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THIN-LAYER CHROMATOGRAPHIC QUANTITATIVE ANALYSIS

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SUMMARY

In this review, the various methods available in quantitative thin-layer chromatographic analysis are discussed. Advantages and disadvantages are pointed out with some suggestions to eliminate or minimize errors.

INTRODUCTION

There are many factors that can affect the quantitative results obtained by means of thin layers[1]. In doing quantitative thin-layer chromatographic (TLC) work there are certain parameters which affect all methods whether it be by direct densitometry, fluorimetry, an elution method, or otherwise.

METHODS*

Application of the sample

To all intents and purposes, the accuracy of the analysis begins with the application of the sample to the plate.

The size of the initial spot should be kept as uniform as possible, since the size of the sample spot can have an effect on the results when measuring the final spot by densitometry, fluorimetry, reflectance, or spot area.

Another large source of error during application of the sample is that due to creep back on the tip of the syringe[2–4]. Part of the drop will curl back around the tip of the syringe and some of it will remain after the drop is discharged. This amount varies. Also there is evaporation taking place thus concentrating this solution that remains behind; subsequent drops can wash this material off, increasing the concentration in that drop significantly. Errors from this source can be mimimized by using as fine a tip as possible and by coating it with silicone. Coating the tip appears to be very effective[4]. Samuels[5] has recommended that the shape of the microsyringe needle be modified by grinding a reverse bevel so that the needle is pointed in the center instead of at the side.

You cannot avoid this error of creep back by touching the tip to the layer, because you then introduce an error caused by the capillary attraction of the layer withdrawing additional fluid from the syringe tip.

* Abbreviations used: CPZ = chlorpromazine; DANS = 5-dimethylaminonaphthalene-1-sulfonyl; DNP = dinitrophenyl; PTH = phenylthiohydantoin.

Equipment such as the Chromatocharger of Camag and the Chromaplot of Burkard (Rickmansworth, Herts.), which eject the drops, avoid both the curl back and the capillary problem.

Brain[6] has investigated the "operator effect" in the application of samples for quantitative work. Using a group of students, he tested the microcap disposable micropipette (the microcap is a capillary cut to hold a precise amount) under various conditions of use and a repeating dispenser using a Hamilton syringe.

He found a great deal of variation among individual operators. Of the various tests that were run, best results were obtained with the repeating dispenser. With unskilled operators, the mean coefficient of variation was 5.6% but with skilled operators this dropped to 3.5%. In contrast to this, the single application of the contents of the microcap resulted in a mean coefficient of variation of 9.5%, with individual coefficients of variation ranging from 1.2% to as high as 53.4%. Various attempts to decrease the error with the microcap such as (a) rinsing out the micropipette, (b) applying the single pipette charge as a series of five applications instead of one continuous application, and (c) expulsion of the contents by means of a rubber bulb, all resulted in increased error. Fairbairn and Relph[4] investigated the "operator effect" with ten experienced operators in applying samples with various instruments and found errors as large as $\pm$ 25%.

Brain also found that the error varied with the type of layer used, ranging from a mean coefficient of variation of 6.8% for silica gel to 13.7% for polyethyleneimine cellulose.

Brain makes the comment: "It is essential that any person needing quantitative sample application should check carefully the errors in the application of samples, by themselves under their experimental conditions, and not simply accept the published figures of other workers."

Another very important factor in quantitative work is the correlation between the final measurement and the amount in the initial application, and also the variation

TABLE I

REPRODUCIBILITY OF SCANNING OF DIFFERENT SPOTS IN THE SAME AND ON DIFFERENT CHROMATOGRAMS

(Time delay between drying and scanning was standardized in each case.) (From Pataki[7], reproduced with permission of the author and Friedr. Vieweg & Sohn GmbH.)

Substance	*On the same chromatogram*		*On different chromatograms*	
	Peak area (mm^2) mean values (n=6)	*s (%)*	*Peak area (mm^2) mean values (n=6)*	*s (%)*
PTH-Proline*	2300	5.8	1820	11.9
DNP-Proline**	2080	7.4	2400	8.6
DNP-Proline***	640	4.4	630	5.4
DANS-Proline§	1550	3	1710	9.1
DANS-Proline§§	2045	6.2	2640	14.4

* 2 μg; quenching (Silica Gel F).
** 2 μg; quenching (silica gel).
*** 2 μg; reflectance (silica gel).
§ 2 μg; fluorescence (silica gel).
§§ 2 μg; fluorescence (silica gel), after spraying with triethanolamine–isopropanol (1:4).

from plate to plate. Good correlation can be obtained when using a single plate, but variations can occur between different plates. As can be seen in Table I[7] the deviation between spots measured on the same plate was less in each than the deviation between spots on different plates. To guard against this source of errors, standards should be run on each plate, including two quantities, one a multiple of the other. In this way, the regression line relating the amount measured in the final spot to the amount applied can be checked.

Once we have the sample on the layer, it goes without saying that the development must be in the same solvent, under the same conditions, in order to avoid any slight variation which could affect the results. Choosing the correct solvent for development is another problem. The most desirable is a single component solvent that will give an R_F value between 0.25 and 0.75 (ref. 8). Higher R_F values are apt to run into interference from adsorbent impurities carried by the solvent front. Also, the higher the R_F value, the greater is the amount of diffusion that occurs in the spot.

Graham *et al.*[9] have pointed out that in the case of mixed solvents if demixing occurs and the sought for compound travels with the β-front, then lateral diffusion of the spot occurs with a consequent decrease in precision. A further consideration here might be adsorbent impurities which could be carried by the β-front.

Having obtained the final chromatogram, we are faced with a choice of methods; let us examine the pros and cons of some of these so as to be able to pick the one most suited to our purpose.

Direct densitometry

First, let us examine direct densitometry. Direct densitometry has the advantage over elution methods in being faster. However, the measurement is affected by a number of factors not encountered in solution densitometry.

The light falling on the plate is split up in a number of ways; some is reflected, some is scattered, and the remainder passes through to be absorbed more or less by the sample. Not only must the layers be uniform in thickness, but each layer must be made homogeneous by thorough mixing of the adsorbent and the binder without air bubbles. Care must be taken to remove all traces of developing solvent.

If the spot area has to be treated with a reagent to produce a colored product, then certain conditions must be observed; the colored product must not diffuse out into the surrounding area, and the amount of color produced must be proportional to the amount of sample and must be reproducible and stable. Likewise, the reagent should leave the background white, or at least be as contrasting as possible with the spot. There may also be a time factor involved as shown in Fig. 1 from Pataki[7]. This is in reference to reflectance measurements of DNP derivatives of amino acids, but the same principle holds for densitometric measurements. As can be seen, there is a steady drop in reflectance for the first 100 min and then it is fairly constant for a period of time. In this case, the obvious thing to do is to work on a definite time schedule, preferably measuring in the area where the change has leveled off or where the rate of change is insignificant.

In applying the reagent, care must be taken to see that it is distributed uniformly across the plate as can be seen from the results of Graham *et al.*[9] shown in Table II, where uniform amounts of standard solution were applied across the layer. One way to diminish this source of error is to apply alternate spots of sample and stan-

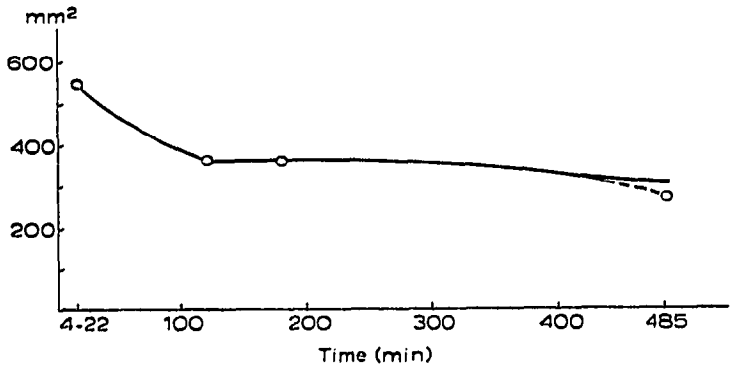

Fig. 1. Influence of time on reflectance (DNP-Gly), measured with the Chromatogram-Spectrophotometer (360 nm). (From Pataki[7], reproduced with permission of the author and Friedr. Vieweg & Sohn GmbH.)

TABLE II

IMPRECISION CAUSED BY UNEVEN SPRAYING OF THE PLATES

Eight spots of Zn(II) standard solution were applied across each plate. (From Graham *et al.*[9], reproduced with permission of the authors.)

Plate number	*Standard deviation (%)*
1	4.3
2	3.2
3	5.2
4	3.9

dard solutions as the average difference between adjacent spots was 2.5% compared to a maximum of 5.2% across the plate.

When it comes to the actual measurement, this can be approached in several ways. First let us consider the size of the beam. Shellard and Alam[10] found that when the slit length was smaller than the width of the spot, the coefficient of variation was smaller than when the slit length was greater than the width of the spot. Also, slightly better results were obtained with a slit width of 0.5 mm than with one of 0.3 mm.

Likewise, the position of the slit with relation to the spot is important. Fig. 2 shows the variation in the peak area caused by shifting the slit 1 mm to each side of the optimum point. As pointed out by Pataki[7], errors of this type can be avoided by running scans after shifting the slit slightly to each side of the initial scan in order to make sure that the initial scan is at the real peak maximum.

The spot may be scanned in the direction of development, or 90° to this direction. In scanning parallel to the development direction, the baseline may not return to the same level. This occurs if (a) the solvent leaves a narrow trail in the center, (b) the spots are not completely separated, or (c) impurities are deposited between the separated spots. Thus, conditions for scanning will vary for different determinations, and the direction of scan should be chosen to suit the occasion. Fig. 3 shows the results of scanning in the two directions. On the left two curves obtained by Turano and Turner[11] in scanning 180° to the flow of the solvent and on the right

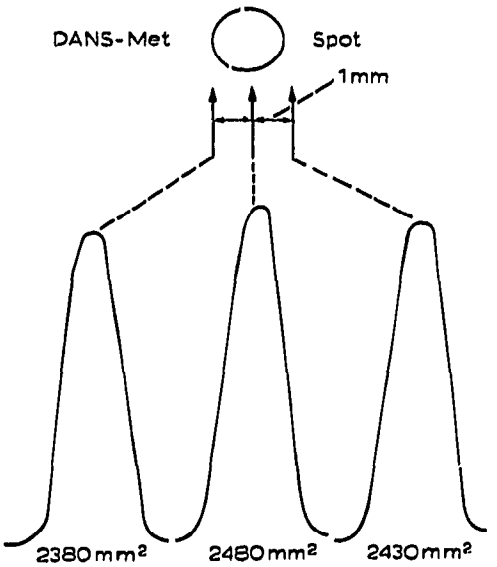

Fig. 2. Influence of the positioning of the scanner (Y-axis). Note the error caused by a variation of ± 1 mm. (From Pataki[7], reproduced with permission of the author and Friedr. Vieweg & Sohn GmbH.)

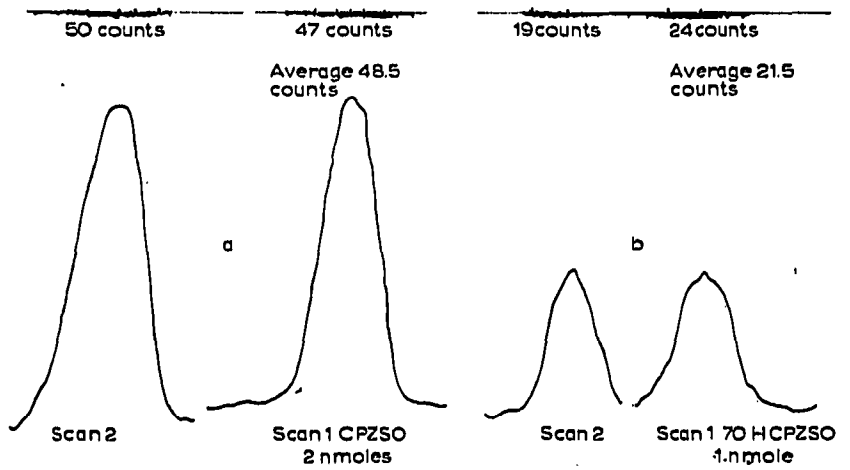

Fig. 3. Segment of integrator chart obtained on scan of one phenolic and one non-phenolic CPZ metabolite. (a) In direction opposite flow of solvent, (b) 90° to (a). (From Turano and Turner[11], reproduced with permission of the authors.)

the resulting curves from a perpendicular scan. These workers found it advantageous to scan in both directions with subsequent averaging of the two results. In this respect, it might be pointed out that by repeated scannings the results can be averaged to decrease the error in this phase of the determination (Table III).

For scanning a spot, a recorder may be fitted with an integrator which automatically gives a count which is proportional to the area of the curve. However, if the recorder pen does not return to the original base line because of any of the factors

TABLE III

SCANNING OF THE SAME SPOT SEVERAL TIMES

(From Pataki[7], reproduced with permission of the author and Friedr. Vieweg & Sohn GmbH.)

Measurement No.	*Peak area** (mm^2)	*Peak area*** (mm^2)
1	1570	620
2	1610	590
3	1510	580
4	1590	600
5	1570	580
6	1500	570
7	1560	600
8	1580	600
9	1520	590
10	1580	570
11	—	580
12	—	600
mean	1560	590
s (%)	2.4	2.2

* 5.8 μg AMP-5′ (quenching measurements with the Camag/Turner Scanner).
** 2 μg DNP-Gly (reflectance measurements with the Chromatogram-Spectrophotometer of Zeiss).

mentioned previously, the count and consequently the area will be in error. In these cases, it is better to use a planimeter to measure the area even though it takes more time. Here again, error in use of the planimeter can be minimized by averaging repetitive measurements. Other methods of measuring curve area may be used but they are less accurate.

Fluorescent and quenching measurements

If a compound fluoresces when exposed to light of a given wavelength, then this phenomenon can be used to quantitatively determine the amount of compound present, because the amount of light emitted is proportional to the amount present. Furthermore, in contrast to direct densitometry, there is not the great loss, because of scattering when the beam passes through the adsorbent and glass layers.

Aside from those factors which have already been mentioned, there are others to be taken into account when measuring fluorescence. One of these is the wavelength of the exciting light. Fig. 4 shows that there can be a change of linearity of the spot area *vs.* the amount when different exciting wavelengths are used. In this case there is a good linearity at an exciting wavelength of 310 nm, but this changes with decreasing wavelength. It is therefore necessary to make sure that an exciting wavelength is used that will give linear results.

Direct fluorescent measurements are more sensitive than density or reflectance measurements so that amounts in the nanogram range can be measured in contrast to micrograms. It is interesting to note also that the adsorbent can affect the amount of fluorescence. Pataki and Wang[12] found for DANS and DNP amino acid derivatives that polyamide layers gave a higher sensitivity than silica gel layers. However,

Fig. 4. Change of linearity due to change in exciting wavelength. ●, 310 nm; △, 300 nm; ■, 320 nm; ○, 290 nm. (From Touchstone *et al.*[8], reproduced with permission of the authors and the American Chemical Society.)

they also found that the resolution using different brands of polyamide was subject to variation.

The moisture content of the layer has an influence on the fluorescence so that there is a time effect as illustrated in Fig. 1. In addition to standardizing the time to negate this effect, the plate may be sprayed with triethanolamine–isopropanol (1:4) (ref. 13) (also shown in Fig. 1).

With fluorescent measurements, one can expect a relative standard deviation of 4 to 6% on the same plate and 8.6–12% on different plates, that is, provided conditions and technique are carefully standardized.

Along with the subject of fluorescence, we can discuss the use of fluorescent quenching measurements. In this case, the compound spot absorbs the exciting wavelength of light and thus prevents the layer from fluorescing at this point. The compound appears as a dark spot on a fluorescent background.

Since only a fraction of the depth of the spot is used in quenching and also in

TABLE IV

INFLUENCE OF LOADING VOLUME AND SIZE OF ORIGIN ON PEAKS OF DNP-PROLINE (3 μg, QUENCHING)

(From Pataki[7], reproduced with permission of the author and Friedr. Vieweg & Sohn GmbH.)

Plate No.	*Volume (μl)*	*Peak area (mm²)*
1*	2	1700
	5	1680
	10	1870
2**	1 × 2	1580
	2 × 2	1700
	3 × 2	1560

* The volumes given are applied to the layer followed by drying with a stream of cold air.
** Intermediate drying with a stream of cold air after application of 2 μl.

fluorescent measurements, it is important that the size of the spots be consistent and the application of the sample be made in a consistent manner. Table IV, taken from Pataki's work, shows the influence of loading volume and size of the origin on the peak areas measured by the quenching effect of dinitrophenylproline. The same quantity (3 μg) was applied at each origin. In the first set of samples the entire volume was applied at once with subsequent drying. In the second case the samples were applied in 2-μl increments with drying between each increment.

TABLE V

INFLUENCE OF DEVELOPING DISTANCE AND LAYER THICKNESS ON PEAKS OF DNP-PROLINE (3 μg, QUENCHING)

(From Pataki[7], reproduced with permission of the author and Friedr. Vieweg & Sohn GmbH.)

	Peak area (mm^2)
Developing distance, cm	
12	1600
10	1520
8	1450
Layer thickness, mm	
0.25	1665
0.5	1740

Similarly, since with increasing R_F value diffusion increases, resulting in larger spots, we can expect a greater distance to affect the results, and this is illustrated in Table V. As can be seen, an increase in developing distance, equivalent to higher R_F values and hence larger spots, results in greater peak area using the same initial quantity in the spot. This same table also shows the effect of layer thickness on peak quenching area.

Reflectance measurements

Frei has written two review articles[14,15] on reflectance spectroscopy; some of the important points to be considered in relation to TLC will be touched on below.

In reflectance measurements, the incident beam which strikes the layer penetrates to a certain depth and then that which is not absorbed or scattered beyond the range of the measuring system is measured as reflected light.

There are two ways to carry out reflectance measurements: (a) by removal of the spot and packing in a cup before measuring, or (b) by direct measurement on the layer. In the first case, the spot together with sufficient additional adsorbent to give a constant weight is intimately ground in a mortar and then packed uniformly in a cup or cell[16]. The corresponding blank consists of adsorbent treated in the same manner. This method is more precise, but is more time consuming.

In using direct reflectance measurements on the thin-layer plate, the thickness of the layer does not have as great an effect on the measurement as in the case of direct densitometry; however, the coating procedures should be standardized to give as uniform and reproducible plates as possible. Even then, it is advisable to use a white background beneath the layer during measurement[17,18]. Fig. 5 shows the calibration curves relating square of the area to concentration for the unbacked plate and for one

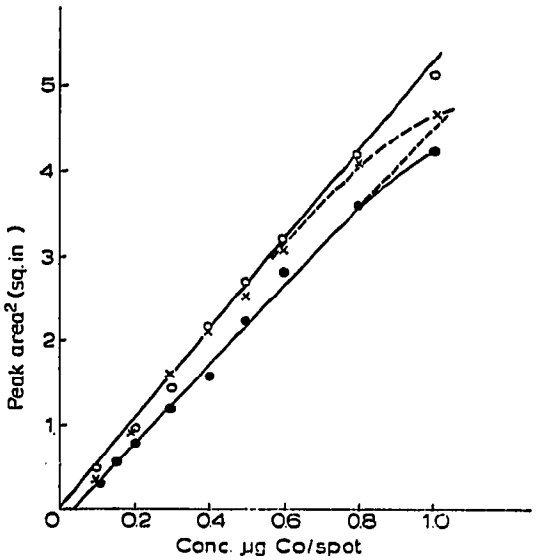

Fig. 5. Calibration curves showing square of the area *vs.* concentration. ×—×, Obtained by reflectance spectroscopy without backing sheets; ○—○, for infinite layer thickness (two backing sheets); ●—●, by transmission spectroscopy. (From Frei[18], reproduced with permission of the author.)

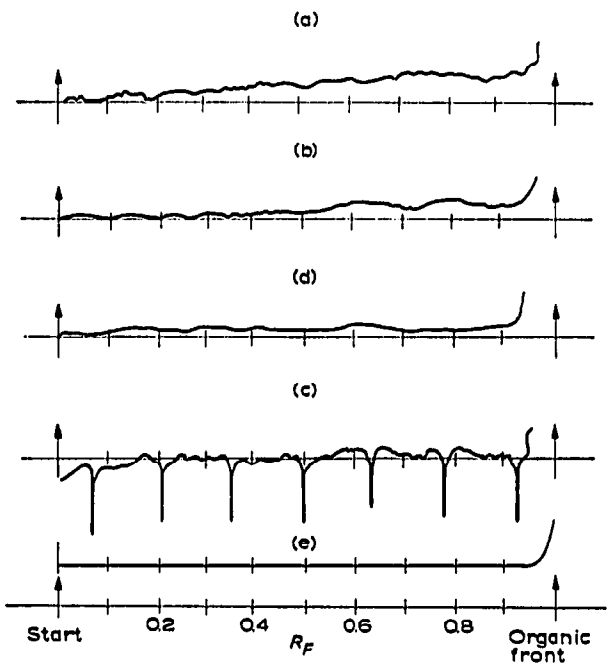

Fig. 6. Scans of reagent background (580 nm) at different conditions with the Zeiss instrument. (a) By transmission measurement; (b–d) by reflectance measurement: (b) on black chromatogram stage, (c) with infinite layer thickness (two empty sheets), (d) on black stage with white lines; (e) double-beam recording the Farrand instrument. (From Frei[18], reproduced with permission of the author.)

backed by two coated layers to produce infinite thickness. Fig. 6 shows the background effect under different conditions.

If everything is carefully standardized, standard deviations of 1–5.3% can be expected in reflectance spectroscopy on the same chromatogram and of 4–6% on different chromatograms.

Spot area measurements

The measurement of spot areas as a means of quantitative determination will be considered here, because it is one of the simpler methods which does not require expensive equipment.

There are a number of ways to measure the area: by means of the planimeter, by photographing and then cutting out the spot and weighing, or transferring to square millimeter paper and counting the squares. Of these, the planimeter can give the best results.

The relationship between the area and the amount of compound has been the subject of considerable discussion. Petrowitz[19], in examining some insecticides, found a straight-line relationship between the area of the spot and the quantity of insecticide as did Seiler[20] in the determination of inorganic ions. Aurenge *et al.*[21], in working with various phenols, obtained a straight-line relationship by plotting the square of the surface area against the weight of the sample. Purdy and Truter[22,23], in examining sixteen different compounds, found a linear relationship between the square root of the area and the log of the weight of the compound. Other workers, not finding a strictly linear relationship with the compounds they were working with, compromised and used only those short sections of the curve that were linear.

The work of Nybom[24] throws some light on this wide variation. He found that different relationships existed between the area and the weight of the material depending on the thickness of the layer. A thin layer gave a linear relationship between log

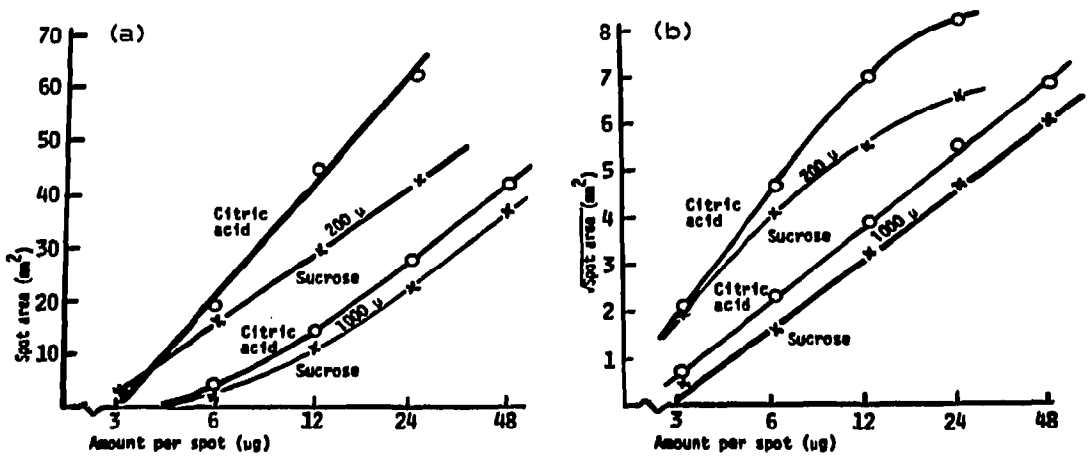

Fig. 7. (a) The relationship between amount of substances per spot, on a logarithmic scale, and the resulting spot area. ○—○, Citric acid; ×—×, sucrose. Bent curves are obtained for the thick layer (1,000 µ) and a straight line for the thin layer (200 µ). (b) The same material as in (a) but with square of the spot area along the ordinate. The thicker layer now gives straight lines, in contrast to the bent curves obtained for the thin layer. (From Nybom[24], reproduced with permission of the author.)

weight and area in contrast to a thick layer, which showed a linearity between weight and the square root of the area (Fig. 7).

Another factor which Nybom discovered was that the visualizing agent had an effect on the area–weight relationship. In Fig. 8 we see that for β-alanine there is a straight-line relationship between the log of the area and the log of the amount when revealed by isatin. However, if these same quantities are revealed by ninhydrin, as shown in Fig. 9, we find a straight-line relationship between the area and the log of the amount.

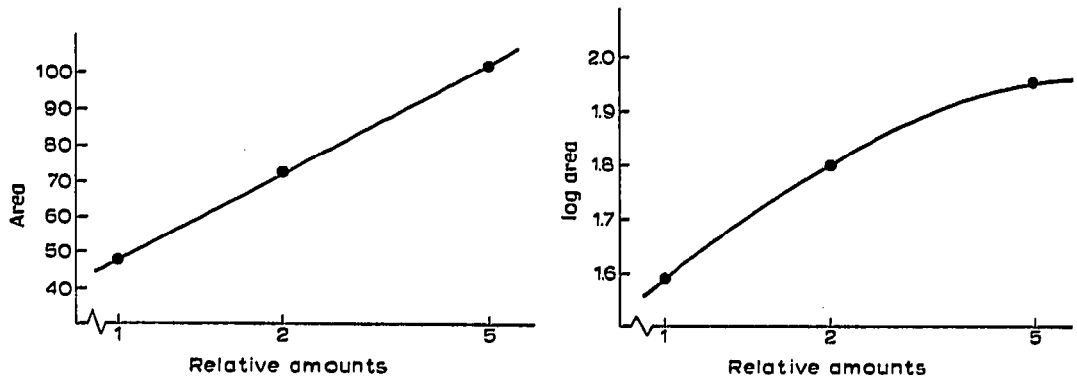

Fig. 8. Three different amounts of β-alanine on a logarithmic scale give a straight-line relationship with area visualized with isatin. (From Nybom[24], reproduced with permission of the author.)

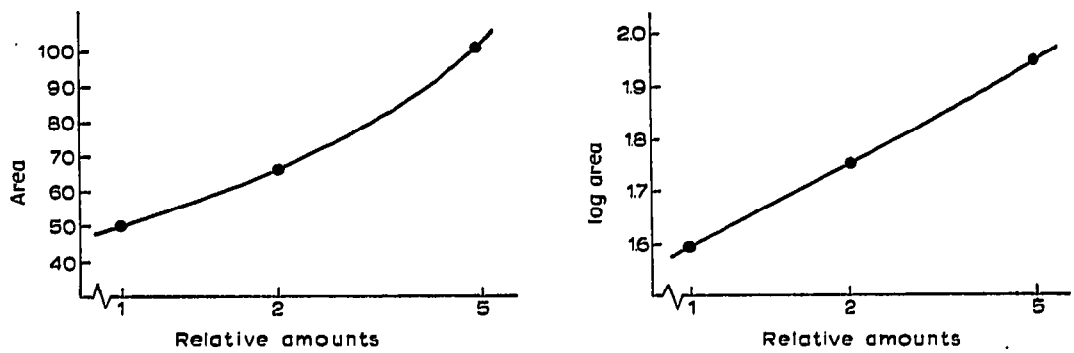

Fig. 9. Three different amounts of β-alanine when visualized with ninhydrin give a straight-line relationship between log amount and the log areas of the spots. (From Nybom[24], reproduced with permission of the author.)

Certainly, area measurements related in some way to the quantity of material are not the most accurate way for quantitative analysis of thin-layer plates, but they do provide some answers where more elaborate equipment is not available.

Elution techniques

Let us take a brief look at elution techniques for quantitative determinations.

Presumably nothing could be simpler, a suitable separation is worked out, the spot is scraped off, the sample is eluted, and then measured by one of a number of methods, *i.e.*, colorimetry, spectrophotometry, analysis for a specific element such as phosphorus, etc. However, it is never quite that simple.

To illustrate some of the problems encountered, let us draw on the first recorded use of TLC for quantitative work. This was the determination of biphenyl in citrus fruit and fruit products[25]. The sample obtained by steam distillation of the fruit was diluted with hexane and applied to chromatostrips[26]. These were thin-layer strips 13 × 136 mm coated with a fluorescent silica gel containing a starch binder.

What problems were encountered? For one, the adsorbent contained ultraviolet absorbing impurities which were eluted with the sample spot to be subsequently measured at 248 nm. It was possible to decrease this effect by pre-developing or washing the layer with alcohol prior to applying the sample. After this purification procedure, the layers were dried quickly and care was taken not to allow them to become contaminated again by adsorption of vapors from the laboratory. However, it was still necessary to run a blank and subtract the value from the sample run. This blank was taken from an area of plate corresponding to the test sample, and Harris *et al.*[27] found that best results could be obtained by using an equal weight of adsorbent from the corresponding area rather than an equal area. This latter fact again points up the need to have very uniform layers.

In some cases there are inorganic ions, iron for instance, present in the adsorbent that interfere with the determination. There is available acid-washed silica gel; or regular commercially prepared plates may be given an initial development with methanol–concentrated hydrochloric acid (9:1) with subsequent drying and reactivation.

How good are the results with elution methods? Fig. 10 shows the standard curve which was constructed for the determination of biphenyl. This was constructed by adding known quantities to various citrus samples and running them through the

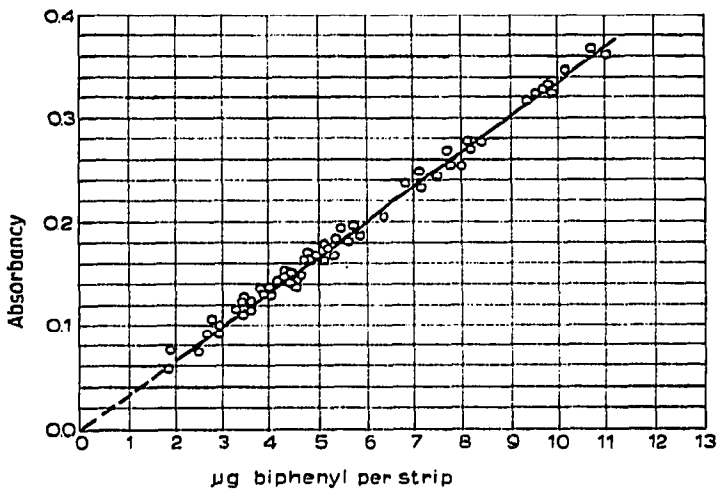

Fig. 10. Spectrophotometric standard curve for determination of biphenyl by means of chromatostrips (λ = 248 nm; 10-mm cell). (From Kirchner *et al.*[25], reproduced with permission of the American Chemical Society.)

entire analysis. In this way one can compensate for the losses which occur during various steps. Seldom can a 100% recovery be made from an adsorbent layer, especially one as strongly adsorbent as silica gel.

In this particular instance we found that it was very essential to have uniformly thick layers, otherwise ultraviolet absorbing materials (in spite of the pre-washing) caused too great an error. To obtain optimum results in the preparation of uniform layers, the glass supporting strips were selected with a micrometer so that they were absolutely uniform.

Hara *et al.*[2] have pointed out that it is necessary to control the quantity of water very carefully in making a slurry so that a slurry suspension of definite density is prepared. The second point they emphasized was the absolute dryness of the applicator and the glass plate. Any moisture present will change the density of the slurry.

If a suitable colorimetric method is available, there is a good chance of having a great deal less interference from impurities extracted from the adsorbent, since in general the determination is shifted to light of longer wavelength. Instead of eluting the compound and then carrying out the reaction to form a colored complex, it may be advantageous to carry out the reaction directly on the silica gel prior to elution.

Of course, in all cases where elution is required, care must be taken to remove all particles of adsorbent.

Radioactive methods

The advantages of using radioisotopes in quantitative TLC lie mainly in the great sensitivity with which they may be detected.

There are a number of methods of detecting and measuring radioactivity that can be applied in thin-layer work.

In autoradiography, an X-ray film is placed in contact with the carefully dried chromatoplate and placed in the dark. The plate should be "carefully dried" in order to prevent the formation of artifacts from traces of solvent left in the layer. Even vapors from the other compounds on the layer may cause artifacts. The possibility of these effects can be decreased by covering the surface of the layer with a plastic spray. However, this also decreases the sensitivity of the detection for radioactive compounds and the plastic spray cannot be used with tritium-containing compounds.

"No-screen medical X-ray safety film" may be used for recording radioactivity, but for tritium, a more sensitive film coated with "Nuclear-track emulsion, Kodak NTB" should be used.

The audioradiographs can be measured by photodensitometry; however, this procedure is a time-consuming operation, and fails for very wide ranges of radioactivity on the same layer.

Instruments are available for measuring the radioactivity directly on the thin layer. Thin-window Geiger-Müller tubes, gas-flow Geiger-Müller tubes, or photo-tubes are used as detectors. These direct scanning instruments are useful if the activity is high enough, but they fail when small quantities of weak β-emitters are present. The photo-tube detector may be used for measuring tritium activity by impregnating the layer with a scintillation solution.

Radioactive compounds may also be eluted and measured in conventional counters.

The most sensitive and precise method for measuring radioactivity is by removal of the spots for liquid scintillation counting. One of the problems encountered here is the quenching of β-energy absorbed by polar compounds. This quenching effect can be corrected by the use of special scintillation solutions and by controlling the size of the adsorbent particles. However, if the quenching effect is too great, the sample may be ignited so that the activity of the carbon dioxide may be determined.

Recently Rapkin and Reich[28] have described an automatic combustion apparatus for igniting all types of samples from which the CO_2 is trapped in a scintillator solution containing β-phenylethylamine; water is also trapped so that tritium can be counted. This apparatus is now available commercially.

Scintillation methods have the disadvantage of making it difficult to recover compounds for further chemical work.

There is one other problem that should be mentioned in connection with the use of radioactive quantitative thin-layer work. This is the problem of assuring the safety of personnel from radioactivity which, adsorbed on finely powdered silica gel can be all too easily dispersed about the laboratory.

Miscellaneous methods

Analogous to the use of X-ray film with radioactive compounds, photographs may be made of suitable spots, and the resulting negatives measured in a densitometer. Caster and Andrews[29], using this method and a specially designed optical integrator, obtained results in the range of 5–10% relative error.

Peterson and Edgington[30] used a bio-autographic method for quantitatively measuring the fungicide benomyl on a thin-layer plate. The area of inhibition of growth of a penicillium mold was linearly related to the log of the amount of fungicide.

Szakasits *et al.*[31] describe a thin-layer scanner in which a thin-layer strip on a narrow metal plate, 3 mm wide, is passed directly between the nozzles of a dual-jet flame ionization detector. The signals are fed to an electrometer, a recorder, and a digital integrator. Very small samples may be used and sample runs showed deviations from 0.1 to 1.8%.

CONCLUSION

In undertaking a quantitative analysis by means of TLC one must take into account: (1) the nature of the substance to be analyzed; (2) the manipulative ability and technique of the operators, especially in applying the sample to the plates; (3) the equipment available; (4) the time factor; and taking into account all of these factors, (5) assess the merits of alternative methods to obtain the desired accuracy.

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