THE PRECISION OF SOME PROCEDURES IN PHARMACEUTICAL ANALYSIS

PART II. TITRATIONS

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Estimates have been made of the variation of the results obtained by students in eight schools of pharmacy and by analysts in five industrial laboratories performing simple titrations. Coefficients of variation in the range 0·1 to 0·2 per cent were typical of the industrial analysts. The corresponding figures for the students were mostly in the range 0·2 to 0·5 per cent.

IN Part I[1] of this series, estimates were made of the magnitude of the variance of the volumes delivered by 10- and 20-ml. pipettes in the hands of a number of students. The total figure was the sum of the between-students variance and the between-pipettes variance. Similar estimates were made of the magnitude of the variance of the volumes delivered by burettes. Again the total figure was the sum of contributions from the students (errors of reading) and the apparatus (errors of calibration). The between-pipettes and between-burettes variances found in Part I can be only an approximate guide to the variances of other batches of volumetric apparatus, but the between-students variances are probably good estimates of between-analysts variances for these operations.

In the present paper, the total variation associated with the use of a pipette and a burette has been compared with the variation of titres found when students and industrial analysts take by pipette an aliquot portion of a sample and titrate it with reagent added from a burette. The objectives were to discover whether the reproducibility of results obtained by students is similar to that of results obtained by industrial analysts and if so, at any rate for some titrations, whether the variation is significantly greater than that which can be attributed to variation associated with calibration and correct usage of the apparatus. A high variation could be attributed in part to difficulty in detecting the end point.

There are comparatively few reports of the precision with which volumetric analysis is normally carried out on a routine basis, as in testing for compliance with pharmacopoeial specifications. Of these, some relate to titrations which involve weighings or to back-titrations, and so are not directly relevant to the subject of this paper. Bishop[2] has stated that the accuracy and precision of routine volumetry and gravimetry are about the same, roughly 0·1 to 0·5 per cent.

Some figures relating to titrations made by large numbers of students have been published. Students of Farquhar and Ray[3] beginning their laboratory course in chemistry gave results which indicated a coefficient of variation, that is, a relative standard deviation, of about 3 per cent for the assay of a sample of vinegar with the use of standard 0·25N acid and approximately 0·25N alkali. Cooper[4] published results obtained by

students in their first semester of quantitative analysis, who achieved a coefficient of variation of about 0·9 per cent for the assay of samples of sodium hypochlorite solution. Chapman[5] found that beginning students were able neither to obtain closely agreeing results in volumetric analysis nor to obtain close agreement with the results obtained by other students; coefficients of variation of about 0·5 per cent were reported for simple acid-base titrations. Park[6] reported that beginning students in quantitative analysis obtained a coefficient of variation of about 0·3 per cent for the volumetric determination of chloride by the Mohr and the Fajans methods.

RESULTS OBTAINED BY BRIGHTON STUDENTS

For several years, detailed records have been kept of all classwork in practical quantitative analysis made by first-year degree and diploma students of the Brighton School of Pharmacy. Some results obtained
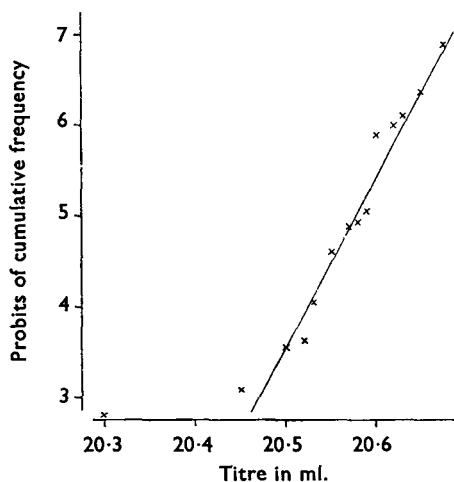

FIG. 1. Distribution of 70 results for the titration of 20 ml. of 0·05M sodium edetate with 0·05M lead nitrate.
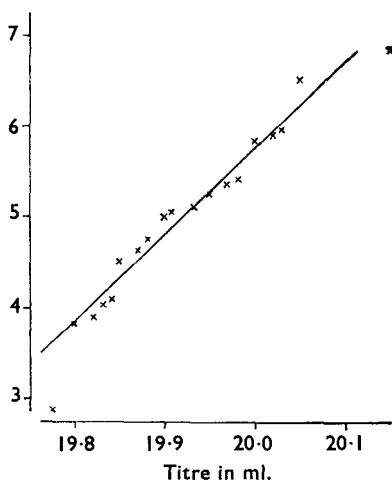
FIG. 2. Distribution of 58 results for the titration of 20 ml. of 0·1N iodine with 0·1N sodium thiosulphate.

by second-year students were also available; they showed no improvement over those obtained by first-year students. The figures were taken directly from the students' original laboratory notebooks, and the nature of the supervision was such that all results obtained by the students were recorded and not merely the "two best" or any other selection. All calculations, including subtractions of burette readings, were checked.

The figures used were the titres obtained when each of a large number of students (30 or 40) took by pipette an aliquot portion of a given solution and titrated it with a given volumetric solution run in from a burette. Each student used his own pipette and burette. The titrations were made in duplicate, although occasionally a student would record the result of three or four replicate titrations, and occasionally only a single result would be available.

Variances were calculated from standard deviations estimated from the reciprocal of the slope of a plot of probit of cumulative frequency against titre[1,7]. Note that a plot on ordinary graph paper of probit of cumulative frequency against titre is equivalent to a plot on probability paper of cumulative frequency against titre. When the plot was slightly curved, a straight line was drawn by eye through those points that came within the range of probits four to six, although attention was also paid to the trend of points outside this range. When the plot was highly curved or S-shaped, the results were discarded; over one-third of the Brighton results were discarded for this reason.

A typical graph is shown in Figure 1, which is derived from the 70 results obtained by 35 students titrating 20 ml. of 0·05M sodium edetate with 0·05M lead nitrate with xylenol orange as indicator. (I am grateful
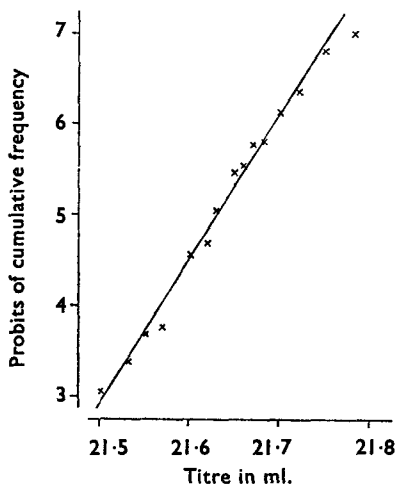

FIG. 3. Distribution of 76 results for the titration of 20 ml. of 0·5N hydrochloric acid with 0·5N sodium hydroxide.

to Mr. C. A. Johnson for suggesting this titration which has a very sharp end point.) The standard deviation calculated from the graph is 0·056 ml., which is equivalent to a coefficient of variation of 0·27 per cent; the co-efficient of variation calculated directly from the 70 titres by summing squares of deviations, and so on, is 0·36 per cent. The graphical method of estimation is preferred because it gives less weight to the "outliers" (results a long way from the mean) which probably arise from mistakes rather than from an accumulation of small normally distributed chance errors.

Figure 2 shows a graph which is a good approximation to a straight line, but the coefficient of variation of 0·51 per cent seems high for such a simple titration, namely, the titration of 20 ml. of 0·1N iodine with 0·1N sodium thiosulphate. By contrast, Figure 3 shows a graph constructed from results for the titration of 20 ml. of 0·5N hydrochloric acid with 0·5N sodium hydroxide with methyl orange as indicator; the coefficient of

variation is 0·28 per cent. Over 30 graphs of this type are now available based on the results of Brighton students.

It is customary in the schools of pharmacy to require a student to make every titration in duplicate, and to hand in two separate results. This procedure sometimes has the advantage of showing a student that it is possible to get close agreement between replicate determinations, and it also provides additional practice in the various exercises. On the other hand, close agreement of replicates may blind a student to the possibility of bias in the procedure or in the way in which it has been carried out. An analyst in an industrial control laboratory will normally perform a
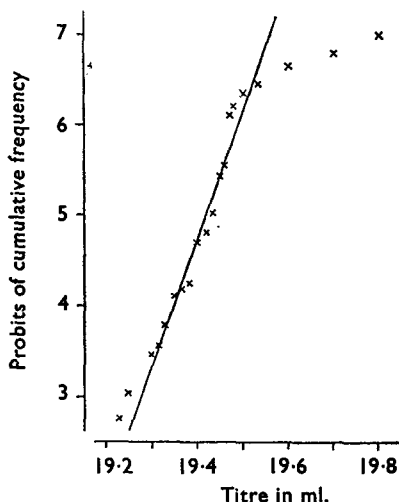

FIG. 4a. Distribution of 80 results for the titration of 20 ml. of 0·1N silver nitrate with 0·1N ammonium thiocyanate. Each student made two titrations.
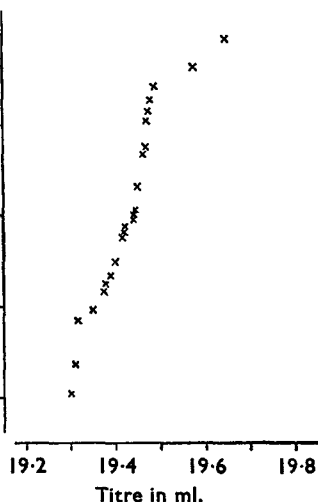
FIG. 4b. Distribution of 40 mean results obtained as in a.

single determination, and replicate analyses will be done only in special circumstances, and then preferably by another analyst on another occasion. A replicate titration made concurrently with or immediately after a first determination by the same analyst in the same laboratory adds little to the accuracy of the result. It may provide a check against a gross error or mistake, and, if the two duplicate results differ, show that further titrations are necessary[8,9].

The variation within students will almost always be less than the variation between students. The coefficient of variation calculated from all of the results will therefore probably be an underestimate of the variation between students and it might seem best to use the students' mean results as the basis of calculations. Although it is always possible to form an estimate of the coefficient of variation from the means of the students' results, the departure from a normal Gaussian distribution is then usually greater than when all the individual results are used, and so the estimate

may be very inaccurate. An example of this is illustrated in Figure 4. Figure 4a shows a graph constructed from the 80 results obtained by 40 students, each of whom made in duplicate the titration of 20 ml. of 0·1N silver nitrate with 0·1N ammonium thiocyanate with ferric ammonium sulphate as indicator. The line is reasonably straight if the three highest figures (19·6, 19·7 and 19·8 ml.) are ignored. The coefficient of variation calculated from the slope of the line is 0·36 per cent. Figure 4b shows the graph constructed from the 40 mean values, and even if a few "outliers" are ignored, it is still not possible to draw a straight line of good fit and the coefficient of variation cannot be estimated at all accurately.

Unless otherwise stated, all coefficients of variation quoted in this paper have been calculated from the individual results, despite the limitations of this procedure.

The coefficient of variation of results found for a given type of titration varied considerably from one group of students to another, and a value as low as 0·2 per cent could be regarded as unusually good. For the easier titrations, a coefficient of variation of 0·3 per cent was typical, and for the more difficult about 0·5 per cent. Of a total of 34 estimated co-efficients of variation, distributed between 13 types of titration, five were below 0·3 per cent, eight were between 0·3 and 0·4 per cent, eight were between 0·4 and 0·5 per cent and 13 were above 0·5 per cent. Another 28 sets of results were discarded because of marked curvature of the probit-titre graphs.

An example of the difference between one group of students and another is the following set of coefficients of variation for the titration of 10 ml. of 0·6 per cent hydrogen peroxide solution with 0·1N potassium permanganate, obtained by seven groups of students: 0·27, 0·28, 0·23, 0·61, 0·44 and 0·19 per cent.

It is likely that these widely differing results reflect differing abilities of different groups of students to adhere to established and approved manipulative techniques. Observations in class and during examinations showed that even after 2 years of training many students would still mis-use a pipette or a burette or would fail to add the correct reagents in the correct amounts. Gregorczych[10] has stated that the main sources of analytical errors are the disregarding of analytical instructions and carelessness in performing the work. This view is supported by preliminary study of the results of students performing gravimetric exercises or titrations in which the sample is taken by weight.

It has therefore not been found possible, as was hoped, to draw up a list of titrations and the corresponding estimates of coefficients of variation found with Brighton students.

RESULTS OBTAINED BY STUDENTS IN OTHER SCHOOLS OF PHARMACY

Titration results obtained by students in seven other schools of pharmacy were collected. (I am grateful to the lecturers concerned for their kind co-operation.) The general pattern of figures was similar to that found at Brighton. For example, the coefficients of variation of the results in the titration of 20 ml. of 0·6 per cent hydrogen peroxide solution with

0·1N potassium permanganate were 0·33, 0·36, 1·27 and 0·34 per cent, respectively, from four groups of students in three schools.

Of a total of 14 estimated coefficients of variation, distributed between eight different types of titration, three were below 0·3 per cent, six were between 0·3 and 0·4 per cent and five were above 0·5 per cent. Another eight sets of results had to be discarded, because of marked curvature of the probit-titre graphs. The group size of students ranged from 10 to 38; usually each student provided two results.

RESULTS OBTAINED BY ANALYSTS IN INDUSTRIAL LABORATORIES

Titration results obtained by analysts in the control laboratories of five pharmaceutical manufacturers were collected. (I am grateful to the Chief Analysts concerned for their kind co-operation.) Eight sets of figures were obtained, of which one set had to be discarded because the distribution was far from normal. The seven remaining sets were either acid-base, (a) to (f), or chloride-silver, (g), titrations in which 25 ml. of solution was taken by pipette and titrated with reagent. The coefficients of variation were estimated as (a) 0·13 per cent, (b) 0·15 per cent, (c) 0·09 per cent, (d) 0·14 per cent, (e) 0·26 per cent, (f) 0·09 per cent and (g) 0·18 per cent. The numbers of analysts per group were (a) 38, (b) 7, (c) 10, (d) 10, (e) 4, (f) 5 and (g) 19. Each analyst provided two results, except that each analyst in (g) provided one result only.

Because of the small group sizes and the close agreement of the results, it was not certain that use of the probit-titre graph was always the best way to estimate the coefficient of variation, and so direct estimates were also made in the usual way by summing squares of deviations, and so on. As already stated, this direct method may overestimate the amount of variation. The direct estimates were (a) 0·19 per cent, (b) 0·17 per cent, (c) 0·10 per cent, (d) 0·13 per cent, (e) 0·27 per cent, (f) 0·09 per cent and (g) 0·23 per cent.

DISCUSSION

It is clear that the hope expressed by Saunders and Fleming[11], that the data for calculating the percentage standard deviation of the different assay methods of the B.P. is available in the schools of pharmacy, cannot be fulfilled. The coefficients of variation of the students' results are often greater than those of the industrial analysts by a factor of two or three, and there is little agreement from one school to another or even from one group of students to another group within the same school.

If the figures obtained for calibration and use of pipettes and burettes and reported in Part I[1] are accepted, then a coefficient of variation within the range 0·12 to 0·20 per cent might be expected in a titration with a sharp end point where 25 ml. of solution taken by pipette gives a titre of 25 ml. of a colourless reagent, according to whether one or many pipettes and burettes are used. The industrial results mostly fall within this range, and so the estimates of Part I are thought to be reasonably accurate.

It seems unlikely that much useful information on the precision of such procedures in pharmaceutical analysis as titrations involving weighings

and gravimetric assays can be obtained from the results of students' work, although very large quantities of data are available. Some useful results might come from the work of individual experienced teachers of pharmaceutical analysis, but the main hope lies with the industrial laboratories. By the fairly frequent but irregular submission of suitably disguised "standard" test samples to some or all of the analysts in a laboratory, estimates can be made of the within-analysts and between-analysts variances, and the performance of each individual can be checked[12-14].

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After Mr. Rogers presented the paper there was a DISCUSSION.