THE PRECISION OF SOME PROCEDURES IN PHARMACEUTICAL ANALYSIS

PART I. USE OF A PIPETTE AND A BURETTE

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Estimates have been made of the variance associated with the calibration of a number of 10- and 20-ml. pipettes and 50-ml. burettes of Grade B quality, and of the variance associated with their use by a class of students. Comparison with the variance of the results of students performing simple titrations with this apparatus indicates that the chief components of the latter variance have been identified.

THE tolerances for the purity of official drugs are framed to take into account variations due to the sampling and assay procedure used, as well as variations due to manufacturing processes. Saunders and Fleming[1] have pointed out that "it would be extremely useful if the percentage standard deviation of the different assay methods of the British Pharmacopoeia could be published in the monographs. The data for calculating them is available in the schools of pharmacy and probably also in a number of industrial analytical laboratories".

During the last few years, a number of workers[2–4] have studied the precision and accuracy of the weighing and measuring operations of extemporaneous dispensing. In those experiments where the accuracy of dispensing is checked by a physical or chemical assay, it is necessary to establish that the assay errors are insignificant in relation to the variations in the dispensing. Although this should always be established within the experimental pattern of the dispensing measurements, it would be helpful in designing and planning these experiments if an "external" estimate of the precision and accuracy were already available.

A detailed discussion of errors associated with the use of volumetric apparatus has been given by Conway[5]. In this paper, an attempt is made to estimate the variation associated with the use of a pipette and a burette by students. The appropriate sum of the variances of these individual operations will be an underestimate of the variance of a complete titration, because not all of the sources of variation will have been identified and measured. The total variance of a titration, estimated by this "synthetic" process, should therefore be compared with the variance of the results obtained in practice. This approach is similar to that adopted by Capper and Dare[4] in an investigation of the precision of measuring and weighing operations in dispensing.

It is important to note that it is not the accuracy (or "correctness") but the precision or reproducibility of analytical operations that is being studied in this series of papers. Nevertheless, since each member of the class of students used a different pipette and burette in the experiments where a complete titration was performed, the accuracy of calibration of

these pieces of apparatus was checked so that appropriate allowance could be made for this source of variation.

Use of a Pipette

About 80 students and members of staff of the school were instructed to proceed according to the following method[6], on one occasion with a 10-ml. pipette, on another with a 20-ml. pipette. Fill the pipette by suction from the vessel, which contains distilled water at 21·0° ±0·5°, to about 2 cm. above the mark. Close the upper end of the pipette with the tip of the dry finger, and wipe any adhering water from the outside of the lower stem. Allow the water to run out slowly by slightly relaxing the pressure of the finger. Hold the pipette vertically so that the mark is at the same level as the eye, and tighten the finger on the mark when the meniscus just reaches the graduation mark. Remove any drops adhering to the tip by stroking against a glass surface. Allow the water to run out into the (already tared) weighing bottle, the tip of the pipette touching the wall of the bottle. When the continuous discharge has ceased, hold the jet in contact with the side of the bottle for a further 15 seconds. Then remove the pipette from contact with the bottle, thus removing any drop adhering to the outside of the pipette.

The weighing bottle was weighed to the nearest 0·1 mg. on a Sartorius "Selecta" semi-micro balance by the author before and after the delivery of the water, and the volume of water which had been delivered was calculated. All weighings were performed within 3 hours of the pipetting, and the bottles were kept stoppered except while being used. The same 10- or 20-ml. pipette was used by every worker taking part in the experiment. The pipettes complied with British Standard, 1583 : 1950.

The standard deviation of the volumes was calculated after rejection of those results, about 10 per cent of the total, where it was observed that the person using the pipette had either not allowed 15 seconds for after-drainage, or not held the jet of the pipette against the side of the bottle during the drainage period, or both.

As a check of this method of estimating the standard deviation, a straight line was drawn by eye through those points on a plot of probit of cumulative frequency against volume of water that came within the range of probits four to six, although attention was also paid to the trend of points lying outside thise range; the reciprocal of the slope of this line was taken[7] as an estimate of the standard deviations of those volumes that had been pipetted according to the established procedure. Tests for "outliers" in observational data have been described[8], but in this procedure the suspected "outliers" were not rejected outright; instead, less weight was attached to them than to the rest of the results. Almost identical standard deviations resulted from the application of these two different procedures, namely 0·0092 ml. for a 10-ml. pipette and 0·0204 ml. for a 20-ml. pipette. The contribution towards the total variance of a titration made by the use of a pipette, assumed to be correctly calibrated, was therefore taken as 0·00008 ml.^{2} for a 10-ml. pipette and 0·00042 ml.^{2} for a 20-ml. pipette.

The raw data are plotted sequentially in Figures 1 and 2. In each diagram, the continuous horizontal line represents the best estimate of the mean, and the dotted lines represent one standard deviation above and below the mean, respectively.

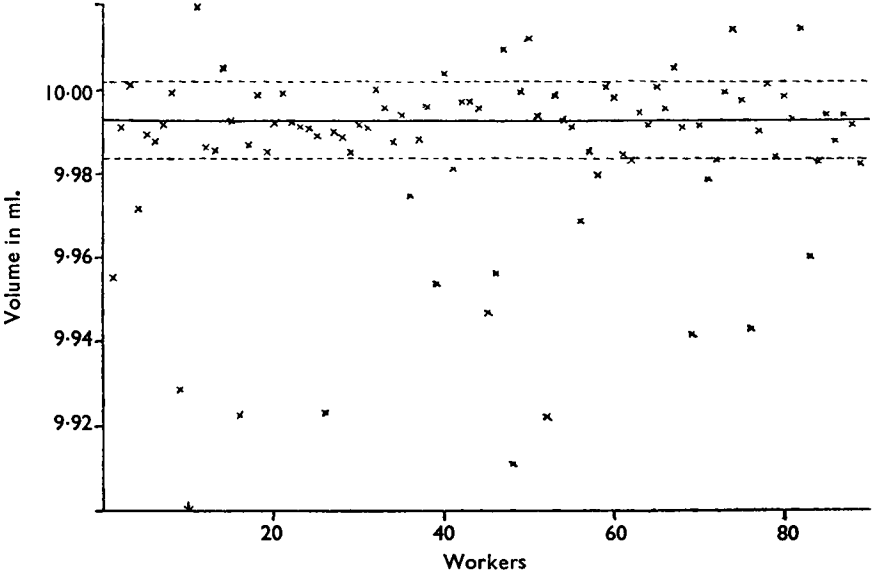

FIG. 1. Volumes of water delivered by different workers using the same 10-ml. pipette. —— Mean. - - - - One standard deviation above and below the mean.

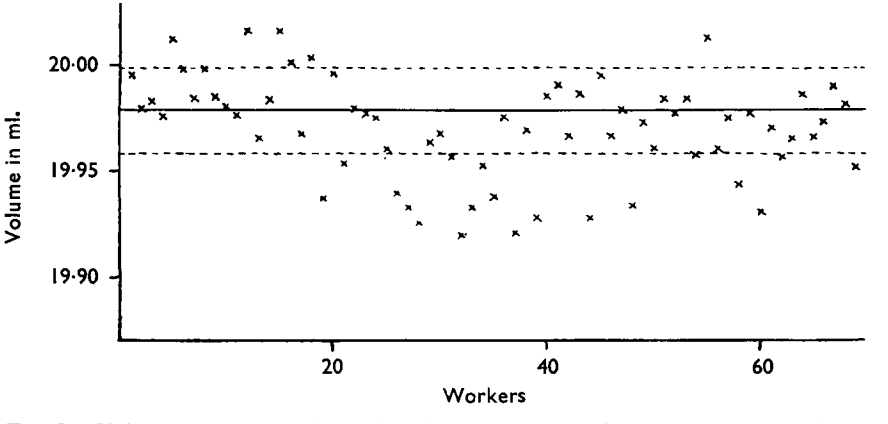

FIG. 2. Volumes of water delivered by different workers using the same 20-ml. pipette. —— Mean. - - - - One standard deviation above and below the mean.

USE OF A BURETTE

About 90 students and members of staff were instructed to read six burettes and record the level of the liquid in each. The burettes were all sealed, top and bottom, and one contained a thermometer which

showed that the temperature of the contents stayed at 21·0° ±0·5° while the experiment was in progress. Four burettes contained distilled water and two contained 0·1N aqueous potassium permanganate. The liquid levels were arbitrary, around the 5-ml. mark in all cases.

In a small number of instances, less than 1 per cent of the total, a gross mistake in reading the burette occurred, for example 5·93 ml. was recorded instead of 4·93 ml.; in these the recorded figure was corrected. No corrections of this type were made unless the correction was exactly 1·00 ml. or, in one reading, 0·50 ml. Mistakes less than 0·50 ml. in magnitude, if they occurred, were included and may have contributed towards the total variance.

For each person collaborating, the average of the readings of the four burettes with water was calculated. A histogram of the distribution of these averages showed that there were two peaks to the frequency distribution (see Fig. 3), which were separated by about 0·04 ml. These are

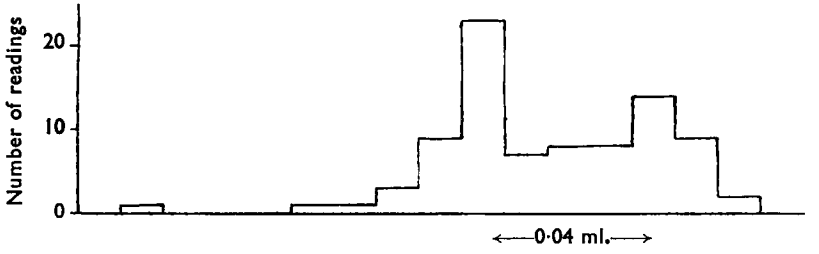

FIG. 3. Histogram showing frequency distribution of average burette readings with water.

presumably caused by some workers observing the "true" meniscus and others the "false" meniscus[2]. For aqueous potassium permanganate, the histograms of the average burette readings showed two quite distinct peaks separated by about 0·15 ml. (see Fig. 4). It is thought that these are caused by some workers observing the top of the meniscus and others the bottom of the meniscus.

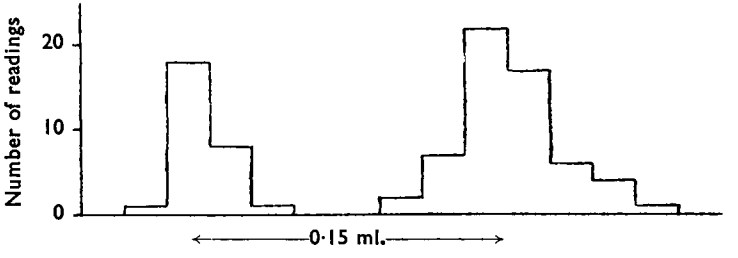

FIG. 4. Histogram showing frequency distribution of average burette readings with 0·1 N aqueous potassium permanganate.

It was clear from the raw data that workers were almost without exception consistent in reading either the top or the bottom of the meniscus, or the "true" or the "false" meniscus. This is important when it is remembered that in practice a burette reading is one of a pair of

readings, and it is the difference between the pair of readings that must be known accurately. Mechanical aids to reading a burette are quite commonly used in practice, and this also would help to ensure that the meniscus is observed in a consistent manner.

The six sets of burette readings were taken in pairs, namely two pairs for water and one pair for aqueous potassium permanganate. For each pair, the probit of the cumulative frequency was plotted against the value of the difference between the two burette readings. The standard deviation of the difference between a pair of burette readings was estimated from the slope of a line through the points in the manner described above. The results for water were 0·0264 ml. and 0·0238 ml.; these gave an F-value of 1·234 with 86 and 85 degrees of freedom, so the estimates were pooled to give a standard deviation of 0·0252 ml. for water. The result for aqueous potassium permanganate was a standard deviation of 0·0314 ml.

The raw data showed that in almost all cases the burettes had been read to the nearest 0·03 ml. Since the true distribution of frequency was continuous, and the frequency tapered off to zero in both directions, the variance calculated from the discontinuous data was corrected for the grouping effect by subtraction of one-twelfth of the square of the class-interval, 0·03 ml. (Sheppard's correction[9]). The contribution towards the total variance of a titration made by reading the burette, assumed to be correctly calibrated, before and after the titration was therefore taken as 0·00056 ml.^{2} for transparent aqueous solutions and 0·00091 ml.^{2} for aqueous potassium permanganate.

Calibration of Pipettes

The calibration of the forty-one 10-ml. pipettes used by the class of students were checked by the author by the procedure described under "use of a pipette". All were of grade B quality (as labelled by the manufacturers) or better, in calibration. In addition, the volume of one pipette was determined 29 times in replicate, so as to obtain an estimate of the variance of the calibration procedure; this was found to be 0·00003 ml.^{2}

The apparent variance of the forty-one 10-ml. pipettes was 0·00034 ml.^{2}, so the corrected estimate of the contribution towards the total variance of the titrations of a class of students made by faulty calibration of the 10-ml. pipettes was taken as 0·00031 ml.^{2}, corresponding to a standard deviation of 0·018 ml.

Similar experiments were made on the 20-ml. pipettes. The apparent variance of the 20-ml. pipettes was 0·00086 ml.^{2} and the variance of the calibration procedure was again 0·00003 ml.^{2}, so the corrected estimate of the contribution towards the total variance of the titrations of a class of students made by faulty calibration of the 20-ml. pipettes was taken as 0·00083 ml.^{2}, corresponding to a standard deviation of 0·029 ml.

It is appreciated that the figures reported in this section are of little interest outside the context of this paper. The calibration of a pipette

is a simple and rapid matter, and it would be expected that analysts in general use pipettes of Grade A quality or better, so that the usual variance from this source may be less than reported here.

Calibration of Burettes

A small random sample of burettes used by the class of students was taken, and the calibrations checked at 2-ml. intervals by the author according to the following procedure[6].

Fill the burette with water to a short distance above the zero mark, and slowly run out water until the meniscus is exactly on the zero mark. Remove the drop of water adhering to the jet by bringing the jet into contact with a glass surface. Allow the burette to discharge freely into the (already tared) bottle. When the meniscus of the water is about 1 cm. from the line to be tested, reduce the rate of outflow so that the motion of the water surface is brought under complete control, and adjust the meniscus exactly on the mark. Remove the drop adhering to the jet after the setting has been made by bringing the side of the bottle into contact with the jet.

In each instance the discharged water was weighed as described before. The volumes were calculated and the discrepancies between the observed and the theoretical volumes and hence the variance of the discrepancies were calculated. In addition, the variation of the calibration procedure was estimated by replicate determinations to be 0·00002 ml.^{2} The variances of the errors of the graduations of the seven burettes studied, corrected for the calibration procedure variance, ranged from 0·00023 to 0·00061 ml.^{2} The application of Bartlett's test[10] showed the absence of heterogeneity of the various estimates so they were pooled to give a variance of 0·00039 ml.^{2}

Because a burette is read twice in a complete titration, the corrected estimate of the contribution to the total variance of the titration results of the class of students made by faulty calibration of the burettes was taken as $2 \times 0{\cdot}00039 = 0{\cdot}00078$ ml.^{2}, corresponding to a standard deviation of 0·028 ml. The estimate is based on rather a small sample of the 41 burettes used by the students, but it is thought not to be seriously in error. All of the burettes tested were found to be of grade B quality, as labelled by the manufacturers, in calibration, and apart from one burette they were only just outside the grade A tolerances. This is a fortunate occurrence in view of the length of time required to check the markings of a burette.

Discussion

Records have been kept during the present session of all classwork in quantitative analysis performed by the 40 or so first-year degree and diploma students whose pipettes and burettes had been checked. It is hoped in Part II of this series of papers to present a detailed report of the reproducibility of their results in titrations, and to discuss the value of the results in estimating the relative standard deviations of the official

assay procedures. Here it is sufficient to say that in the easiest and most accurate titrations, such as peroxide—permanganate and some acid—base titrations, which involve one pipetting operation and the use of a burette, a coefficient of variation (relative standard deviation) of results of about 0·25 per cent was found.

Taking the variances for the operations to be those given earlier in this paper, the coefficient of variation of results in a titration where 20 ml. of solution, taken by pipette, gives a titre of 20 ml. of a colourless reagent would be,

$$\sqrt{\frac{0{\cdot}00042 + 0{\cdot}00083}{20^2} + \frac{0{\cdot}00056 + 0{\cdot}00078}{20^2}} = 0{\cdot}0025 \text{ or } 0{\cdot}25 \text{ per cent.}$$

Similarly for 10 ml. of solution giving a titre of 35 ml. of 0·1N aqueous potassium permanganate, the coefficient of variation of the results would be,

$$\sqrt{\frac{0{\cdot}00008 + 0{\cdot}00031}{10^2} + \frac{0{\cdot}00091 + 0{\cdot}00078}{35^2}} = 0{\cdot}0023 \text{ or } 0{\cdot}23 \text{ per cent.}$$

These figures are of the same order of magnitude as the experimental results for the titrations performed by the class of students using the apparatus in those cases where there were no special difficulties or other large sources of variation or error. The discrepancies which do exist can be attributed to (*a*) difficulty in deciding on the indicator colour to select as the end point, (*b*) temperature differences, (*c*) use of dirty apparatus, (*d*) incorrect use of pipette and burette, (*e*) irregularities in the amounts of other reagents added, (*f*) loss by splashing, and (*g*) other factors not identified. The effect of factor (*a*) will vary from one type of titration to another, but it is probably very small in the most favourable instances. For most aqueous solutions, factor (*b*) is small enough not to be significant, though correction must be made with solvents such as glacial acetic acid. Factors (*c*) to (*f*) are difficult to measure, and since they are "mistakes" that should not occur, no attempt to estimate their magnitude has been made.

It is concluded that the chief sources of variation in titrimetric results, with the exception of the "indicator blank", have been identified and measured.

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DISCUSSION

The paper was presented by the AUTHOR.

The CHAIRMAN. Moran had shown that duplicate analyses made at the same time were not truly random. Would the Author's results have been different if the readings had not been made on the same day? How was the apparatus cleaned?

DR. D. C. GARRATT (Nottingham). Would the Author present figures from industrial laboratories together with his other results in future papers?

DR. J. G. DARE (Leeds). It had been found that the standard deviation in the analysis of sulphuric acid by industrial analysts was 0·3 per cent which was close to the author's own figure for a simple titration of 0·25. He had found that when students expected to obtain the same answer in a duplicate determination they tended to do so.

MR. H. D. C. RAPSON (Betchworth). He had collected similar data and often found skew results. If statistical methods were applied, a skew parameter as well as standard deviation should be considered.

DR. L. SAUNDERS (London). He had expected a reliability of about 0·2 per cent in a volumetric analysis, a standard deviation of 0·25 per cent seemed high. Was it correct to add the figures for the calibration variants at this stage?

MR. C. A. JOHNSON (Nottingham). In his experience the greatest source of error in a volumetric determination was the recognition of the end point.

MR. G. R. WILKINSON (London). Figures obtained by a number of skilled and unskilled analysts for the factor of sulphuric acid using the same reagents and apparatus, varied from 0·998 to 1·002.

MR. ROGERS. Chromic acid was used for cleaning burettes, with 20 washings with tap water followed by 3 or 4 with distilled water. Skewness was showing up all the time, sometimes one way, sometimes the other, even in the same assay, but he thought he knew the cause. Many of the experiments were spead over three or four days. The figure of 0·25 per cent had been quoted based on unpublished work. He included calibration figures so that in a future paper he could be sure that the figures achieved using the apparatus were not markedly discrepant from their best results. He hoped that industrial firms would publish their figures.