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Sources of variation during collaborative evaluation of in vitro dissolution tests for two solid preparations

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Collaborative in vitro dissolution tests on a sample of commercial tolbutamide tablets and a sample of oxytetracycline capsules were carried out in eight laboratories. The two preparations tested showed differences between the products in release characteristics, particularly in the disintegration phase. This may have caused the difference in the pattern of variance in the two trials. In the case of tolbutamide tablets the value of the repeatability standard deviation was small, and therefore the major contribution to the variance was in the difference between laboratories. With oxytetracycline capsules the major contribution to the variance lies in the random errors common to all laboratories (i.e. the within-laboratory variance). One major source of inter-laboratory variance was identified as the level of vibration at the side of the dissolution flask. Another source of variation was found to be due to using a stated extinction coefficient instead of comparing the absorbances of the samples to those of a solution of a reference substance.

In vitro dissolution tests are being developed for publication in the British Pharmacopoeia. A vital part of the development of such tests is their collaborative evaluation in a number of different laboratories to determine the robustness to variation of the proposed procedures and equipment. Some collaborative trials have been reported using the United States Pharmacopeia rotating basket method (Kristensen et al 1973; McGilveray et al 1976) but none have been published using the equipment detailed in the British Pharmacopoeia 1973: Addendum 1977 apparatus for Dissolution Test for Tablets and Capsules. This apparatus, although similar in principle to the method described in the United States Pharmacopeia XIX has a number of differences—and particularly the use of a flat bottomed dissolution flask. The present study was undertaken to determine the repeatability and reproducibility of the proposed dissolution test procedure for tablets and capsules. An attempt was made to relate the different values for repeatability and reproducibility in the two tests to differences in the release characteristics of the two preparations determined using the equations proposed by Kitazawa et al (1975, 1977). Some of the possible reasons for the inter-laboratory variation were investigated separately. The level of displacement vibration at the dissolution flask has been found by Hanson (1975, 1976, 1977) to be important in the United States Pharmacopeia rotating basket method. A study of the level of vibration at the dissolution flask in some of the collaborating laboratories suggests that it may also be critical in the British Pharmacopoeia dissolution test.

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MATERIALS AND METHODS

Materials. A sample of commercial uncoated tolbutamide tablets B.P. were obtained directly from Berk Laboratories Ltd. These were from Batch 605047A and had a label claim of 500 mg tolbutamide. A sample of commercial hard shell gelatin oxytetracycline capsules B.P. (Imperacin) were obtained directly from ICI Limited. These were from Batch HH293 and had a label claim of 250 mg oxytetracycline hydrochloride.

Samples of oxytetracycline hydrochloride B.P. and tolbutamide B.P. were obtained from ICI Pharmaceuticals Ltd and Berk Laboratories respectively. These were used as reference materials to determine the possible variation in the spectrophotometric assay methods used to determine the release of tolbutamide and oxytetracycline from their dosage forms.

Dissolution procedures. The release of drug from the tablets and capsules was determined using the equipment detailed in the Dissolution Test for Tablets and Capsules in Appendix XIXF of the 1977 Addendum to the British Pharmacopoeia 1973. This consisted of a rotating stainless steel mesh basket rotated in a flat bottomed 1 litre glass dissolution flask.

900 ml of 0·1M HCl at 37 °C was used as the dissolution medium for oxytetracycline capsules. The basket rotation speed used was 100 rev min^{-1}. Sampling was effected using a glass or stainless steel canula. Each 10 ml sample was filtered through a 1·2 μm pore size 13 mm diameter cellulose acetate/nitrate membrane filter in a holder. The filter was discarded between samples. The samples were removed after 5, 10, 20 and 30 min. Each sample was replaced with fresh 0·1 M HCl at 37 °C to keep the volume of dissolution medium constant.

900 ml of phosphate buffer (containing 2·04% w/v disodium hydrogen phosphate and 0·139% w/v potassium dihydrogen phosphate) at 37 °C was used as the dissolution medium for tolbutamide tablets. The basket rotation speed used was 100 rev min^{-1}. Sampling was effected using a glass or stainless steel cannula, and filtered as described above. 10 ml samples were removed at 10, 20, 30 and 45 min. Each sample was replaced with fresh buffer at 37 °C.

Each collaborating laboratory carried out the dissolution test on 5 tolbutamide tablets and 5 oxytetracycline capsules.

Analytical procedures. The amount of oxytetracycline hydrochloride in solution was determined spectrophotometrically (after dilution as necessary with 0·1 M HCl) taking 282 as the value of A (1%, 1 cm) at the absorbance maximum at about 353 nm.

The amount of tolbutamide in solution was determined spectrophotometrically (after dilution as necessary with buffer) taking 417 as the value of A (1%, 1 cm) at the absorbance maximum at about 228 nm.

Each collaborating laboratory was asked to determine the apparent ('as is') A (1%, 1 cm) as indicated in Appendix IV I of the British Pharmacopoeia 1973 and the wavelength of maximum absorbance for the reference samples of tolbutamide and oxytetracycline hydrochloride.

Characterization of the dissolution profile of the tablet and capsule samples

The dissolution test was carried out as described above in the British Pharmacopoeia Commission Laboratory using the samples of tolbutamide tablets and oxytetracycline capsules. Samples were taken more frequently however (every minute for the first few minutes) to enable the dissolution profile to be determined in detail. As before each sample was filtered immediately and the dissolution volume was kept constant by replacing each sample with fresh medium at 37 °C.

Collaborating laboratories. The following laboratories took part in the trials.

Laboratory 1. Quality Assurance Laboratory, The Wellcome Foundation Ltd, Dartford

Laboratory 2. Product Development Laboratory, The Wellcome Foundation Ltd, Dartford

Laboratory 3. Analytical Development Laboratory, The Boots Company Ltd, Nottingham

Laboratory 4. Quality Control Laboratory, Pharmacy Department, Norfolk and Norwich Hospital, Norwich

Laboratory 5. Department of Pharmacy, University of Aston, Gosta Green, Birmingham

Laboratory 6. Department of Pharmaceutical Sciences, Pharmaceutical Society of Great Britain, Edinburgh

Laboratory 7. Formulation Research Section, ICI Ltd (Pharmaceutical Division), Alderley Park, Macclesfield

Laboratory 8. British Pharmacopoeia Commission Laboratory, Stanmore, Middx.

The equipment used by the collaborating laboratories is detailed in Table 1.

Table 1. Equipment used by the collaborating laboratories.

	Equipment used by laboratory							
Item	1	2	3	4	5	6	7	8
Motor drive	Hanson 72R	Heidolph RZR1	Parvalux	Citenco KQTS11	Citenco KQTS9	Hanson 72R	Hanson 72B	Hanson 72R
Single or multihead stirrer	Multi	Single	Single	Single	Single	Multi	Multi	Multi
Presence of guide plug	Guide plug	None	Guide plug	Guide plug	None	None	Guide plug	None
Sampling system	Not known	Stainless canula	Stainless canula	Stainless canula	Stainless canula	Pipette	Glass canula	Glass canula
Spectrophotometer	Pye SP500	Pye SP1750	Coleman 55	Pye SP1700	Unicam SP800	Cecil CE272	Pye SP8000	Cecil CE272

Measurement of vibration at the dissolution flask

The velocity of displacement vibration was measured at the side of the dissolution flask in each laboratory's equipment by attaching an accelerometer with molten paraffin wax. The accelerometer was connected by a cable to a Bruel & Kjaer type 2511 vibration meter capable of measurement in the frequency range 0·3 Hz to 15 kHz. The meter was used in the RMS (root mean square) mode with 10 s time constant. The vibration meter was used in conjunction with a Bruel and Kjaer type 1621 tunable band-pass filter. This consisted of a tunable single pole butterworth filter with switchable band width thus enabling the frequency to be selected. The accelerometer, vibration meter and tunable band-pass filter were used to determine the vibration frequency spectrogram at the side of the dissolution flask. The stirrer motor, water bath and stirrer were all operated as used in the collaborative trial. Where the dissolution equipment consisted of a multiple spindle drive and a number of dissolution flasks, the accelerometer was positioned on the side of the flask nearest the circulating water pump (if one was used). Thus the flask likely to be susceptible to the maximum vibration was evaluated.

RESULTS

The mean dissolution profile found by each collaborating laboratory for the two preparations are shown in Fig 1. To illustrate the variation of the results for individual tablets and capsules, the grand mean dissolution profile and range of results for the two preparations are shown in Fig. 2.

The results from the study in Laboratory 8 to characterize the dissolution profile were calculated and plotted according to the theories of Kitazawa et al (1975, 1977). Two straight regression lines were obtained from these plots and the slope of the first line being K_1 and that of the second K_f. From the slopes of the straight lines the dissolution rate constants were calculated and are shown in Table 2 and Fig. 3. The times at which the two lines intersected was called t_i and the results are again given in Table 2 and in Fig. 3.

The results of the collaborative check on the reference samples of oxytetracycline hydrochloride and tolbutamide are shown in Table 3.

The mean tolbutamide dissolution result from each collaborating laboratory at each time was used to provide a Kitazawa plot—assuming that the final (45 min) dissolution result represented essential completion of the dissolution process. The same was done for the 10 and 20 min oxytetracycline capsule data (assuming that the final 30 min dissolution result represented essentially complete dissolution). The slopes of the regression lines represent K_f the final phase of the dissolution process. These are

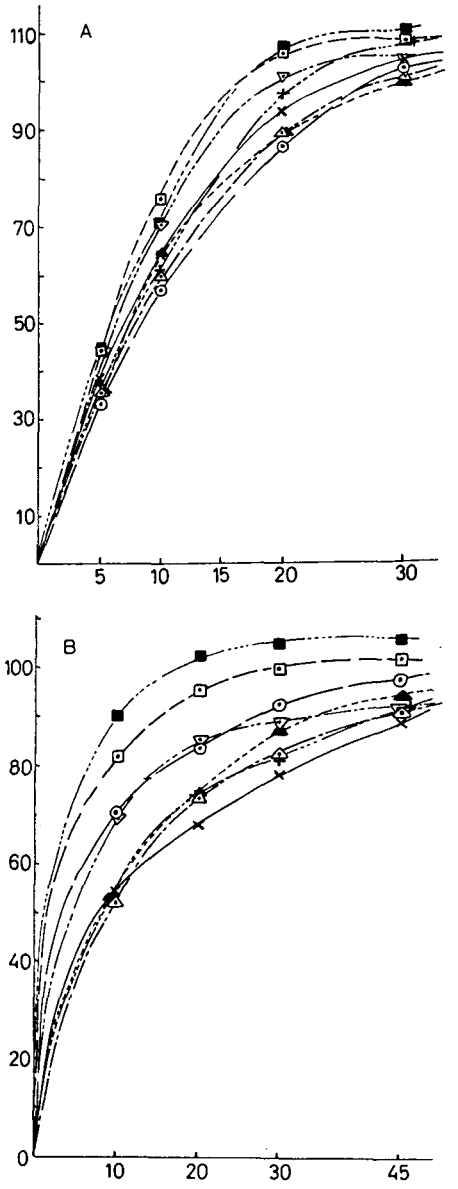

FIG. 1. Mean dissolution profiles for (A) oxytetracyline capsules (B) tolbutamide tablets determined by each collaborating laboratory. ×——× Laboratory 1, ○— —○ Laboratory 2, □ - - - - □ Laboratory 3, △ — - — - — △ Laboratory 4, ◁ — - - — ▽ Laboratory 5, + — - - - — + Laboratory 6, ■ — - - - — ■ Laboratory 7, ▲ - - - - - - - - - ▲ Laboratory 8. Ordinate: Mean percentage label claim drug released. Abscissa: time (min).

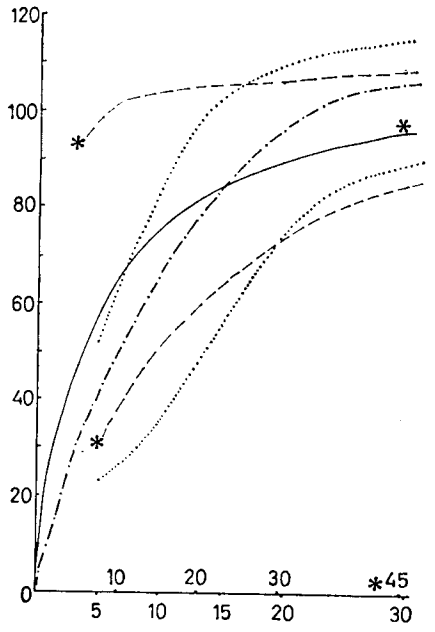

FIG. 2. Grand mean dissolution profiles and range of results for individual tolbutamide tablets (—— ✳, - - - ✳) and individual oxytetracycline capsules (— · —, · · · ·). Ordinate mean percentage label claim drug released. Abscissa: time (min).

shown in Fig. 4, and the values of K_f are given in Table 4.

The results of the vibration tests are summarized in Table 5 as the wavelength of the 3 main peaks and the velocity of RMS displacement in mm s^{-1} at each peak. To illustrate the type of results obtained typical spectrograms are shown in Fig. 5 for the results from Laboratory 7 and 8. It will be noted that the velocity of displacement vibration at 35 Hz in Laboratory 7 is over 14 times greater than that in Laboratory 8. This difference was not evident from inspection of the equipment whilst operating. The vibration data in Fig. 5 is shown on a logarithmic scale. It was not possible to measure vibration levels in all of the collaborating laboratories. Thus there are no results for laboratory 5 or 6.

Table 2. Dissolution characteristics of the tolbutamide tablets and oxytetracycline capsules (determined on the data in the characterization study in Laboratory 8).

Preparation	K_1 (min^{-1})	K_f (min^{-1})	t_i (min)
Tolbutamide tablets	0·11	0·082	9·8
Oxytetracycline capsules	0·066	0·13	9·1

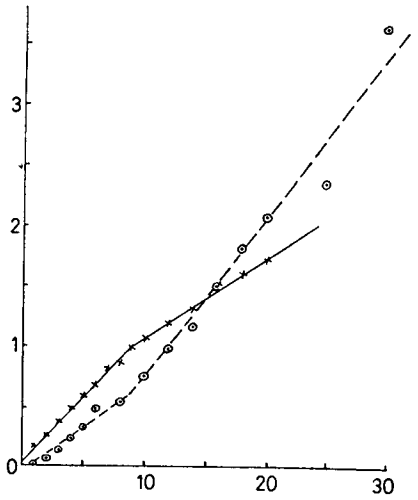

FIG. 3. Mean dissolution profiles for tolbutamide tablets and oxytetracycline capsules plotted according to Kitazawa et al—plots of $\ln W\infty/(W\infty - W)$ versus time (min), where W is the amount of drug dissolved at time t and $W\infty$ is the amount dissolved at time $t\infty$, that is the total amount that will dissolve. ×——× tolbutamide tablets, and ○— — —○ oxytetracycline capsules.

Statistical analysis

The data were analysed for each product by carrying out an analysis of variance on the data at each sampling time. The repeatability and reproducibility were also determined. These are defined (ISO, 1966; Youden & Steiner 1975) as follows:

Reproducibility: closeness of agreement between individual results obtained with the same method on identical test material but under different test conditions (i.e. different operators, different dissolution apparatus, different laboratory, different times, etc).

Repeatability: closeness of agreement between successive results obtained with the same method on identical test material and under the same conditions (same operator, same apparatus, same laboratory and same time).

Table 3. Results of collaborating laboratories determinations of the apparent A (1% 1 cm) and absorbance maxima for reference samples of tolbutamide and oxytetracycline hydrochloride.

Drug and parameter	Results from Laboratory							
	1	2	3	4	5	6	7	8
Tolbutamide								
A (1%, 1 cm)	422	413	420	438	428	430	457	—
λ max (nm)	227	227	227	226·5	225	227	226	228
Oxytetracycline								
A (1%, 1 cm)	286	279	281	283	288	281	291	—
λ max (nm)	354	356	353	353	353	352	353	353

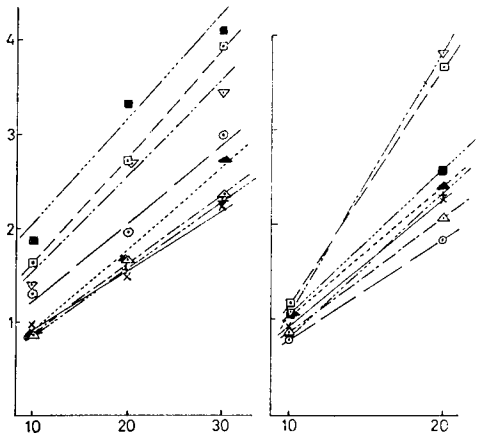

FIG. 4. Mean dissolution profiles for (left) tolbutamide tablets (right) oxytetracycline capsules determined by each collaborating laboratory plotted according to Kitazawa and others—plots of ln $W\infty/(W\infty - W)$ versus time (min). Laboratory Codes as FIG 1.

The values for the reproducibility standard deviation σ_x and the repeatability standard deviation σ_0 are calculated from the data at each time for each product and are given in Tables 6 and 7. The respective coefficients of variation C_x and C_0 (i.e. the standard deviation expressed as a percentage of the mean) are also given in the Tables.

DISCUSSION

The dissolution of the sample of tolbutamide tablets plotted according to Kitazawa is shown in Fig. 3. It proceeds in two phases—dissolution during the disintegration phase (when the initial rate of dissolution K_i applies) and the phase of dissolution from particles of the disintegrated tablet (dissolution rate K_f). The process is in two phases—a fast dissolution during disintegration and a slower second phase of dissolution from the disintegrated tablet. Presumably this is due to the particles of disintegrated tablet sinking to the bottom of the dissolution vessel where the intensity agitation is lower and therefore the rate of dissolution decreases.

Table 4. Calculated values of K_f dissolution rate constant in min^{-1} for tolbutamide tablets and oxytetracycline capsules.

	Mean value from laboratory						
1	2	3	4	5	6	7	8
Tolbutamide							
0·06	0·08	0·14	0·07	0·10	0·07	0·13	0·09
Oxytetracycline							
0·13	0·11	0·25	0·12	0·27	0·15	0·16	0·13

Table 5. Summary of vibration frequency maxima (Hz) and RMS velocity of displacement (in mm s^{-1}) at the maxima.

Lab.	Freq.	Vel.	Freq.	Vel.	Freq.	Vel.
1	9	0·0038	35	0·031	100	0·046
2	12	0·053	50	0·040	100	0·043
3	14	0·0076	30	0·036	100	0·024
4	10	0·042	35	0·035	90	0·011
7	14	0·028	35	0·57	100	0·028
8	12	0·010	35	0·040	100	0·014

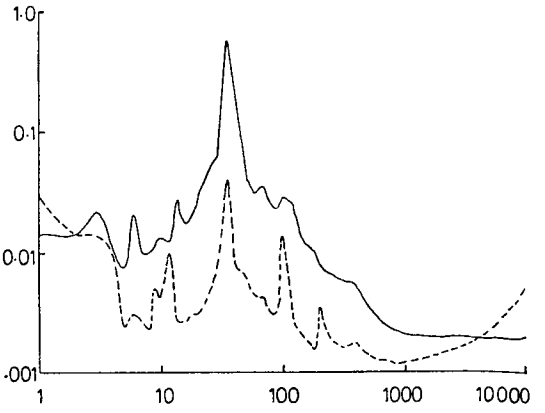

FIG. 5. Vibration frequency spectrograms measured at the side of the dissolution flasks in Laboratories 7 and 8. ——— Laboratory 7, - - - - - - - - - - - - - Laboratory 8. Ordinate: Velocity of displacement (mm s^{-1}). Abscissa: Frequency (Hz).

Table 6. Repeatability standard deviation (σ_0), reproducibility standard deviation (σ) and coefficients of variation (C_0 and C_x) for the tolbutamide tablet dissolution data.

Sampling time (min)	σ_0	C_0 (%)	σ_x	C_x (%)
10	6·98	10·6	16·2	24·6
20	6·76	8·3	13·5	16·4
30	5·38	6·0	10·3	11·5
45	3·67	3·9	7·1	7·5

Table 7. Repeatability standard deviation (σ_0), reproducibility standard deviation (σ_x) and coefficients of variation (C_0 and C_x) for the oxytetracycline capsule dissolution data.

Sampling time (min)	σ_0	C_0 (%)	σ_x	C_x (%)
5	8·9	22·6	11·0	27·8
10	10·7	15·2	11·6	17·7
20	8·4	8·8	10·4	10·9
30	4·9	4·7	6·2	5·9

The dissolution of the sample of oxytetracycline capsules is shown also in Fig. 3. It again proceeds in two phases—a slow disintegration and a faster second phase of dissolution from the disintegrated capsule. The capsule plug contents when disintegrated are more finely divided and move freely in the dissolution flask. In this case the second dissolution phase (where the surface area exposed to the dissolution medium had increased rapidly after disintegration) is faster than the disintegration phase.

The different patterns of dissolution for the two products may be decisive in determining the differences in variance found in the collaborative trials. The greater overall variance of the tolbutamide tablet data particularly at early sampling times is shown in Fig. 2. This can be compared with the oxytetracycline data in Fig. 2. The data were calculated according to the theories of Kitazawa et al (1975, 1977) and the resultant plots are shown in Fig. 4. This shows that for oxytetracycline capsules there is very little difference between the results in different laboratories during the disintegration phase. Fig. 4B showing the second phase of dissolution indicates that at the start of the second phase there was very little difference between laboratories. The disintegration phase is not shown since no early time samples were taken during the collaborative trial. Fig. 4B representing K_f shows a 2-fold range in mean dissolution rate between the different laboaratories. For tolbutamide tablets there are clearly differences between the laboratories in both the disintegration phase (not shown in the Fig.) and the second phase of dissolution from the particles of disintegrated capsule. From Fig. 4A it can be inferred that there was at least a two-fold range between laboratories during the disintegration phase. The data in Fig. 4A shows the dissolution in its second and final phase and represents a further widening of the range of results.

The statistical analysis of the collaborative data is summarized in Tables 6 and 7. With the tolbutamide tablet data the reproducibility standard deviation is very much greater than the repeatability standard deviation. Thus the major contribution to the variance lies in the difference between laboratories. As the dissolution test proceeds the magnitudes of the values of the standard deviations fall. The reproducibility of the test is best at the last sampling point. Thus it is preferable with any single sampling point dissolution test to choose a sampling time when the dissolution process is complete or nearly so.

With oxytetracycline capsules the reproducibility standard deviation is larger than the repeatability standard deviation—but only marginally so. Thus in this case the major contribution to the variance lies in the random sources of error in operation of the method common to all laboratories (i.e. the within laboratory variance).

Part of the variation between laboratories is clearly due to the differences in analytical results by using a stated extinction coefficient. The coefficient of variation calculated from the data shown in Table 3 on the collaborative check on the extinction coefficients of tolbutamide is 3·35%. This represents a considerable contribution to the total variance. This source of variation would be reduced if instead of being calculated using a stated extinction coefficient, the dissolution samples were read on the spectrophotometer in comparison to a solution of a reference standard of tolbutamide. With oxytetracycline collaborative extinction data in Table 3 the calculated coefficient of variation is 1·5%. This contributes a smaller but still an important part of the total variance.

The British Pharmacopoeia 1973, Addendum 1977 Dissolution Test advises that care should be taken to avoid displacement vibration. Displacement vibration in the 30 to 60 Hz range has been identified by Hanson (1975, 1976, 1977) as an important factor in determining both between-laboratory variation and within-laboratory variation. The results in Table 5 of the test for vibration at the dissolution flask in the equipment of the collaborating laboratories identified a large peak at 35 Hz in the data from Laboratory 7. This laboratory showed a consistently high rate of dissolution in both the collaborative trials which could possibly be explained partly by the effects of displacement vibration. The complete spectrogram from Laboratory 7 is shown as a comparison in Fig. 5 with that of Laboratory 8. However Laboratory 3 also showed dissolution rates which were high and not related to large displacement vibration levels. So this is only one possible causative factor in determining inter-laboratory variation.

Other factors which may cause intra and inter-laboratory variation include variation in temperature of the dissolution medium, dissolved air in the medium, filter adsorption, release of interfering substances from the filter, drive motor speed variation, stirrer shaft eccentricity, basket dimensions, and basket distortion.

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