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# MINIATURE THIN-LAYER CHROMATOGRAPHY: A RAPID TECHNIQUE WITH OPTIONAL SPOT EVALUATION BY DENSITOMETRY

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SUMMARY

A technique of micro thin-layer chromatography on 75 × 75 mm plates has been established and compared with that on the conventional 200 × 200 mm size. The main advantages of the smaller plates are saving in cost, economy and speed of processing through all the stages, *i.e.* loading, drying, developing, and detecting the spots. An example compares a development of pentagastrin, a pentapeptide carboxylic acid, for 10 min on the smaller with 60 min on the larger plates. Attention to loading, development systems and sensitivity of detection enables limits of minor zone detection of less than 1% to be achieved. Stability tests on a 0.25 mg/ml isotonic solution of pentagastrin can detect 0.06 $\mu$g of a decomposition product, and in practice with a load of twice 5 $\mu$l this limit amounts to 2.5% decomposition. For a more precise evaluation of small chromatograms an optical method of flying-spot scanning followed by data-processing is described. Glass plates are transparent down to 320 nm; below this synthetic fused silica plates are required. Although recent improvements and applications to scanning at 240 nm will be described later, it is shown in earlier work that the coefficient of variation (% standard deviation) within one plate is 1.9%.

## INTRODUCTION

During our work on the quantitative densitometry of thin-layer chromatograms in the visible and UV[1] we developed a technique based on small square glass or fused silica plates of 75 × 75 × 1.1 mm. Although this smaller size was primarily dictated by the high cost of fused silica, the method now in operation offers many advantages over the use of the normal 20 × 20 cm size. The cheapness and uniformity of the glass, the ease of preparing and storing the thin-layer (TLC) plates, the speed of loading and development, and the modest space required for multiple operation are strong points in favour of work on this scale, as detailed below. An additional facility is the use of a small vacuum vessel attached to a "Drikold" trap and high-capacity vacuum pump for rapid removal of solvent from sets of spots (usually six or seven simultaneously after loading) and from the developed chromatogram. When the vacuum equipment is set up with a large Dewar flask this can be charged with sufficient coolant for three days at one filling. Thus the facility and mildness of the method of loading offers obvious attractions over the alternative of multiple spottings

(or overspottings) on 20 × 20 cm plates with evaporation by warming under nitrogen. The removal of solvents by pumping from the TLC plates also facilitates uniformity in surface appearance after spraying with colour-forming reagents or treating with iodine vapour.

Since these chromatograms are developed in about one fifth to one tenth of the time required for the 20-cm run, it follows that the separation between adjacent spots is smaller. Although this fact is sometimes put forward as a disadvantage and in certain cases an unacceptable limitation, it is usually not difficult to adjust the loadings and separating systems to suit the dimensions available. The operator has a useful path length of about 50 instead of about 150 mm in which to achieve separations.

Limit tests against adjacent reference standards can be set up in the well known way, with a prospect of quantitative densitometry to follow should this be necessary. In the pharmaceutical industry, limit testing is especially useful for defining quality and stability of new drugs acceptable to the registration authorities, before marketing can proceed.

## PROCEDURE

### *Special apparatus*

*Plates.* (a) Micro-plates, size 75 × 75 × 1.1 mm ±0.1 mm; individual plates uniformly thick to within ±0.01 mm are obtainable in microscope slide quality glass cut to size, *e.g.* from Chance Bros., Smethwick, Birmingham, Great Britain. Price at three gross rate is about 1 p each. (b) Optically worked synthetic fused silica is available as 'Spectrosil' from Thermal Syndicate Ltd., P.O. Box 6, Wallsend, Northumberland, Great Britain. Price at tens rate is about £10 each. (c) When the sandwich-chamber or iodination technique is used a set of glass cover plates 75 × 70 × 1.1 ±0.1 mm is required.

*Coating apparatus.* A brass bedplate is machined flat to the dimensions of Fig. 1a. This accommodates four plates plus leading and finishing micro-slides for the start and finish of coating. A brass slurry box is machined to the dimensions of Fig. 1b. The bedplate and slurry box should be washed in cold water immediately after use. As the plates may be required for quantitative determination the bedplate is machined flat to within ± 0.03 mm, and is checked occasionally to see that it has remained flat. Any curvature is removed by scraping by a skilled fitter.

*TLC plates coated with adsorbent.* Clean four of the plates. Normally a final wipe with a linen cloth moistened with methanol is satisfactory. If there is a subsequent tendency for tracks of gypsum to crystallise out of the spread layer, the plates should be cleaned with chromic acid, which prevents this effect. Place the brass bedplate on a level surface. Cover the brass surface with four clean plates and a micro-slide at each end. The plates form a continuous surface bounded by the sills on the brass. Check that the slurry box (Fig. 1b) can be slid along over the plates without contacting the glass edges, and that the glasses are prevented from moving by the sills. Leave the slurry box above the first micro-slide.

To 11 ml of water in a small glass mortar stir in with the pestle about half of a 10-g quantity of Silica Gel G. Add another 11 ml of water and stir in the remainder of the silica gel. Grind slowly for about 15 sec and try to avoid creating bubbles. If

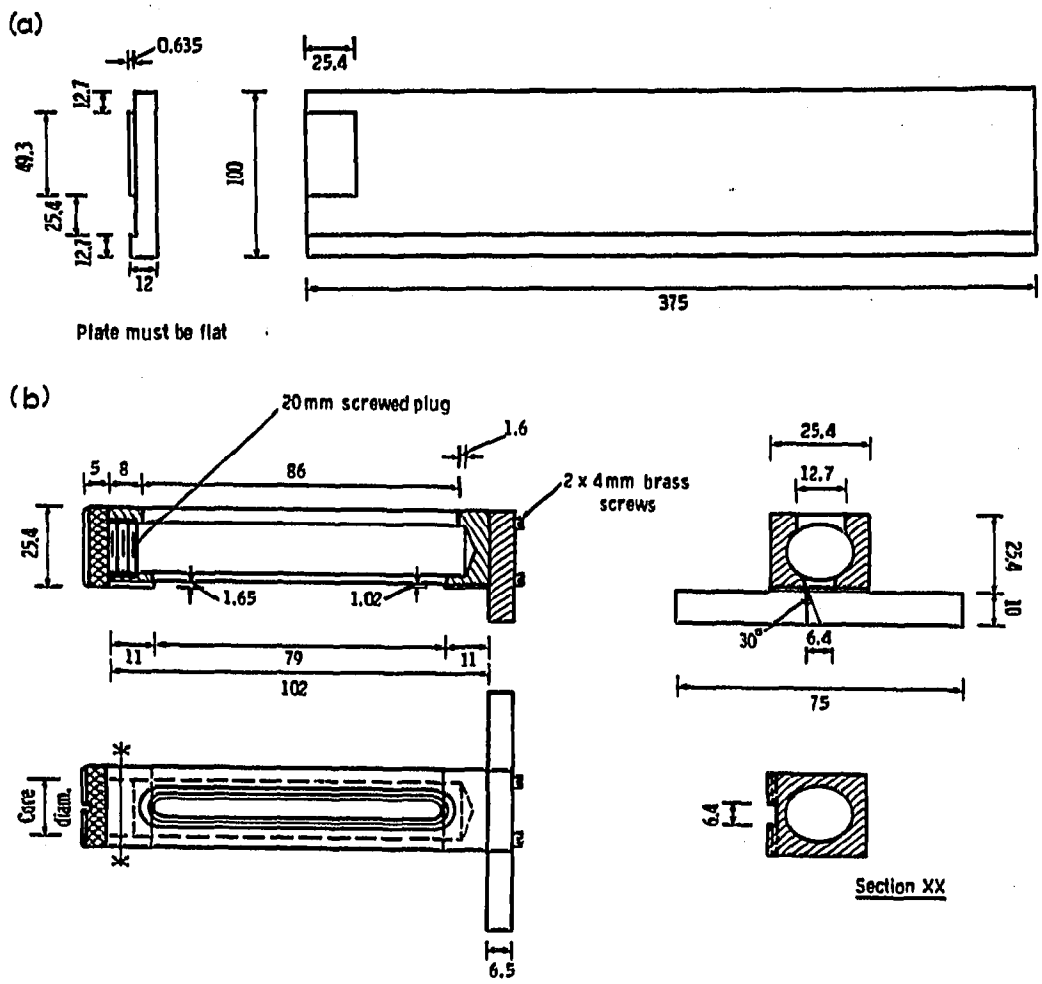


Fig. 1. (a) Brass bed-plate for glass coating. (b) Brass slurry box for glass coating. Dimensions in all figures are in mm.

these appear, transfer the slurry to a 100-ml round-bottomed flask, place the flask in the vacuum vessel and evacuate at the pump for a few seconds till boiling commences and the bubbles burst on releasing the vacuum.

Immediately pour the slurry into the brass slurry box and pull it at a uniform speed of about 2 cm/sec across the plates until it passes off the final micro-slide on to a polythene sheet. If the spread surface is not smooth, thump the bench beside the bed-plate using the clenched fist and repeat the action about ten times. The dispersion and spreading of the silica gel must be accomplished before the slurry starts to set, *i.e.* over a time scale not exceeding about 2 min. Protect the spread layers from dust and after about 10 min, when set, push the plates on to a 20 × 20 cm glass using a PTFE strip to avoid scratching the brass surface. Allow the plates to dry overnight in a horizontal position protected from dust. Activation at 105° may then be applied but is usually not necessary.

*Micropipettes.* 1-, 2- and 5-$\mu$l capillary micropipettes were used (Drummond Scientific Co., Broomall, Pa., U.S.A.). Insert each pipette transversely at the tip of a 30-mm length of silicone rubber tubing (approx. 1 mm bore × 1 mm wall). This comprises a flexible holder facilitating gentle contact with the adsorbent surface.

*Note*: Capillary pipettes cease to function if they pick up a slug of air. This may be cleared by sucking at the bench vacuum tap or blowing via a polythene tube. To maintain rapid action the capillary should be cleaned occasionally by filling with concentrated sulphuric acid in which a few crystals of potassium permanganate have been dissolved.

*Glass vials.* Glass vials, 37 × 12 mm O.D., with screw tops to contain sample and standard solutions were used. To contain the glass vials a rubber block, 180 × 105 × 40 mm, bearing 9 × 5 holes of size 15 × 35 mm deep was necessary.

*Spotting template.* A spotting template for six and seven spots was constructed in Perspex to the dimensions of Fig. 2.

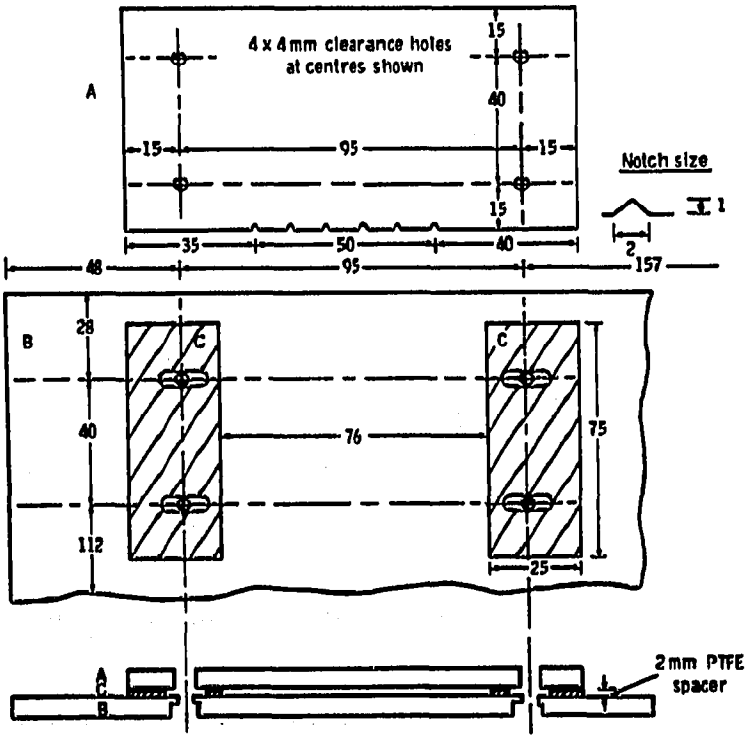


Fig. 2. Spotting template (A), base plate (B) in 6-mm Perspex and PTFE spacers (C) for loading TLC plates.

*Vacuum pump.* A high-capacity vacuum pump was used, the vacuum trap containing dry ice and methanol was connected to a vacuum vessel 135 mm high and 100-mm flange bore via a flat flange lid (100-mm bore). Quickfit FR 700F and lid DA 5/100 from Stone, Staffs, Great Britain, are suitable. The vessel requires a ring stand.

*Developing tank.* The developing tank is to contain 75 × 75 mm plates. Suitable tanks are: Eastman Chromatogram developing jar 6079, 78 mm wide × 80 mm high × 30 mm deep (Eastman Kodak Co., Rochester, N.Y. 14650 U.S.A.) or Shandon Elliot Chromatank 8022, I.D. 100 × 110 × 50 mm (Shandon, 65 Pound Lane, London, N.W.10, Great Britain).

*Densitometer.* If quantitative densitometry of the spots is required, a flying-spot scanning densitometer is necessary, constructed as described in ref. 1. This pre-supposes that off-line data processing facilities are available as described later.

*Procedure*

Weigh sufficient sample in a glass vial to form a concentration of about 0.5 mg/ml of the compound of interest. If this is a minor constituent of the sample, increase the concentration in proportion, if a suitable solvent is available. Up to five samples and two standards may be accommodated on one plate. Position the prepared plate in the template assembly so that when a capillary pipette is held in the locating notches, the loads will be delivered along a line approx. 10 mm above the bottom edge of the silica. Fill a suitable capillary micropipette (*e.g.*, 2 $\mu$l) via the top end with standard solution. Hold it at notch 1 and discharge it via the lower end by gently touching the silica. Charge the capillary with wash liquid via the top end and discharge via the lower end on top of the spot. Avoid excessive pressure, which will dig a hole in the substrate. In a similar manner deliver the second standard and wash at position 6 or 7, followed by the appropriate number of samples in the intermediate positions. Transfer the plate to the vacuum drying vessel and evacuate to 1 mm via the Drikold trap. Continue the evacuation until the spots are no longer visible. With common solvents and water, about 3 min will be required. It may be advantageous to remove the plate after 2 min and warm the back of the plate below the spots with the palm of the hand, so that on re-evacuation the rate of evaporation is maintained.

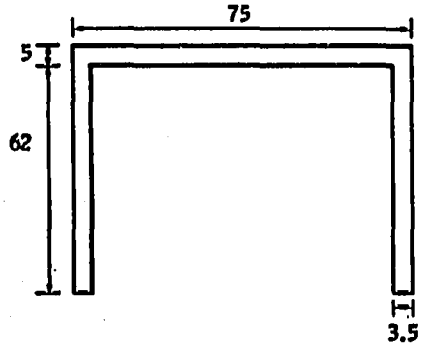


Fig. 3. Gasket for TLC sandwich chamber, cut from PTFE (2 mm thick).

Pour approx. 8 ml of developing solvent into the small developing tank. If required, the silica plate is converted to a "sandwich-chamber" by removing a 5-mm width of gel from the top and sides, placing thereon a PTFE Π-shaped gasket (Fig. 3) and a cover plate (C), and clamping together at each side with two office fold-back clips. Place the plate in the tank in a near vertical position so that the foot is immersed to a depth of about 3 mm. Cover the tank with the glass lid and allow development to proceed until the solvent front has risen to the desired height, *e.g.* within 10 mm of the top. Remove the plate from the tank, dissemble the cover if present, warm the back of the plate to about 50° during 2 min, then transfer to the vacuum vessel and pump at approx. 1 mm until all volatile solvent has been removed. (It is not possible to remove glacial acetic acid quickly in this way.)

Remove the plate from the vessel and apply a suitable method of locating and estimating the spots, *i.e.* either by spraying, by absorbtion of iodine vapour (ref. 2) or by scanning in the flying spot densitometer (ref. 1).

*Scanning with the flying spot microdensitometer*

This apparatus and its use has already been described in ref. 1. The following additional instructions are based on experience.

The glass plates mentioned under *Plates* (a) are suitable for scanning down to 320 nm and in view of the low cost compared to synthetic fused silica (b) they should always be used for preliminary work. The adsorbent layer should be inspected by transmitted light and should be free from "pin-holes" or obvious sudden changes in thickness in the regions where scanning is to be made.

The most conveniently variable reference standard to calibrate the densitometer is dilute Indian ink. To prevent mould growth about thirty drops of the ink are dispersed in 600 ml of dilute aqueous mercuric chloride (2 mg %) and filtered. The absorbance and spectrum of a 1:5 dilution is determined in a conventional spectrophotometer. As the dispersion obeys the Lambert–Beer Law, the primary solution is used to prepare a set of four reference standards in dilute (2 mg %) mercuric chloride. For example a set of four in the absorbance range 2.7–1.6 at 335 nm is convenient for use with Silica Gel G layers in the range 240–450 nm.

Very recently it has been found that the UV source in Fig. 5a of ref. 1, a water-cooled deuterium lamp, is less readily focussed than an air-cooled lamp of lower output, so that the light spot from the latter is more intense at the point of scan.

The analysis is usually in the form of a quantitative comparison of one or more test spot(s) with standards on the same plate, and the procedure is to scan across the direction of $R_F$. The length of individual spots should not exceed 7 mm, but more than twice this length, *i.e.* 16 mm of scanning excursion, is allowed to cover individual variations of $R_F$. It is convenient to mark the scanning boundaries just above the start and below the finish of the scan. In favourable circumstances a fluorescent glass placed in contact with the silica gel layer will locate UV absorbing spots as dark areas which do not transmit UV light of $\lambda = 350$ nm. The boundaries of invisible zones can also be determined by manual operation of the scanning carriage with the scan-motor switch in the "off" position. To do this the plate is inserted in the jaws of the carriage with the plate side facing the source, and orientated so that the up-and-down motion is across the $R_F$ direction. The background Silica Gel G reading is adjusted to about the mid-scale position (500 on the output meter). The plate is then scanned by rapid manual operation of the carriage in both vertical and horizontal directions. A sudden fall in the meter response defines the position of each spot. A mechanical selector (centre, Fig. 3 of ref. 1) is operated to limit the scanning traverse to 16 mm and the plate is then positioned and marked so that all the spots are completely scanned when the plate is moved vertically about the 16-mm limits.

Manual scanning is also useful to check that the maximum spot attenuation is not in excess of a factor of ten (*i.e.* 500–50), *i.e.* one absorbance unit, which is the computing limit.

After these preliminaries, a 6 × 6 mm triangle of silica is cleaned from a suitable corner of the plate such that when the mechanical selector is released this clear triangle can be interposed in the light beam. The plate is then returned to the scanning carriage, and with H.T. voltage at 1000 V or less the slit width is adjusted so that the silica gel background registers about 500 on the output. The detector time-constant is set at 30 msec. The slit width must not be altered thereafter. An orange-coloured plastic sheet is inserted before the first Cassegrain condenser, a

grey reference standard is placed in its holder behind the second Cassegrain condenser, and the TLC plate is moved out slowly by the selector knob until the clear triangle is in the beam. The orange-coloured screen is removed and the reading of the standard noted. This should be in the 100–300 range, if not it should be replaced by one which conforms to these limits. The plate is returned to the start of the 16-mm scanning traverse, the grey standard and orange filter removed and the tape-punch and scan motor power switch are switched on. Scanning is commenced automatically by switching on the scan (auto) switch. Scanning is at 63 msec per reading, unless this is reduced by the delay potentiometer.

A bell indicates completion of scanning, when the scan (auto) switch, scan motor and punch are thrown to "off". The grey standard is recorded once more in the above manner.

The tape is edited as follows. A heading bearing the following information is spliced on: Data title, code No. of tape, number of spots to be computed, $L/S$ ratio, absolute transmission of grey standard $\times$ collection efficiency ($\simeq 3$), standard readings at start and finish. The end of the tape bears the symbol $-1$.

Since the publication of GOLDMAN AND GOODALL[1], the computation has been transferred to an IBM 360, which does not directly accept a tape input. Thus the data tape first goes through a conversion to card images, which are deposited in a magnetic tape store. The computation follows using 200 K in the central processing unit. A maximum of six tapes (about 22,000 data points) may be computed in one sequence. The computer provides total $\mu$g $\times$ absorbtivity together with a point-by-point contour density map of each spot, thus one can see at a glance the shape of the scanned area and whether there is any overlap on other zones of different $R_F$.

## RESULTS

*Comparative test on 20-cm and 7.5-cm plates*

Pentagastrin (N-*tert.*-butyloxycarbonyl-$\beta$-alanyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine amide, ref. 5), 5 mg, was dissolved in 1 ml of 0.1 *M* ammonium hydroxide solution. Standard material and products under test were prepared at this concentration for side-by-side comparison.

In addition limit test spots representing approximately 2 % and 1 % levels of impurity on the above loading were prepared by diluting 40 $\mu$l of the 5 mg/ml standard solution to 2.0 and 4.0 ml, respectively.

A set of 5-$\mu$l spots comprising the standard 25 $\mu$g, two tests at 25 $\mu$g, and the two limits 0.5 and 0.25 $\mu$g were loaded using templates (A) on a 20 $\times$ 20 cm plate and (B) on a 7.5 $\times$ 7.5 cm plate carrying Silica Gel G approx. 0.25 mm deep and previously activated at 105° for 1 h. After drying, the spotted plates were made into a "sandwich" by clamping on appropriate PTFE gaskets and face plates in the manner described above. A suitable developing tank was prepared with an approx. 3-mm deep layer of developer comprising diethyl ether–glacial acetic acid–water (6:2:1). The prepared plates were developed until the fronts of (A) had advanced 11.5 cm and (B) 5 cm, respectively. The plates were then dried, sprayed lightly with a 10 *N* sulphuric acid solution and heated at 105° for about 3 min until purple spots appeared. Fig. 4 shows a sketch of the comparative appearance of each plate together with the distance and time figures. The obvious advantage of the smaller size is that 50 min

have been saved in development time. Further savings in the time of handling and processing are also effected but these have not been numerically assessed. Much time is also saved by drying all the spot loads together in the vacuum vessel during about 3 min. The author tested thirty samples in the above way during less than 4 h.

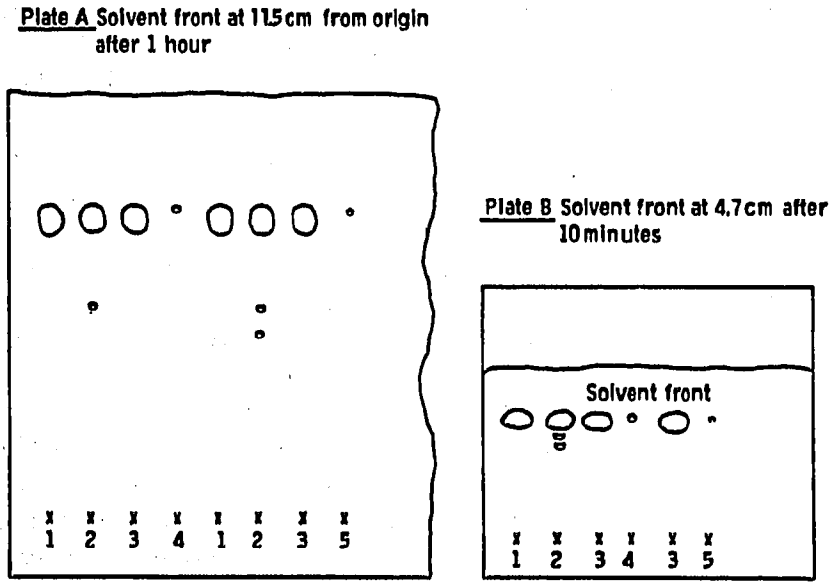


Fig. 4. Comparative tests on 20- and 7.5-cm Silica Gel G plates, drawn on the same scale. Each plate A and B carries similar loads. 1, 2 and 3 are loaded with 5 $\mu$l $\equiv$ 25 $\mu$g of various pentagastrin samples, 4 and 5 are reference limits, loaded from 5 $\mu$l of 1/50 and 1/100 dilutions of standard pentagastrin. Impurity spots as in 2 appear on heating at 105° after a 10 $N$ $H_2SO_4$ spray. These are assessed visually against the limits.

*Limits of sensitivity in the pentagastrin test*

Although pentagastrin is stable in the solid state aqueous solutions tend to decompose on prolonged storage at, or above, room temperature, or on prolonged exposure to light.

A dilute solution of pentagastrin (0.25 mg/ml) is injected into patients for the diagnostic testing of gastric secretion, and it is therefore important to be able to determine whether decomposition products are being formed on storage.

The pentagastrin is not extractable from aqueous solutions by immiscible solvents, nor is concentration by lyophilic drying attractive because the 0.9 % isotonic salt present interferes in subsequent solution. Thus it is a question of pressing the colour reaction to the limits of detection. It was found on the 75-mm plates that the limit of detection after chromatography and colour development as above was 0.063 $\mu$g when viewed by transmitted light. The reference limit had to be applied as a 1-$\mu$l load to obtain a small compact spot comparable in diamter to the decomposition product, and indeed use of higher load volumes produced negative results. The appropriate load for the test was 2 $\times$ 5 $\mu$l with vacuum drying after the first and second applications. The decomposition product has an $R_F$ value of 0.22 in the above system and with the aid of the micro TLC limit tests applied to accelerated and long-term testing a shelf-life of several years when stored in the refrigerator has been established.

*Standard deviations within and between plates*

A solution of acetone dinitrophenylhydrazone (0.254 mg/ml) in toluene was loaded from a 1-$\mu$l Drummond micropipette on to a Silica Gel G plate. The micropipette was washed with one charge of ethanol delivered on to the spot as described under *Procedure*. Using the template and further dilutions of the hydrazone, the plate was loaded with six 1-$\mu$l spots comprising three at 254 and one at 191, 127 and 64 ng, respectively. The plate was dried and then developed with toluene through 5 cm. The plate was dried and then scanned at 365 nm. The experiment was repeated four times: the results are shown in Table I.

TABLE I

STANDARD DEVIATIONS WITHIN AND BETWEEN PLATES

| *Load of DNPH (ng)* | *Found for each spot ($\mu g \times a$)* | | | |
|---|---|---|---|---|
| | *Plate 1* | *Plate 2* | *Plate 3* | *Plate 4* |
| 254 | 27.4 | 28.0 | 28.7 | 28.9 |
| 254 | 25.9 | 27.2 | 28.8 | 28.9 |
| 254 | 26.6 | 27.1 | 28.0 | 29.4 |
| 191 | 21.3 | 20.8 | 20.8 | 20.7 |
| 127 | 14.0 | 14.0 | 14.6 | 13.0 |
| 64 | 6.6 | 7.1 | 7.4 | 6.3 |

Mean $\mu g \times a$ for twelve points at 254 ng = 27.9

| | *Standard deviations* | |
|---|---|---|
| | *Within plates* | *Between plates* |
| On 254 ng (12 replicates) | 0.52 | 1.08 |
| Coefficient of variation | 1.9% | 3.7% |
| On all data points | 0.81 | 0.70 |
| Coefficient of variation | 2.9% | 2.5% |

The experiments in Table I were made in 1969. A repeat of the series using the recent instrumental improvements is amongst future experimental work, which is expected to show significant reductions in the standard deviation.

## DISCUSSION

The preparation of 75-mm plates is a simple matter, given the special spreading equipment. However, the use of the 200-mm size may be more attractive because these are commercially available in the "prepared" state. It is possible to cut these prepared plates to 75 mm but limitations are as follows. Prepared plates are spread on glass or a plastic film base usually with an organic binder. They absorb light strongly

below 320 nm and so are not suitable for work at the lower UV wavelengths. The uniformity and high abrasion resistance of prepared plates is offset by the fact that the binder modifies slightly the behaviour of the prepared layer by comparison with Silica Gel G containing 12 % gypsum. With polar solvents both loading and developing may be twice as prolonged by a reduction in the capillary "suction" effect. The diameters of the spots tend to be wider than on conventional plates of similar thickness. The binder may also modify the adsorbent behaviour, although this may be exceptional. A case in point is the displacement development of foodstuff colours with acidic methanol[3].

When the analyst is confronted with large numbers of tests, *e.g.* in stability or process development studies, the usual strategy is to do a preliminary qualitative "screening test", so as to reduce the number of precise analytical determinations required. It is here that the use of 75-mm plates is particularly attractive in terms of speed and convenience. For example, thirty pentagastrin formulations were screened for stability in less than half a day. In general, if the work load is high, a target of 100 tests per day should not be difficult to organise. The template can be revised to position eight loads at 9-mm intervals with 6-mm margins. Thus if the developed spots are kept less than 8 mm in transverse diameter, 96 chromatograms could be accommodated on twelve plates. The template margins in Fig. 2 are determined by the fact that the vertical oscillation of the scanning carriage is not quite symmetrical about the optical centre of the light beam.

When fractional percentages have to be detected on these smaller plates it is an advantage to start by finding a sensitive colour reaction, as this saves either preliminary treatment or the use of high loads of the test mixture. Absorption of iodine is often quite satisfactory and a suitable technique will be described in ref. 2. Charring with sulphuric acid, or alkaline permanganate oxidation, is also suitable for revealing sub-microgram quantities. Spraying with fluorescein or using fluorescent silica gel is another sensitive alternative.

The limits of determination in the flying-spot scanning apparatus ultimately depend on the peak absorbtivity of the compound, and the precision required. Experience to date is that an analysis range of 10–500 ng can be expected. The determination of substances in the further UV and recent modifications to the scanning device will be detailed in a forthcoming paper[4].

Capillary micropipettes in a flexible holder enable a variety of sample solutions to be loaded very rapidly. Aqueous solutions, particularly of high surface tension as in pentagastrin may require that 1- and 2-$\mu$l pipettes be cleaned by rinsing with sulphuric acid–permanganate after loading each plate. 5 × 10 $\mu$l pipettes do not tend to "stick" when delivering aqueous solutions. 10-$\mu$l pipettes tend to deposit the load too rapidly and could be improved by constricting the exit.

Further experiments are required to determine whether precision is better served by washing out the capillary with solvent on to the spot—thus forming an annular ring or by using a larger pipette with no washing. The comparison required after development is that of scanning a larger less intensely absorbing zone or a smaller more intensely absorbing zone with a scanning light beam which has recently been reduced in diameter by improvements in focussing.

There is possibly a strong economic argument in favour of the small plates (see below). Whether they are suitable for a particular job is a decision for the chemist/

analyst and not for the laboratory controller. There is a tendency to dismiss small plates as either showing inadequate resolution or less sensitivity because of the lower loadings of minor components. The micro TLC technique bears some analogy with micro-weighing, *vs.* macro-weighing. Some effort is required to master the technique. Once this is acquired separations are apparent over smaller distances. The sensitivity argument can also be specious because the limits are determined not by the total amount in a spot, but by the "concentration" of the spot. Thus what is important is that the load should occupy the smallest possible diameter, and where spots of impurity are likely to be near the limit of detection, the diameter of such spots should be controlled to the smallest practicable. This requires that the $R_F$ value be kept low, and that diffusion during development or subsequent processing for detection be kept down by minimal time of development or processing in contact with polar solvents.

In this context the limit of detection of pentagastrin was shown to be 0.063 $\mu$g when applied as a 1-$\mu$l spot which had travelled approx. 3.3 cm. It was not found possible to reach this limit on the 200-mm square plates where the time and distance travelled allowed the reference limit to spread by diffusion.

The economic argument applies to large establishments where many thousand TLC plates may be used annually. A tentative estimate of the money saved by changing to the smaller size is (25 − 5) = 20 pence per plate, i.e. £ 200 per thousand. At a consumption of 100,000 plates per annum a saving of £ 20,000 becomes significant. The operative time reduction is a more economic saving but a rational costing is beyond the scope of this paper. If it is assumed that 50 min/plate are saved as in pentagastrin and that one quarter of this is working time, the saving in cost could be £ 0.6 per plate. Once more large savings follow for the user of many thousands of plates, especially in countries where laboratory salaries are higher than in Britain.

## CONCLUSION

Considerable saving in cost, time and materials is possible by the use of 75-mm instead of 200-mm plates. Since development times are greatly reduced, the distance between separated zones is naturally less, but this does not signify a loss of resolution. Attention to the detail of the technique and the use of a sensitive method of detection enables limits of below 1 % of impurity zone to be achieved.

The simplicity and speed of the qualitative procedure permits a high rate of sample throughput. Thirty samples of pentagastrin are readily chromatographed within 4 h. In general, with four tanks and twelve plates each bearing eight spots, 96 samples per day should be within the capability of one operator. Sample weighing and preparation is not included in this estimate.

For precise evaluation of chromatograms the authors' technique of flying-spot microdensitometry is proposed. The 75-mm glass plates transmit light down to 320 nm, below this synthetic fused silica plates are available. A coefficient of variation of 1.9 % within plates is recorded in early work, recent amendments to the optics are expected to lead to improved precision. Data processing in a large computer using a sophisticated programme is required to deal with the large number of data points recorded and to compute the effect of variation in layer thickness on the plate.

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