FACTORS WHICH INFLUENCE PAPER CHROMATOGRAPHIC R_F VALUES

INTRODUCTORY REMARKS

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SUMMARY

As an introduction to the discussion on the reproducibility of spot positions in paper chromatography, attention is drawn to the role of paper, the volume and composition of solvents (eluents and stationary phases), the atmosphere of the tank (the problems of conditioning and temperature control), development procedures, and additional components of the sample. Correction of the experimental data by means of reference substances and R_M calculations is also mentioned.

Although we have gathered here to discuss reproducibility, let me begin by admitting that exact reproducibility of R_F values is not always absolutely necessary. In fact, it has been often sacrificed for the sake of simplicity of operation. The overall pattern, for example in the case of the usual amino acids, or the use of standard samples, will suffice for identification in simple mixtures even if the positions of the spots vary.

There are two fields of chromatography in which reproducibility seems to be of special importance. By mentioning them, I recall the two main subjects of the first symposium, which was held here six years ago[1], namely the relationship between the chemical structure and chromatographic behaviour on one hand, and the so-called systematic qualitative analysis on the other. These subjects have remained among the most popular topics of fundamental chromatographic study ever since then. This illustrates the importance of these two fields, but at the same time it is a reflection on the difficulties encountered in these studies. There is no doubt that exact reproducibility would be of great help in just these two spheres. It would make it possible to pool R_F values obtained for various substances in different laboratories; so far, no serious worker would consider this more than a dream.

I shall only briefly sketch the factors which are known to influence the relative positions of the spots and the quality of separation. Obviously, the list is not complete. It would be easier to ensure reproducible conditions if only known factors were involved.

The first group of variables concerns the *paper*. The last Liblice symposium[2] was entirely devoted to the stationary phase, so I shall not speak too much about it.

Even a reliable and well-established manufacturer and a specific brand number may not be a guarantee of reproducible material, and batch variations have often been reported. During the last few years, possibly due to improvements in manufacture and packaging, complaints as to batch variations have ceased to appear. Batch variations are to be expected, especially in some special cases, such as that of ion-exchange celluloses. Storage is an important and often underrated variable. It is well known that swelling and shrinkage of cellulose is a slow process. Thus the atmospheric humidity and temperature prevailing on the day on which the paper is used is not sufficient to define the water content, and the whole previous history may exert its delayed effect.

Solvent systems, the purity of their components, their mutual chemical interactions (such as esterification) or differential evaporation of the more volatile constituents, will probably be discussed in the papers to follow. There are still some mysteries in the problem of ageing; some reported changes of multicomponent mixtures cannot be explained by known chemical interactions.

The influence of the fluid volume in the trough has been known for a long time. Unequal sorption of different components on the paper and differential evaporation are the most likely causes of this phenomenon.

If the stationary phase is established by impregnation, another set of variables is introduced, including the amount and composition of the impregnating liquid, its acidity and humidity. If these factors are standardized, chromatography in such systems is fairly reproducible.

The composition of the *atmosphere* of the tank, especially its humidity, has a very great influence. Reproducible humidity of the paper and a satisfactory chromatographic pattern can be assisted by adding the calculated amount of water to the tank and using a fan to distribute it evenly in the atmosphere[3] or by equilibrating over salt solutions which act as "constant humidity agents"[4].

There is a considerable amount of literature on whether and how to equilibrate (condition) the papers before development. A great deal has been published on the so-called BUSH systems for steroids in which saturation with certain components of the mobile phase is critical. I am also one of those who, after several months of attempts, have failed to achieve separation just because of difficulties of this kind. Controlled evaporation from the paper has been used intentionally in a procedure called exatmochromatography by its authors[5]; so far it has not found wide application.

Stabilization of *temperature* is closely connected with the question of the atmosphere in the tank. GOCAN AND LITEANU[6] have tested an equation to account for the effect of temperature gradient on the R_F values. As expected, the influence of temperature was found to be more marked in the case of partition than ion-exchange chromatography[7].

Naturally the various *development procedures* (ascending, descending, horizontal, on strips, sheets, disks and wedge-shaped lanes) lead to different R_F values and present different gradient problems.

Additional components of the sample often greatly modify the R_F values. Among their non-specific effects I mention those of viscosity and flow geometry. Generally speaking, I would place little reliance on the position of a spot in biological samples, unless co-chromatographed with a standard.

Obviously, standardization is not an easy problem. No wonder that methods have been devised to obtain comparable R_F values by suitable *correction* of experimental data. Referring the relative positions to a standard substance X, instead of to the solvent front, is a common practice. R_X values may help, if the spots under comparison are not far apart, *i.e.* for R_X values close to unity, or if R_M values are made the basis of comparison. Even here, gradient effects tend to deform the R_M scale, and therefore the R_C scale[9,10] and similar devices have been investigated. An empirical linear formula has been suggested[11], but any deviations which lead to changes in the sequence of spots can hardly be corrected by a general formula.

It is to be expected that in the papers presented at this symposium, various influences on the R_F values will be analysed and standardization and correction procedures recommended. In general, there are less variables in PC than in TLC. According to circumstances this may be an advantage or disadvantage. But even in PC, gradient phenomena and the influence of the tank saturation on these may become critical[12], especially in the case of the adsorption mechanisms.

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