being used. The color will, however, be readily apparent when viewed under the microscope.

Results

A mixture of four sterols: cholesterol, lanosterol, ergosterol and 7-dehydrocholesterol were spotted on silver nitrate-impregnated Silica Gel G and the plate developed in chloroform. The average developing distance was about 2.5 cm and the approximate R_F values, which remained fairly constant over several runs, were: lanosterol, 0.95; cholesterol, 0.85; ergosterol and 7-dehydrocholesterol, 0.40 and 0.40 (same type of dienoid system). Although lanosterol and cholesterol ran fairly close together, there was a complete separation in all cases. For a comparison, a regular-sized thin-layer plate was run under the same conditions with 20 γ of sterol mixture. The same degree of separation was obtained for this plate as with the micro plate except that the total migration of the spots was less. When a duplicate regular plate was developed in chloroform–acetone (90:10), very similar results were obtained as with the micro plate except that the ergosterol–7-dehydrocholesterol spot traveled closer to the cholesterol spot with an R_F value of about 0.5. The lower limit of detection for each of the sterols on the micro plate was 0.01 γ.

Discussion

It is suggested that the lower limit of detection of compounds by this procedure may be lowered even further if ways are found to produce scratches of comparable or better quality and in notably smaller dimensions. The adsorbent may be obtained in a somewhat lesser degree of particle size. If these problems can be effectively overcome, then the advantage to the worker, who must deal with very small quantities, need not be explained.

The author wishes to express his thanks to Professor JACQUES LENOIR for samples of the sterols used in this experiment.

Sir George Williams University, Chemistry Department, 1435 Drummond Street, Montreal 107, Que. (Canada) DUART EDGAR

Received May 12th, 1969

CHROM. 4168

A modified loop for sample application in thin-layer chromatography

For quantitative procedures or where the application of a large volume of sample to a chromatogram is required micropipets or microsyringes and syringe drive assemblies[1] may be necessary, but for qualitative work such as urine screening programs where only a few microliters are to be spotted on a thin-layer chromatogram it is much quicker and more convenient to use a wire loop. A loop can be quickly filled,

the aliquot delivered and then be quickly cleaned between samples by flaming. The typical circular loop of wire is unreliable, however, since they tend to be very erratic in the amount of liquid which they deliver when touched to the adsorbent service. The modified loop reported here has the form of a surface capillary, is easily constructed and is far more reliable for the routine application of aqueous solutions to a chromatogram.

Methods

Construction. The loops can be made from small diameter (B&S Gauge No. 24) platinum or Nichrome wire. The latter is not only less expensive but is more rigid and less likely to be deformed through frequent use. Jewelers pliers and forceps are used

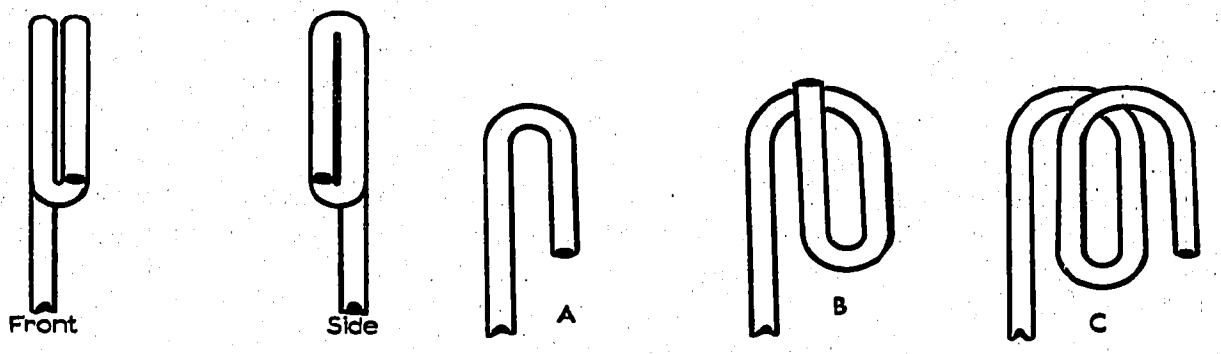

Fig. 1. Diagrammatic appearance of the spotting loop; front and side views.

Fig. 2. Three steps in the construction of the spotting loop. Bends are shown open for clarity but are actually as seen in Fig. 1.

to bend the loops to shape. The exact dimensions and the volume delivered can be varied. The general shape, however, is like that of two adjacent bent fingers in which the fluid is retained by the space and grooves between the knuckles (Fig. 1).

Approximately 2 mm from one end of the wire a bend of 180° is made and the wire is pressed flat (Fig. 2). This bend is then doubled over and pressed flat again so that the end of the wire is pointed in the original direction (Fig. 2b). The double bend of wire is bent over again (Fig. 2c), great care being taken that the two "knuckles" are exactly even. The wire is pressed firmly and tightly together to provide rigidity and the construction of the loop is finished (Fig. 1). For use it can be mounted in a standard commercially available loop holder or inserted in a cork or wooden handle. A loop of the dimensions given delivers approximately 0.33 μl of an aqueous solution. By varying the length of the bends, the volume delivered can be increased or decreased.

Evaluation. The volumes delivered by the loops were measured by three different techniques: (1) measuring the size of a dye spot delivered by a loop, (2) colorimetric determination of the amount of dye delivered by a loop, and (3) measurement of the amount of a radioactive solution delivered by a loop.

Spot area was determined by delivering a 1% aqueous solution of Crystal Violet (C.I. No. 42555) onto Whatman No. 40 filter paper. Spot diameters were measured with a Pickett No. 419M aluminum ruler graduated in hundredths of an inch using a Bauch and Lomb 7 × magnifier. A standard curve was prepared by incrementally

spotting 1, 2, 3, 4 and 5 μl of dye with a 1 μl Lang–Levy micropipet. (Bie and Bernsten, Denmark). The volume of dye applied to filter paper can be regarded as having a cylindrical form with the thickness of the paper equal to the height of the cylinder. This is expressed mathematically as $V = \pi r^2 h$ where V is the volume of dye, r is the radius of the spot and h is the thickness of the paper. For this work, the thickness of the paper is essentially a constant and different dye spots can be simply compared by means of the equation:

$$\frac{V_1}{V_2} = \frac{r_1^2}{r_2^2}$$

Five loops were calibrated by this technique.

The dye dilution and radioisotopic methods for loop calibration were quite similar in that in both cases the test solution was delivered onto a 6 mm (1/4 in.) circle punched from cellulose acetate membrane (Gelman Sepraphor III) and the membrane and applied material dissolved in *p*-dioxane. In this way there was no possibility of incomplete elution of the applied material. The 1% Crystal Violet solution was used for the dye dilution procedure. A standard curve was prepared using a 2.0 μl Lang–Levy micropipet (Microchemical Specialties Company, Berkeley, Calif.) and a series of serial dilutions of the original dye solution. The membrane circles with the spotted dye were dissolved in 1.0 ml of *p*-dioxane and the absorbancy read at 590 nm. Four loops were calibrated by this method.

TABLE I

EVALUATION OF THE LOOPS

Values are the means plus or minus the standard deviations calculated from six replicate measurements. The methods are described in the text.

Loop	*Spot area* (μl)	*Dye dilution* (μl)	*Isotopic count* (μl)	*Average variation* (%)
A	0.31 ± 4	0.31 ± 4		± 9.7[a]
B	0.33 ± 5		0.33 ± 6	±15.1
C	0.24 ± 2	0.25 ± 5	0.26 ± 2	± 8.1
D	0.35 ± 3	0.32 ± 4		± 7.6
E	0.50 ± 3		0.45 ± 5[b]	± 8.5
F		0.47 ± 4	0.51 ± 2	± 5.3

[a] Average deviation from the mean divided by the mean × 100. Overall average is ±9.1 %.
[b] Mean of 12 determinations.

For radioisotopic calibration a solution of [3-^{14}C]L-serine* was spotted onto the cellulose acetate membrane circles and dissolved in a dioxane-based counting fluid[2] and assayed in a liquid scintillation counter (Beckman Model No. LS-200B). For standardization three different 1 μl pipets were used to spot a series of nine membrane discs. Five loops were calibrated by this method.

* Calbiochem, Los Angeles, California, 25 μC/1.0 ml, 25 μC/μmole; 5.52 × 10^4 c.p.m./μl.

The protocol and calibration data are summarized in Table I. All recorded figures are the averages of six replicate determinations.

Discussion

For quantitative work a volumetric device which delivers with a variability of ±10% would be quite unacceptable but for routine qualitative work this level of reproducibility is quite satisfactory. Combined with the speed, convenience and ease of cleaning, loops of this design have been found to be the most suitable technique for sample application in a urine screening program based on TLC methods[3]. With a delivery volume of approximately 0.33 μl the origin spot is kept quite small and the drying time is very brief. Repeated flaming of the wire undoubtedly has some effect on the volume delivered but this is probably minimal and within the permissible limit of error. Loosening of the wider bends can be serious and ideally should be prevented by soldering the bends together. If the wire has been bent tightly and is not subject to abnormally hard use, loosening can be minimized. Loops made to deliver a small volume are more compact in design and consequently more rigid and less susceptible to deformation during use.

Loops to deliver substantially larger volumes can be made by adding additional bends of wire to the basic design, again, taking care that they project evenly. Heavier wire could also be used.

Optimally the wire should be dipped in the spotting solution only enough to fully cover the loop. Most of the loop variability was probably due to errors in this since the technique used was more routine than optimal.

The three different calibration techniques used here can be generally applied to the calibration of other spotting devices. The isotopic counting technique is certainly the most elegant but really offered no advantages over the spot area method which required less than $10.– worth of apparatus.

In view of the accepted imprecision of the loop, it was not necessary to establish more exact volumetric standards. The calibration measurements were primarily to determine reproducibility rather than to measure the true volumes delivered. The similar variances of the three calibration techniques—which were of widely different sensitivity—indicate that the variation was in the loops and not in the assay techniques.

This work was supported by a grant from the American Institute for Mental Studies which is gratefully acknowledged.

The generous help of Dr. PAUL MANOWITZ in the radioisotopic procedures is greatly appreciated.

The Metabolic Laboratory for Mental Retardation,
Department of Psychiatry and Neurology,
New York University Medical Center,
550 First Avenue, New York, N.Y. 10016 (U.S.A.)

S. SAMUELS
P. L. SHANK

1 S. SAMUELS, *J. Chromatog.*, 32 (1968) 751.
2 H. WERBIN, I. L. CHAIKOFF AND M. R. IMADA, *Proc. Soc. Exp. Biol. Med.*, 102 (1959) 8.
3 S. SAMUELS, P. L. SHANK AND G. VELIZ, in preparation.

Received May 5th, 1969