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QUANTITATIVE METHODS IN THIN-LAYER CHROMATOGRAPHY

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In 1954 I and my associates published the first quantitative determination, using thin-layer chromatography (TLC). This was for the determination of biphenyl in citrus fruits and fruit products. Actually two methods of measurement were presented, one a semi-quantitative method based on the minimum amount detectable in a spot, and the other a quantitative method based on elution of the biphenyl and subsequent measuring at 248 m μ . The latter had an average error of $\pm 2.8\%$, and demonstrated the reliability of TLC at that time.

In quantitative TLC, there are a number of choices. Initially we can divide these in two: measurement on the plate, and elution before measurement. In the former case, we have a choice of transmission measurements or reflectance and in addition, that of radioactive scanning and fluorescent measurements.

There are a number of factors that affect direct densitometric measurements, and these include: the nature of the layer, the thickness of the layer, the moisture content of the layer, the R_F value of the compound, the solvent system, the rate of flow, the direction of scanning, the presence of other components, and the shape, size and accuracy of initial sample application.

In addition, if the plate is treated with a reagent to make the spot visible, other factors are brought into play such as the degree of coloring of the background due to excess reagent or diffusion of the spot caused by the reagent solvent.

Reflectance spectra have the advantage of requiring very small quantities of sample material, 0.2–5 μg usually being sufficient. Many of the factors just mentioned affecting transmittance results also affect reflectance measurements. However, differences in the thickness of the layer are not as important as in the case of direct densitometry.

PRODYMA *et al.*¹ have shown that the spots may also be scraped from the plate and packed in special cells for reflectance measurements.

In all cases of analyses using electronic instruments, there is the factor of noise and I understand POLLAK AND BOULTON (p. 87) will have some pertinent comments on this subject as to sources of noise and means of eliminating or compensating for them.

Indirect densitometric methods can also be used. In this case photographs of the spots are taken and the resulting negative is measured in a densitometer. In this respect, it has been shown that Polaroid film of the type 46-L projection film can be used to advantage, as it eliminates the wet processing step for the film. This film provides a positive image and precludes the necessity of image reversal. Needless to

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say that in this as well as all other quantitative methods, standard samples should be run.

While on the subject of films, of course X-ray films can be used in like manner for the quantitation of radioactive spots.

Fluorescent measurements can be considered as a special case of reflectance in which the reflected light is of a different wavelength from that of the incident light. Under this category also comes the use of scintillation phosphors for radioactive materials. These may be sprayed on the plate or mixed with the powdered material scraped from the plate.

With the indirect measurements, those where the spot is eluted before measuring the quantity present, some of the same factors affect the results. Of course the accuracy of the initial sampling is one of these which is very important and which is too often taken for granted. That is to say one picks up a micro-pipette or burette and dutifully applies let us say 10 μ l. However, as pointed out by FAIRBAIRN AND RELPH², there are sources of error here. Creep-back can cause compound build-up on the needle of a syringe or micro-burette which ultimately may be washed off by a somewhat larger drop. Similarly, contact of the needle with the absorbent causes capillary withdrawal of excess sample. These errors can be minimized by proper selection of equipment and attention to details.

The adsorbents are a source of contamination because of their tendency to adsorb impurities from the air. Prewashing of the plate with alcohol prior to sample application helps to minimize this problem, however, the eluate from a sample blank of silica gel still contains ultraviolet absorbing material. It is therefore necessary to use very uniform layers and to elute identical size sample and blank areas. Careful attention must be given to the activation of the plate so that uniform R_F values are obtained.

Naturally the developing solvent should be free of any interfering contamination.

In the indirect approach to analysis, there are a number of alternative methods: One can use the spectrophotometer, or the colorimeter if the compound is colored; with colorless compounds one can use a reagent to form a colored complex which can be formed either prior to or after elution. Use of a colored complex helps to avoid the interference from ultraviolet absorbing impurities eluted from the adsorbent. Needless to say, the complex must obey the Lambert-Beer law within the limits of the assay.

There are of course other methods of determination which can be applied, such as spot area measurement, gravimetric methods, titration of eluted material, polarographic methods, and injection of the eluted sample into a gas-liquid chromatography unit. These all have their own problems and limitations which I will not attempt to discuss at this time.

I have pointed out a few of the problems which confront the thin-layer quantitative analyst and the talks today will show how some of these can be solved as well as presenting some of the other problems involved in TLC analysis.

REFERENCES

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 - 2 J. W. FAIRBAIRN AND S. J. RELPH, *J. Chromatogr.*, 33 (1968) 494.
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