DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 5(3), 277-291 (1979)

PRACTICAL ASPECTS OF DISSOLUTION TESTING

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ABSTRACT

The introduction of in vitro dissolution tests into the British Pharmacopoeia necessitated a considerable programme of laboratory investigation and collaborative testing. The importance of minimising variation and identifying and controlling some of the factors causing variation was an important aspect of this programme. Factors which were identified were sampling position, temperature of the dissolution medium, dissolved air, filter adsorption and filter release of interfering substances, drive motor speed variation, stirrer shaft eccentricity, basket dimensions, displacement vibration, and flask shape. Reference is made to automated sampling systems and to the use of Dissolution Calibrators.

INTRODUCTION

The results of analyses of samples taken from a tablet or capsule during the dynamic dissolution process are likely to show more inherent variability than analyses of a defined static sample.

The increased use of in vitro dissolution tests for tablets and capsules by pharmaceutical manufacturers and control laboratories has led to a realisation of the importance of the control of factors which might affect the repeatability (within laboratory variances) and reproducibility (within and between laboratory variances) of the tests.

If laboratories cannot obtain good agreement on test results then an in vitro - in vivo correlation obtained by one laboratory cannot necessarily be applied in all laboratories. Further, where the in vitro dissolution data is used as an assurance of batch bioequivalence uniformity, then it is clearly essential to use standardised equipment producing reproducible results.

Many of the factors causing variation have now been identified. These are discussed here mainly in relation to the rotating basket/flat bottomed dissolution flask equipment specified in the 1977 Addendum to the British Pharmacopoeia 1973[1]. However many of the factors will also apply to other types of test - such as the paddle stirrer or rotating basket/round bottom flask methods adopted in the 4th supplement to USP XIX.

Sampling Position

Withey in 1971[2] drew attention to the different patterns of dissolution obtained from a cube of sodium chloride by sampling (in the USP rotating basket method) at different depths. However the specific gravity of a saturated solution of sodium chloride is greater than water and this undoubtedly exaggerates the effect obtained. In a later paper[3] Withey visualised the flow obtained from the rotating basket by using a strobe lamp. He showed that the fluid near the surface of the medium is barely moving, and the primary pattern of flow is radially from the basket, with little vertical mixing.

The effect of this limited mixing on the variation in concentrations of drug at different depths in the dissolution flask will depend on the properties of the dosage form. If the products disintegrate into small fine particles with a small difference in specific gravity between the particles and the dissolution medium, then samples can be taken at different depths without showing any difference in concentration, since the particles remain suspended uniformly. Products which disintegrate into larger particles, or those with a large difference in specific gravity between the particles and the medium, are likely to show a significant difference in assay. Figure 1 shows the effect of sampling at different depths from commercial Methylprednisolone tablets.

The release of methylprednisolone was monitored spectrophotometrically at 246 nanometres.

The British Pharmacopoeia dissolution tests specifies the sampling position as being half-way up the basket and half-way between the basket and the wall.

Temperature of the Dissolution Medicine

The 1977 Addendum specifies that the temperature of the dissolution medicine shall be between 36.5 and 37.5°C. Hanson[4] has reported that a difference of only 1.5°C in temperature caused a 15% variation in the dissolution of salicylic acid standard wafers.

To maintain the temperature at the dissolution medium in the flask within the required limits it is often necessary to maintain the temperature of the water bath at a 0.5 to 1.0°C higher temperature. Heat losses from the dissolution flask can be dramatically reduced by using a glass or acrylic lid, and in the water

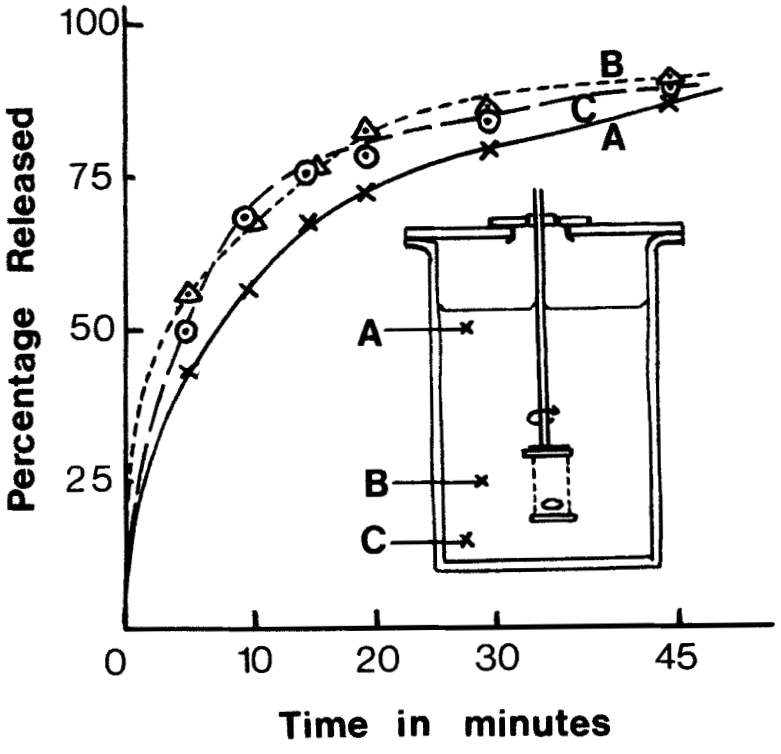

FIGURE 1.

Mean Dissolution Curves For Tests On Methylprednisolone 16 mg Tablets At 3 Sampling Positions.

bath by using a cover or by using floating polypropylene spheres (heat loss reduction of up to 75%).

The time taken for a flask of dissolution medium in a large water bath to achieve temperature from cold is often underestimated - it can often take up to 2 to 3 hours.

It is important to check the temperature of the medium at the beginning and end of the run by using a suitable partial im-

mersion thermometer (to British Standard 593 or equivalent) graduated to 0.1°C.

Dissolved Air

The British Pharmacopoeia specifies the use of de-aerated dissolution media. If an aerated medium is used, air bubbles can form on the surface of the basket mesh which can alter the flow of medium from the basket.

The medium can be de-aerated under vacuum. However, it is often more convenient to immerse the flask of dissolution medium in an ultrasonic bath. This method is less likely than vacuum de-aeration to cause changes in the concentration of the buffer solutions used in many tests.

Filters

Dissolution samples are filtered to remove solid particles of drug present in the sample which would otherwise continue to dissolve and give spurious readings. Also tablet excipients might otherwise cause turbidity which would interfere (for example in a spectrophotometric assay).

It is important to choose a filter which does not significantly adsorb material from solution or which itself will release any material into the filtered sample which would interfere with the assay. Membrane filters in a suitable holder used in conjunction with a sampling syringe are a convenient system. However Liu, Carney and Hurwitz[5] have shown that some drugs can be adsorbed from solution by various membrane filters.

Many membrane filters contain isooctylphenoxypolyethoxyethanol as a wetting agent to enable solutions to be filtered

readily. The surfactant also helps to enable the membranes to be cast during their manufacture. However the surfactant can be eluted from the membrane filter. Figure 2 shows the absorbance at 224 nanometres of successive 20 ml samples of 0.1M HCl filtered through a 25 mm diameter 8 μm pore size cellulose acetate/nitrate membrane filter.

This problem mainly affects drugs assayed spectrophotometrically between 220 and 240 nanometres.

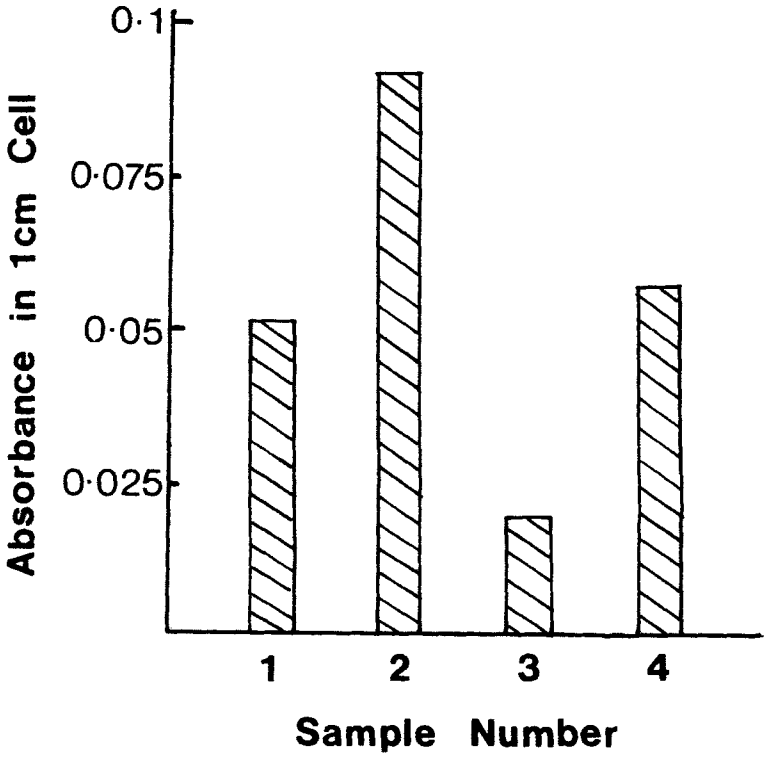

FIGURE 2.

Absorbance At 224 nanometres Of Successive Samples Of 0.1M HCl After Passage Through An 8 μm Pore Size Cellulose Acetate/Nitrate 20 mm Membrane Filter.

Suitable inert membrane filters with a pore size of 1 µm or less with no added surfactant are readily available from commercial sources.

Speed Variation

Many drive motors increase in speed after they have been running for a period, and thus it is essential to run them for a preliminary period before a test is started. The speed alteration in this initial phase can easily place the speed outside the ± 5 specified in the 1977 Addendum to the British Pharmacopoeia.

Some motors are subject to torsional vibration or "cogging", where the mean speed is correct but the motor speed varies over a short time - slowing down and then speeding up. Motors that do this are clearly unsuitable since it could alter the flow of drug particles and solution from the basket - causing surges of material.

One of the commercial multiple head motor drive units can be purchased equipped with a variable speed controller and an indicating tachometer. This provides a very useful indication of speed changes during a test, but should not be used as an absolute indication of speed as it has been noted that the calibration can change over several months.

Motor speeds are commonly checked by attaching a paper "flag" to the spindle and counting with a stop-watch. Many workers tend to overcount by this method and set the speed by up to 4% too low.

It is essential to carry out an independant check on the stirrer speed at the beginning and the end of each test using a suitable tachometer. Mechanical analogue or electronic digital

tachometers are readily available which are actuated by a rubber pad pressed on the end of the stirrer shaft. Digital photo-tachometers can also be purchased which need no contact with the stirrer shaft.

Stirrer Shaft Eccentricity

The 1977 Addendum to the British Pharmacopoeia limits the eccentricity to an imperceptible wobble. The shaft at the current BP basket assembly is 6.0–6.5 mm in diameter. Although the shaft is manufactured to within 0.1 mm eccentricity it can easily be bent if mishandled. Small eccentricities are probably not a problem with the BP basket, but the effect is greater with the rotating paddle method of USP X1X.

To minimise wobble the rotating shaft should be supported in two places. This can be done either by using a hollow spindle drive motor with the shaft pinned in mounts at the bottom chuck and at the top of the motor, or by fixing into the chuck and using a guide-bush with a bearing in the flask cover. No attempt should be made to support the shaft in three places. Where the shaft is fixed in two places in the hollow spindle drive the motor should be adjusted to as near the cover of the flask as practicable (the "close - coupled" mode).

Basket Dimensions

The dimensions of the basket assembly are closely defined in the 1977 Addendum to the British Pharmacopoeia 1973. Although most commercial baskets fall within the range of dimensions quoted, some baskets have been found which are longer or thinner than specified. Deviations from the specification (and particularly that for the overall length of the basket assembly) have been found to cause significant differences in dissolution rate.

The mesh baskets are also quite fragile and easily bent and distorted. Other workers have reported that distorted baskets can have rough protrusions which can abrade the tablet causing an increase in the dissolution rate. Baskets should be carefully stored, regularly inspected and discarded if bent or distorted to a degree which cannot be corrected by simple manipulation.

Displacement Vibration

Hanson[4,6] has identified the effect of displacement vibration. He introduced measured vibration in the 10 to 500 Hz frequency range and found that in the 30 to 60 Hz range there is a linear relationship between the dissolution rate and the horizontal displacement at the dissolution vessels. In a multiple spindle apparatus the effect of displacement vibration is to alter the mean dissolution rate and increase the difference between dissolution vessels.

Sources of displacement vibration are those related to the dissolution equipment itself (including its water bath and agitator), and those external to it. External sources can even be outside the laboratory itself (for example a large pump motor in an adjacent boiler house). More commonly machinery on the same bench as the dissolution test equipment can set up displacement vibration.

The dissolution equipment itself can cause displacement vibration. In particular the pump agitator systems commonly employed in the water baths can give problems. Care should be taken to avoid clamping directly onto the water bath if possible, or to insulate it to obviate the effects.

There are two basic techniques to reduce the effect of displacement vibration - insulation and increasing the mass. Insu-

lation of the water bath for example can be achieved by mounting on foam. The dissolution vessels should be clamped to their covers which in turn should be fixed to a support which itself is mounted firmly on the water bath. Thus the effective mass of the vessels is that of the whole dissolution equipment and any vibration is heavily damped.

The level of vibration at the side of the flask can be measured by attaching an accelerometer to the side of the flask with wax and connecting the accelerometer to a vibration meter. By using a tunable band-pass filter with the meter, a vibration frequency spectrogram can be determined. Using the vibration meter, the effect of using different equipment or of altering the apparatus can be found and the level of vibration minimised.

Dissolution Flask Shape

The USP X1X dissolution vessels have a complex shape and it has been noted that they vary in shape depending on the commercial source. Underwood and Cadwallader[7] have demonstrated some of the effects that vessel shape has on dissolution pattern. The 4th supplement to USP X1X adopted a round bottomed straight sided vessel. Although this is clearly easier to standardise, Cox and his co-workers[8] of FDA have reported that they have found vessels which are not uniform with respect to weight, inside diameter and inside curvative. Statistically significant differences were found when the same product was tested in different vessels.

The use of the flat-bottomed vessel of the British Pharmacopoeia obviated all the problems of vessel shape and eliminates this source of inter-laboratory variance.

Automated Sampling Systems

Many laboratories have chosen to use automated sampling and analysis systems to cope with a large throughput of samples. Many methods have been published giving details of equipment and techniques, but in essence they mormally consist of a sampling probe, a pump and the analytical train (often a spectrophotometer).

The sampling probe usually consists of a narrow bore tube fitted with a filter (often either of sintered glass or a membrane type). Care must be taken to avoid setting up currents in the dissolution flask when sampling.

Figure 3 shows the effect of pump speed through a porosity 4 sintered glass filter on the apparent T50% value (the time taken to release 50% of the drug into solution) of a sample of commercial Cortisone Acetate Tablets 25 mg in water as the dissolution medium with a basket rotation speed of 100 rev min^{-1}.

A fast pumping speed can also lead to a layer of undissolved drug building up on the filter, which is then percolated and extracted by the passage of the dissolution fluid.

One of the main problems of automated sampling and analysis systems is that the system contains in the sample probe, lines, spectrophotometer flow cells etc a certain volume of fluid. This mixes with the next sample aliquot, giving an apparent reading which is higher or lower than the real one. This effect can be minimised by using suitable flow rates, and short runs of small bore (transmission) tubing to connect items. Mixing in the line can also be minimised by using air segmentation[9,10].

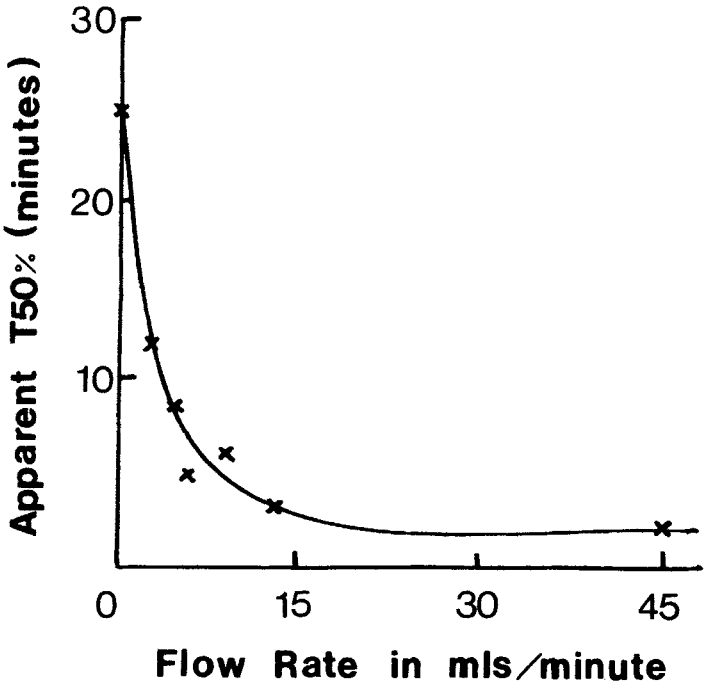

FIGURE 3.

Mean T50% values For Commercial 25 mg Cortisone Acetate Tablets At Different Sampling Flow Rates.

An interesting experiment to evaluate the characteristics of a particular sampling system is to pump blank dissolution medium and then dip the sample probe into a drug solution representing a completely dissolved tablet or capsule. This therefore represents an instantaneous 100% dissolution. The time taken for the sampling and analysis system to reach 100% can then be measured and the system "lag factor" built into any calculation of release characteristics of an actual dosage form. Figure 4 shows this effect.

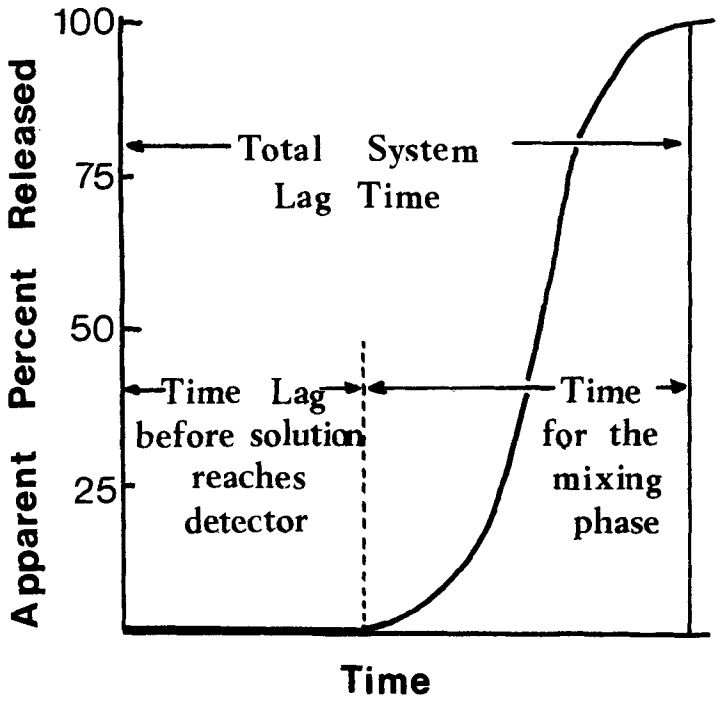

FIGURE 4.

The Lag Phase Of An Automated Sampling And Analysis System.

Analytical Variation

As well as the variation attributable to the dissolution equipment and technique, there are also sources of variation in the analytical method used to determine the concentration of drug in the dissolution medium.

Where spectrophotometric methods are used for the determination, regular checks need to be performed on the instrument wavelength setting with a holmium filter, and on the accuracy of the absorbance setting with dichromate solutions or standards

glass filters (for example those supplied by the National Physical Laboratory, Teddington, Middlesex, England). Where use is made of a quoted extinction coefficient, care must be taken to use an instrument at an appropriate band width in relation to the peak being measured. Normally an instrument with a band width of 2 nanometres or less would be suitable.

Dissolution Calibrators

There is great hope that the best means of evaluating the characteristics of a particular set of dissolution equipment would be to use a reference preparation with known and closely defined dissolution characteristics. The United States Pharmacopoeia has proposed providing Dissolution Calibrators Disintegrating Type and Non-disintegrating Type[11].

Salicylic acid wafers have been widely used as non-disintegrating dissolution calibrators, and recently Rees has proposed using a potassium sorbate wax pellet[12]. However there is evidence that the between laboratory variances can be mainly ascribed to the effect of apparatus variables on the dissolution during the disintegration phase. Thus the provision of Disintegrating Type Calibrators will be a vital part of the standardisation programme.

ACKNOWLEDGEMENTS

Grateful acknowledgement is made of the help and cooperation of the industrial, academic, and other collaborators who provided practical help, and contributed many of the ideas in this paper. Mr J F Chissell of Roussel Laboratories Limited kindly made available the results of the dissolution tests on Cortisone Acetate Tablets which are reported here.

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