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IDENTIFICATION BASED ON MOBILITY

INTRODUCTION

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Suspended like a sword of Damocles above our heads in our chromatography laboratory is a text from a book review written by STEWARD[1] in 1958; it runs as follows: "...while chromatography on paper (...today we may also add thin layers...) has been one of the most powerful of new analytical tools, it has also been said that it is one of the most rapid known ways of reaching the wrong conclusion!"

I believe that this statement still holds true today—for example, in cases where the interpretation placed on insufficient chromatographic data is too sweeping or based on too much wishful thinking. By itself, a single R_F value does not, at first, provide much more information than, for instance, a melting point. It reflects the various energy contributions of the specific interactions between solute, solvent, and carrier. However, if several chromatographically different systems are used in combination, the R_F values obtained do, in fact, guarantee relatively reliable results in cases where identification has to be based solely on mobility.

The reliability of the identification, of course, is much greater if additional chemical and physical properties can be established along with the mobility characteristics. We shall be hearing more about this aspect of the problem later on in the course of our Symposium. At the moment, however, we are concerned with the characteristics of mobility, and with the reasons that prompt us to study the parameters involved. In this introduction of mine I shall try briefly to fix our terms of reference because otherwise we would be likely to spend two days simply defining the symbols employed hitherto to designate theoretical approaches.

In the early 1940s, as you know, MARTIN AND SYNGE[2] achieved a major breakthrough in this extremely confusing and complex domain by developing their theoretical plate model of chromatography. At that time, chromatographic separation—performed by means of partition chromatography—was described quantitatively by reference to two independent experimental parameters: (1) the equilibrium distribution of a solute between bed and solvent, and (2) the number of equivalent theoretical plates in the bed. The MARTIN AND SYNGE model—which, incidentally, facilitated the advent of paper chromatography (PC)—led in the years that followed to the development of quantitative methods of expressing mobility or migration in liquid–liquid partition, ion-exchange, and gas–liquid chromatography. To quote only one example, I would remind you of the method devised by BATE-SMITH AND WESTALL[3] for deriving the R_M value in paper partition chromatography. These theories attempt to correlate mobility in a given system with the sum of the free energies of transition of the constituent groups of a substance from one phase to the other.

The MARTIN AND SYNGE model seemed to be inapplicable to adsorption chro-

matography since the adsorption isotherms in the systems employed at that time were generally non-linear. On the one hand, therefore, an endeavour was made to develop a theoretical basis for a non-linear form of adsorption chromatography—that is, for a form which we today consider to be rather bad; on the other hand, this exploration of theoretical aspects gave rise to improved experimental methods.

Finally, thin-layer chromatography (TLC), which owes its wide propagation to STAHL, signalled a major advance as regards the practical application of chromatography and also fulfilled certain theoretical prerequisites inasmuch as TLC separations normally display linear adsorption isotherms. The alleged superiority of TLC, by the way, is due in large measure simply to the fact that column separations are—or were—usually carried out under conditions which are anything but optimal. The realisation of this fact led, in turn, to improvements in the conditions for adsorption chromatography—that is, to the use of columns with an efficiency at least equal, if not superior, to that of TLC (high-pressure liquid chromatography). Thus, the way was also open for an improvement in the theoretical basis of adsorption chromatography.

If we now return to our key-word "mobility", we are bound to admit that mobility in paper partition chromatography does not mean the same thing as mobility in thin-layer adsorption chromatography. In both cases, it is true, the R_F value can be regarded as a function of the equilibrium distribution coefficient K. However, the extent to which K depends on:

(1) the separation conditions, and
(2) the molecular structure of the solute

is not the same in partition chromatography as in adsorption chromatography.

Let us take, first of all, the *separation conditions*. In paper chromatography, cellulose serves not only as a neutral carrier of the stationary phase but it even constitutes an integral part of this phase. The properties of the stationary phase are therefore also dependent on the structural features of the cellulose—such as its shape, matrix, and molecular structure—and thus influence the bed efficiency. In the case of adsorption chromatography, on the other hand, we all know from our experience with the older liquid column chromatography that bed efficiency here is dependent on a number of parameters, such as: type, activity, and particle size of the adsorbent; velocity and viscosity of the solvent; length, diameter, packing procedure, pressure, and temperature of the column; and time of separation.

It might be expected that quite similar conditions would likewise apply to thin-layer chromatography; in actual fact, however, the quasi-open TLC system exhibits several important differences, to which DALLAS[4] and STEWART[5] also referred recently. For example, the reproducibility of the R_F values is dependent on the following factors, listed in decreasing order of importance:

Adsorbent activity and relative humidity;
Pre-adsorption of solvent vapour by adsorbent;
Ratio of liquid to solid phase on chromatoplates;
Liquid–vapour equilibration in solvent chamber;
Position of origin and distance of travel of solvent front;
Temperature;
Layer thickness;
Amount of material spotted onto the plate;

Angle of plane with vertical;
Nature of adsorbent.

Even though several of these parameters also influence the separation conditions in this case of PC, some of them are nevertheless specific for TLC—for example, adsorbent surface volume or area, adsorbent activity, and adsorption energy of the solvent per unit area of adsorbent surface, *i.e.* the eluent strength of the solvent.

Consequently, it is a definite advantage to be aware of these not unimportant differences between partition and adsorption when making use of mobility for identification purposes; in other words, the differences between the separation conditions should be put to good account by applying *both* principles.

The influence exerted by the *solute molecular structure* on the distribution coefficient K is likewise not the same in PC as in TLC. This is already clear from the fact that the end result of an R_F value is due, in the last analysis, to a complex function of the solute–solvent–carrier or adsorbent system. It is, moreover, precisely these specific interactions which, though greatly complicating the theoretical aspect of the subject, are nevertheless extremely useful for the purposes of separation and identification. In PC, as you know, owing to the lack of additivity of the group constants, we very soon became familiar with the specific effects exerted on the R_F value in a given system by molecular interactions such as: (1) steric effects; (2) intramolecular hydrogen bonding; (3) electronic effects such as electronic displacements effected by substituents, particularly in aromatic systems; (4) chain branching, and so on.

In view of the wide extent to which TLC is used today, I should now like to examine more closely the influence exerted by solute molecular structure on the coefficient K in adsorption. Let us assume here, too, that the separation conditions are known and constant. It would certainly be tempting to discuss in detail the theoretical principles proposed, for example, by SNYDER[6], but this would make my introduction far too long. In connection with these interesting attempts to correlate the effects involved, I will mention here only those interactions which necessitate major corrections of the adsorption energies.

(1) Solute planarity: Non-planar solutes are adsorbed less strongly than planar solutes. Thus, even small groups which favour or hinder the planarity of a molecule can exert important effects on the R_F value.

(2) Steric hindrance to adsorption: The adsorption energy of a polar group—for example, a ring nitrogen atom—will be decreased by adjacent substituents, such as methyl groups.

(3) Chemical interaction of adjacent groups: If two substituents—for example, a hydrogen donor and a hydrogen acceptor—are spatially arranged in such a way that a hydrogen bond is formed, this will result in a decrease in the adsorption energy of each of the two groups.

(4) Electronic interaction between groups: This effect is quite important for aromatic solutes with strongly adsorbing groups; their adsorption energy will, for example, be increased if there is an adjacent electron-donating group.

(5) Concerted adsorption of two adjacent groups: For example, two adjacent carbonyl groups in a ring structure permitting interaction with a *single* adsorbent site are generally much more strongly adsorbed than isomers in which the two groups are non-adjacent.

(6) Localisation phenomenon: Solute adsorption energy is lost if an adsorbent

surface is covered with isolated strong sites for the adsorption of various solute groups. The strongest adsorbing group might prefer localisation over one of these strong sites, and this means that the remaining groups in the solute molecule will be unable to overlap exactly similar strong adsorbent sites. This effect occurs, for example, in the adsorption of polyaromatic hydrocarbons on silica, where one benzene ring localises and the adsorption energy of the remaining fused rings is proportionately lower. Another type of localisation can occur in molecules such as steroids where in a particular configuration of the adsorbed molecule some groups are held away from the surface.

Here again, some of the interactions mentioned may have the same effect on mobility in PC as in TLC, and some may have an opposite effect; in addition, the relative contribution in PC may differ quite appreciably from that in TLC.

On the other hand, we can conclude from this that we should endeavour to achieve optimal separation conditions for both forms of chromatography so as to obtain, from a comparison of the mobilities, more information than has been possible hitherto. Furthermore, we cannot expect the theoreticians to work out, on the basis of imperfect experiments, optimal correlation equations which could serve as pointers to future experimental developments in this field.

I hope that in this respect the ensuing discussions at this Symposium, too, will make a valuable contribution to a better understanding of these highly complex processes.

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