the reasons why we decided to choose this topic for our 4th Symposium. I should like to emphasize at the beginning that we shall speak mainly about identification even if in some sections an overlap with structure investigations cannot be avoided.

The analytical methods most frequently used for the identification of organic compounds can be divided into two groups: (1) methods yielding knowledge about the properties of a molecule as a whole (according to BUSH[2] exclusive information); and (2) methods yielding a partial knowledge about the structure of a molecule. The first group of methods predominantly serve for the rapid identification of a known compound, *e.g.*, the determination of the melting point and also, to a great extent, infrared spectroscopy and paper and thin-layer chromatography. The second group of methods[3] (*e.g.*, elementary analysis, the estimation of functional groups, most of the spectroscopic methods, but sometimes also paper chromatography and thin-layer chromatography) are chiefly used for the structural determination of newly prepared compounds or compounds not expected to occur in the material to be analyzed. Their use for the identification of compounds is often very time-consuming and very expensive, even if sometimes their application is necessary to support the identification.

The main criterion for obtaining any particular information about the compound to be analyzed by the use of chromatographic procedures is the mobility of the compound in a system consisting of two phases, one stationary and one mobile. Paper and thin-layer chromatography belong to that type of chromatographic system in which the stationary phase is arranged as a flat bed. The difference between the two methods is not in the nature of the stationary phase but in the form of its immobility. In paper chromatography the stationary phase is matted into the form of a sheet, whereas in thin-layer chromatography the powdered stationary phase is fixed on an inert support.

The mobility of a compound is determined by the character of forces which influence it in the chosen solvent system. Of these forces ion–ion attraction, the

ion–dipole and dipole–dipole interaction, hydrogen bridging, coordination forces, chelate formation, dispersion forces, etc., should be mentioned.

For the positive identification of a compound the method of expression of its mobility in a chromatographic system is of great importance. The mobility of a compound is frequently expressed by relative R_F or R_X values (see *e.g.* refs. 4, 5) in paper and thin-layer chromatography. As with other chromatographic procedures, the expression of the mobility in paper and thin-layer chromatography poses some problems because there is no value which takes into account all the variables. One of the problems is in the flat bed arrangement itself, which makes it difficult to reproduce the formation of gradients and changes in the composition of the stationary and mobile phases. However, the advantage of methods using a flat bed arrangement lies in the fact that it is not necessary, for most identification procedures, to determine the absolute mobility because the reference compounds can be run on the same chromatogram. All the compounds are then run under the same working conditions and, in cases of equal mobility, the compounds can be considered as identical. The degree of probability of identification is determined by the choice of experimental conditions, by the position on the R_F scale and by the nature of the sample to be analyzed. Even work under strictly reproducible conditions is not always necessary for some purposes. The R_F value then loses the character of a constant and can only be considered as a value expressing the approximate mobility in the solvent system used or as a value that indicates the relative mobility of several substances on a particular chromatogram.

But some cases exist where it is necessary to treat the R_F value as a constant. These are mostly where a large number of R_F values are recorded, *e.g.*, by means of punched cards or tape systems in procedures of so-called systematic analysis, and, furthermore, it is important in experiments dealing with structural studies in which the R_F value or a constant derived from it is split up into partial values. The first section will be devoted to these problems.

As has already been stated, the identification of a compound based on its mobility in one solvent system is only a probable one, as with all types of identification procedure based on the properties of the entire molecule. If some further, usually partial, information is added to this basic information, then the degree of probability of the identification is much higher and even can approach certainty on the basis of the chromatographic procedures. The next sections will be devoted to these possibilities.

Every chromatographic analysis in a flat bed brings, in addition to the basic information, at least one piece of partial information and this is the result of a detection reaction. In paper and thin-layer chromatography mostly colorless compounds are chromatographed so that their detection on the chromatogram is inevitable. For this purpose one can use either physical or chemical methods. Amongst the physical methods detection by short-wave or long-wave UV light is most popular. In the case of chemical methods, reagents which will give a colored reaction product with the compound under analysis are used. Such reactions can be chosen so that they are selective for a particular structure or functional group or are even specific. Here again it is advisable to perform the detection reaction not only with the substance to be analyzed but also with the reference compound. If the reaction is not identical then the compounds are different or the detection of the compound under

analysis has been influenced by the unresolved substances accompanying the sample. In such a case, which is not rare in the analysis of biological materials, it may be necessary to perform a purification operation prior to chromatography or to use two-dimensional development. It is also possible to add the expected standard compound to the sample and to perform the detection reaction with the mixed sample.

Earlier on I mentioned some interactions between the compound to be chromatographed and the components of both phases. It is possible, of course, by choice of experimental conditions to enhance some of these interactions or to suppress others and so substantially change the mobility of the compound in a chromatographic system. From such changes in mobility it is possible to deduce some information about certain functional groups or, in other words, to obtain some partial information about the structure of the compound[6].

Another possibility is demonstrated by the reverse case: *i.e.* by maintaining a constant solvent system and changing the structure of the compound to be analyzed. Such a change is generally performed before spotting, on the origin of the chromatogram or between two developments in two-dimensional chromatography. Here MARTIN's relationship[7] that every change in the character of the functional group or in the number of such groups results in a change of mobility, which is determined in an ideal case only by this change, is applied; in practice the degree of change is influenced by interactions with other functional groups. If this mobility is expressed by R_M values then the difference in the R_M values, the so-called ΔR_M values, before and after the reaction will be the measure of the chemical change. BUSH[2] called this difference the ΔR_{Mr} value. The size of this value will depend on the character of the functional groups and also on their number. The presence of other functional groups in the molecule can of course sometimes influence the mobility.

Another interesting procedure is the so-called diagonal chromatography[8,9]. In this case the compound is subjected to two-dimensional chromatography in the same solvent system, but after the first run the compound is subjected to some chemical, physico-chemical or biological operation directly on the chromatogram. If no reaction occurs then the compound should lie, after the second development, on the diagonal. Any deviation from this diagonal is an indication of the presence of a group which is capable of undergoing a reaction.

A further section will be devoted to the decomposition products of the compound being analyzed. Here the characterization of compounds with a complicated structure should be mentioned primarily, where chromatography following the decomposition procedure does not need to identify the decomposition products but only results in a fingerprint picture[10]. Among such methods pyrolysis[11] and partial hydrolysis in the case of high-molecular compounds should be especially mentioned. In the case of proteins the fingerprint is achieved by the combination of chromatography with electrophoresis. Some other procedures, on the other hand, identify the decomposition products[3]. Thus it is possible *e.g.* to determine the basic skeleton after catalytic hydrogenation or to determine some functional groups, such as O-alkyl or N-alkyl. There is a very extensive group of papers devoted to structural determination methods in the field of high-molecular compounds, such as proteins, polysaccharides, lipids, nucleic acids, etc., but as these papers deal mostly with structural estimation there will be no time to discuss them here.

It is well known that, especially for the identification of a compound in rather

complicated mixtures, it is very important to apply several different criteria. For a long time, liquid column chromatography combined with paper or thin-layer chromatography has been used, the column chromatography being used for preseparation and the paper or thin-layer chromatography for sharper separation and identification. In recent years preference has more often been given to gas chromatography combined with thin-layer chromatography, where the thin-layer chromatography is used for the preseparation and the gas chromatography for further separation or quantitative analysis; it is also possible to reverse the order of procedure. Such combinations showed some advantage as they make use of various interactions between the compounds to be analyzed and the system of phases (*e.g.* ref. 12).

In the last few years the combination of paper and thin-layer chromatography with other physico-chemical methods has become very popular. It is used equally for identification and some structural studies. In these cases, paper and thin-layer chromatography serve as a micropreparative procedure for further spectroscopic analysis (*e.g.* refs. 13–15). By comparison with other preparative procedures, paper and thin-layer chromatography also give a value which can also serve as a criterion for supporting the results obtained.

In the last section procedures for the identification of inorganic compounds will be discussed; these are treated separately because some problems in this field are quite different from those for organic compounds.

An equilibrium state between phases is required for chromatographic procedures and if we compare the problems in our program which should be discussed and the time available, there could be the danger of reaching non-equilibrium conditions. Such a condition is not sound chromatography practice and I should like to ask you to help us to maintain our equilibrium conditions as far as possible and to discuss only the basic and important problems. I am convinced that our Symposium will elucidate some problems connected with identification and could also give us some new ideas for further development in this field.

REFERENCES

1 K. MACEK, *Rev. Cienc. Ind. Farm*, 1 (1969) No. 2.
2 I. E. BUSH, *The Chromatography of Steroids*, Pergamon Press, Oxford, 1961.
3 J. GASPARIČ, *Advan. Chromatog.*, 6 (1968) 3.
4 I. M. HAIS AND K. MACEK (Editors), *Paper Chromatography*, Academic Press, London, 1963.
5 E. STAHL (Editor), *Dünnschicht-Chromatographie*, Springer, Berlin, 1967.
6 I. M. HAIS AND K. MACEK (Editors), *Some General Problems of Paper Chromatography*, Publ. House of Czechoslov. Acad. Sci., Prague, 1962.
7 A. J. P. MARTIN, *Biochem. Soc. Symp. (Cambridge Engl.)*, 3 (1948) 4.
8 S. SVOBODOVÁ, I. M. HAIS AND J. V. KOŠTÍŘ, *Chem. Listy*, 47 (1953) 205.
9 O. MIKEŠ AND V. HOLEYŠOVSKÝ, *Chem. Listy*, 51 (1957) 1497.
10 V. M. INGRAM, *Biochim. Biophys. Acta*, 28 (1958) 539.
11 R. N. ROGERS, *Anal. Chem.*, 39 (1967) 730.
12 J. JANÁK, *J. Chromatog.*, 15 (1964) 15.
13 D. DOSKOČILOVÁ, *Chem. Listy*, 55 (1961) 193.
14 N. NASH, P. ALLEN, A. BEVENUE AND H. BECKMAN, *J. Chromatog.*, 12 (1963) 421.
15 K. HEYNS AND H. F. GRUETZMACHER, *Angew. Chem.*, 74 (1962) 387.