Journal of Chromatography, 82 (1973) 31–36
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CHROM. 6623

THE SCOPE OF THIN-LAYER CHROMATOGRAPHY

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SUMMARY

The broad applications of thin-layer chromatography are discussed, including some of the fundamental aspects and various parameters of the method.

Thin-layer chromatography (TLC) is one of the most widely used separation procedures. There are now well over 10,000 publications on TLC and the number is increasing rapidly. It has gained this position because of its great versatility. Combining the advantages of paper and column chromatography, it is fast, it is simple, and relatively inexpensive compared to some of the other separation techniques.

Let us consider some of the parameters involved in TLC. First, we will consider the support for the thin-layer. By far the most commonly used support is the glass plate, although almost any type of support material is satisfactory as long as it does not react with the solvent or does not interfere in subsequent work. Obviously a metal plate could not be used for TLC direct densitometry on the layer. Uniformity of the supporting plate is important, because this is where the uniform layer starts.

Next we might consider the preparation of the layers. There is still a lot to be desired in the way of uniform layers, both commercially prepared and those made in the laboratory. This becomes evident when you start to do quantitative work and discover the variation in results from plate to plate. Perhaps the ideal of absolute uniformity is not attainable. There are, however, certain steps that can be taken to obtain as great a uniformity as possible. To this end, the equipment and plates must be dry, because traces of moisture will decrease the density of the slurry[1]. The quantities of water and adsorbent must also be carefully measured.

Waksmundzki *et al.*[2] have used a viscometer to measure the viscosity of the slurry and claim uniformity to the extent that they can obtain R_F value reproducibility of $\pm$ 0.02.

Different batches of adsorbent from the same manufacturer may differ so that slightly different amounts of water may be needed to produce the same results.

In drying the layers, care must be taken not to heat them at too high a temperature nor too long even at 110°C; in this case, layers bound with gypsum are meant. Thirty minutes to 1 h are sufficient, as with longer times the gypsum loses its water of crystallization and, consequently, its binding power.

For the application of samples, the least polar solvent that will dissolve the

sample should be used. The solvent should be evaporated from the sample spot, and of course, if the sample is sensitive to oxidation an inert atmosphere should be used. Traces of solvent left at the origin can alter the R_F values.

The use of alcohol as a sample solvent in the separation of terpenes has been found not only to affect the R_F values but also to cause streaking of the spots[3].

The sample size can in some cases affect the R_F value. Cerny *et al.*[4] found that with steroids R_F values were independent of concentration between the values of 50 and 200 μg; below 50 μg the R_F values were affected by concentration.

Another closely related factor that can have an effect on the R_F value is that of making a series of applications allowing the solvent to evaporate between each application instead of a single application. A series of applications in one spot causes some radial chromatography to take place which can affect both the shape and the R_F value of the spot.

The effect of the distance from the solvent level to the point of sample application on the R_F will depend on the adsorbent used, the compounds separated, and the solvent system in use. With mixed solvents, if de-mixing occurs, a definite effect on the R_F values may take place.

While on this subject, let us consider some of the other parameters that affect R_F values[5]. First let us consider those that affect the adsorbent, in this case silica gel. These are: (a) the pore diameter, (b) the pore volume, (c) the particle size, (d) the surface area, (e) the number of surface hydroxyl groups per unit area, and (f) the number of siloxane bands. These parameters vary from one manufacturer's product to the next, because of differences in processing. All of these adsorbent parameters have an effect on the R_F values, and it is not surprising, although regrettable, that different batches from the same manufacturer will vary in characteristics.

Temperature has some effect on R_F value as can be seen from Figs. 1 and 2.

The distance the solvent travels has only a slight effect on the R_F value.

The composition of the sample may affect the R_F value. This is especially so where liquids are chromatographed, for example a group of terpenes. In this case, the solvent composition in the layer is not just the solvent in the tank, but rather the sol-

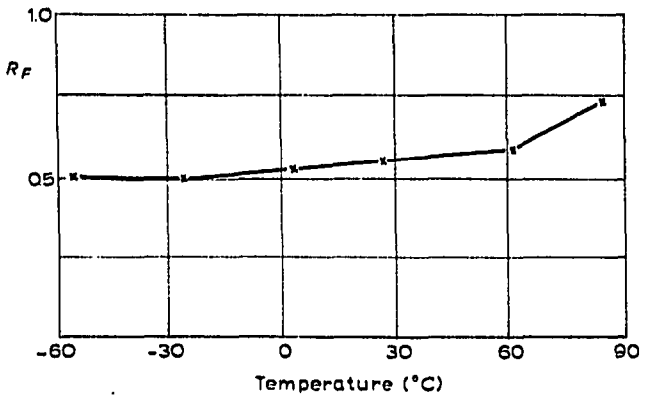

Fig. 1. Effect of temperature on the R_F value of undecanone-2 using 10% ethyl acetate in chlorobenzene as the solvent on silicic acid.

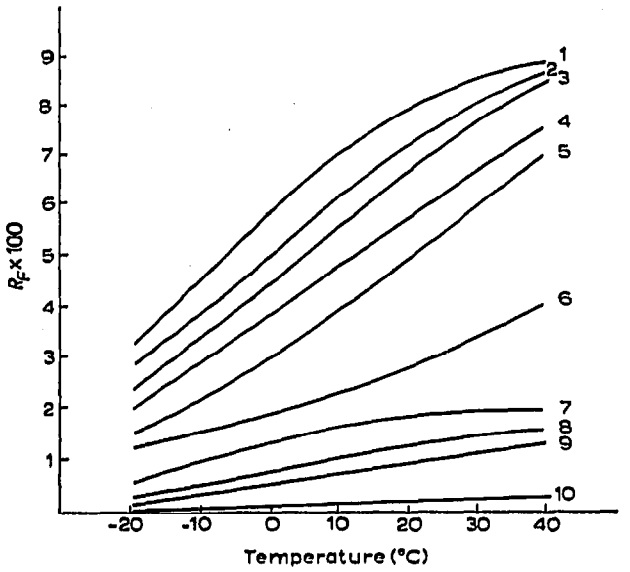

Fig. 2. Temperature effect on the R_F values of some pesticides on silica gel chromatoplates developed in hexane. (From D. C. Abbott and J. Thomson[16]; reproduced with permission of the authors and the Centre for Overseas Pest Research.) 1 = Aldrin; 2 = *p,p'*-DDE; 3 = heptachlor; 4 = *o,p'*-DDT; 5 = *p,p'*-DDT; 6 = *p,p'*-TDE; 7 = heptachlor epoxide; 8 = dieldrin; 9 = endrin; 10 = endosulfan B.

vent plus the components of the sample. In cases like this, the R_F value of a component may be slightly higher than that of the component chromatographed by itself.

Another factor affecting R_F values is that of the saturated tank *vs.* the unsaturated tank. In this case, the unsaturated tank will give a higher R_F value probably for several reasons. In the saturated tank, the plate will quickly adsorb solvent vapors so that not as much solvent will have to pass through the layer by capillarity before reaching the final solvent front. Therefore, the less the flow of solvent, the lower the R_F value. On the other hand, the plate in the unsaturated tank requires more solvent flow to saturate the layer, and also because the chamber is unsaturated, evaporation will take place from the layer, thus requiring even more solvent flow to reach the final solvent front. Added to this effect, of course, will be the heat of adsorption which will help evaporate solvent from the layer.

There has been a great deal of discussion on the relative merits of saturated and unsaturated development, but it is believed that one should use the method which yields the best results for his particular purpose.

There is one other factor affecting not only the R_F value but, what is more important, the resolution. Specifically this is the effect of humidity on the adsorbent. I believe this should be mentioned at this point, especially since Dr. Geiss has been instrumental in working out the details.

Back in 1951[3] working with chromatostrips, where individual strips were removed one at a time from the desiccator, spotted, and immediately placed in the test tube developing chamber, it was noticed that after 10 min of opening and closing

the desiccator, the R_F values were no longer consistent. It was realized, of course, that this was because of the adsorption of atmospheric moisture by the silica gel adsorbent.

In the case where 20 × 20 cm plates are used, the problem becomes more acute, because of the time required to place the sample spots across the plate. Dallas[6] has shown that half the total moisture adsorbed when a silica gel plate was allowed to come to equilibrium in an atmosphere of 50% relative humidity was accounted for in the first 3 min.

In 1963 Geiss and coworkers[7] drew attention to the effect of humidity on aluminum oxide layers and published the design for a chamber for maintaining development under controlled humidity conditions. The ultimate outcome of all this was the development by Geiss and Schlitt[8] of the Vario KS chamber. With this chamber plates may be exposed to atmospheres of controlled humidity, thus producing plates of different but controlled activity. Furthermore, gradient layers may be obtained by exposing sections of the plate to different degrees of humidity.

Not only is there an effect because of the adsorption of moisture, but also from the adsorption of solvent vapors. In 1965 Dallas[6] showed that there was considerable adsorption of solvent vapors by the chromatographic layer. Later De Zeeuw[9] followed up this lead to show that improved separations can be obtained by the proper choice of solvent vapors to pre-treat the layers. This pre-treatment can be carried out in the Vario KS chamber.

With all this emphasis on R_F values, it should be made clear that having identical R_F values does not insure that two compounds are identical —it merely indicates that they might be identical. Other confirming evidence is needed to positively establish identity.

The use of an inert atmosphere for oxygen-sensitive materials has been mentioned, but additional preventative measures can be taken. For example: ascorbic acid may be incorporated in the layer when chromatographing carotenoid pigments[10] and 4-methyl-2,6-di-*tert.*-butylphenol has been used for lipids[11,12] as well as 1,4-dihydroxy-2-*tert.*-butyl benzene[12].

One of the problems that arises in TLC is the fact that silica gel is such a good adsorbent that it picks up impurities out of the air. These impurities are especially bothersome when it comes to quantitative work or when samples are required for infrared (IR) or mass spectrometric (MS) work. For this reason, the adsorbent should not be stored in plastic containers as it picks up plasticizers from this source. Special attention has to be paid to keeping the adsorbent away from all plastic material, as even the brief contact with the plastic tip of a Ritter and Meyer[13] vacuum collector picks up contamination. Amos[14] has investigated this problem very thoroughly, and has also pointed out that in a 10-μg sample on which IR and mass spectra can be taken not more than 0.5 μg of an impurity can be tolerated. In view of this, he found that high-purity solvents were satisfactory for eluting the samples, but in lieu of these expensive solvents, analytical solvents could be used by simply distilling the first 70% in order to eliminate the non-volatile solids. Even then the minimum amount of solvent should be used in eluting the spot.

For IR and MS work all precoated plates are unsatisfactory, not because of the manufacturer's fault, but because the adsorbent picks up impurities from the atmosphere. The adsorbents for the preparation of the layers should be thoroughly purified before the layers are made.

As a further precaution with this type of work, Amos found that it was necessary to clean all glassware in an ultrasonic bath for 30 min using one of three detergents, *viz.* "Extran", "R.B.S. 25", or "Decan 75". "Lab-brite" and "D.D.N. 150" were less satisfactory.

Is there any field where separations are involved where TLC cannot be used to good advantage? Naturally with very volatile compounds there is a problem, although low temperatures can be used to prevent the loss of compounds. However, it is not believed that TLC should be thought of as a cure-all, and these very volatile compounds are better left to gas–liquid chromatography (GLC). It is a field where the two methods complement one another; however, it is very well to repeat Kaiser's[15] warning to the GLC man: "Results may be obtained which were not expected; similarly, results which have been observed with the existing methods when used alone may be found incorrect. These facts may be very irritating and may require additional work." The GLC man will literally see spots before his eyes. There are several fields where TLC has already been of inestimable value. These include lipids, steroids, and nucleic acids. Prior to the advent of TLC, nucleic acid and nucleotide chemistry were mainly dependent on partition chromatography. This has all changed with the advent of ion-exchange materials for use in TLC.

The vast amount of literature that has built up on the use of TLC for lipid and steroid work is testimony to its usefulness in these fields.

One of the latest additions to the list of TLC techniques is that of isoelectric focusing. It can be applied to the analysis of enzymes, hormones, and other ampholytes of biological interest. The layers for this work can be prepared from polyacrylamide gel or Sephadex, in which is incorporated a mixture of "carrier ampholytes". When an electric current is passed through the layer, the carrier ampholytes arrange themselves, so that we have a layer with a pH gradient on it. The sample placed on this layer migrates so that each individual ampholyte in the sample proceeds to that point where the pH is equal to its isoelectric point, and there it becomes concentrated in a sharp zone.

With isoelectric focusing, additional bands have been found in proteins and enzymes wihch were formerly thought to be homogeneous. This technique complements electrophoresis, and two-dimensional work may be implemented using electrophoresis in one direction and isoelectric focusing in another. This technique should find use in the field of taxonomy where thin-layer electrophoresis has already found use in differentiating species.

REFERENCES

1 S. Hara, H. Tanaka and M. Takeuchi, *Chem. Pharm. Bull.*, 12 (1964) 626.
2 A. Waksmundzki, J. Rozylo and J. Zajdel, *Chromatographia*, 2 (1969) 449.
3 J. G. Kirchner, J. M. Miller and G. J. Keller, *Anal. Chem.*, 23 (1951) 420.
4 V. Cerny, J. Joska and L. Labler, *Collect. Czech. Chem. Commun.*, 26 (1961) 1658.
5 J. G. Kirchner, *Thin-Layer Chromatography, Techniques of Organic Chemistry*, Vol. XII, Wiley-Interscience, New York, 1967, p. 193.
6 M. S. J. Dallas, *J. Chromatogr.*, 17 (1965) 267.
7 F. Geiss, H. Schlitt, F. J. Ritter and W. M. Weimer, *J. Chromatogr.*, 12 (1963) 469.
8 F. Geiss and H. Schlitt, *Chromatographia*, 1 (1968) 392.

9 R. A. de Zeeuw, *Anal. Chem.*, 40 (1968) 2134.
10 A. Hager and T. Bertenrath, *Planta*, 58 (1962) 564.
11 J. J. Wren and A. D. Szczepanowski, *J. Chromatogr.*, 14 (1964) 405.
12 T. S. Neudoerffer and C. H. Lea, *J. Chromatogr.*, 21 (1966) 138.
13 F. J. Ritter and G. M. Meyer, *Nature (London)*, 193 (1962) 941.
14 R. Amos, *J. Chromatogr.*, 48 (1970) 343.
15 R. Kaiser, in L. S. Ettre and W. H. McFadden (Editors), *Ancillary Techniques of Gas Chromatography*, Wiley-Interscience, New York, 1969, p. 229.
16 D. C. Abbott and J. Thomson, *Pest Artic. News Summ.*, 12 (1966) 21.