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# Factorial design used for ruggedness testing of flow through cell dissolution method by means of Weibull transformed drug release profiles

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## Summary

Ruggedness testing on dissolution methods yields important information about critical factors. However, separate testing on data from all sampling times is circumstantial and it might be difficult to draw precise conclusions. By Weibull transformation the profiles are described by only two parameters ($\beta$ and $\tau$) regardless of the number of sampling times. $\beta$ and $\tau$ are very suitable parameters for statistical testing by analysis of variance (ANOVA) and the ruggedness test can therefore with advantage be based on a factorial design The ruggedness test described was performed on a calibration dissolution method discussed for use in the US Pharmacopeia for flow-through cell apparatus. The testing subjects for this method are salicylic acid calibrator tablets (non-disintegrating) and the flow rate is 16 ml/min through 12 mm cells The factorial design included four factors: Flow, temperature, with or without degassing of the medium and conductivity in the medium. The statistical evaluation showed that degassing increases the shape parameter ($\beta$) of the profile ($p < 0.01$) since it results in a higher release rate for the last part of the dissolution. The time required for 63.2% dissolved ($\tau$) is affected by degassing ($p < 0.05$) as degasssing decreases $\tau$. Also, the flow rate affects $\tau$ ($p < 0.01$) since increased flow decreases $\tau$. In other words, the dissolution method is rugged against deviations in temperature and conductivity of the medium. In contrast, degassing and a correct flow rate are important factors in order to achieve reliable results

## Introduction

Analytical methods including dissolution testing require validation to ensure the reliability of the results. A suitable dissolution method should be optimised with respect to discriminating power which will probably also make it sensitive to some operational factors. The in vitro ruggedness testing should identify these factors and may lead to special precautions in the performance of the analysis.

For ruggedness testing, dissolution profiles obtained under different conditions should be compared. This can be rather circumstantial as a typical dissolution method includes at least three sampling times. Furthermore, it can be problematic to give a thorough description of differences between profiles especially if they overlap.

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Transformation to the Weibull distribution (also denoted as the RRSBW distribution) of dissolution data makes it possible to describe a profile with only two parameters: the form parameter, $\beta$, and the scale parameter, $\tau$, regardless of the number of sampling times [described further in the following text and by others (Langenbucher, 1976; Koch, 1984)].

Ruggedness testing can with advantage be performed as a factorial experiment and the results should then be statistically evaluated by analysis of variance. $\beta$ and $\tau$ are very suitable parameters for this statistical test, as they vary in accordance with the normal distribution.

The flow through cell apparatus has recently been officially accepted (US Pharmacopeia, 1991) but is still not very widespread. A review of the steps involved in the development of the system has been published (Langenbucher et al., 1989) giving general information on the high level of standardization achieved thus far.

Three collaborative studies (Nicklasson et al., 1987, 1989; Wennergren, et al., 1989) comparing the flow through cell method with the USP basket and paddle methods showed that the former method produces reproducible and corresponding data both within and among the different laboratories.

The results from the first two studies have been summarised (Nicklasson and Langenbucher, 1990) as supporting data for acceptance of the flow through cell apparatus in the US Pharmacopeia. It appears from the data that the salicylic acid calibrator tablets used for the basket and paddle apparatus are also suitable for calibration of the flow through cell apparatus. Among the rates of flow tested, a rate of 16 ml/min, gave more concordant results and is therefore the optimum rate for a calibration test method. As dissolution medium, a phosphate buffer solution pH 7.4 was used corresponding to basket and paddle calibration, and the flow cell apparatus was equipped with 12 mm cells.

The present experiments are based on the above-mentioned method and supplement the collaborative studies. The inter- and intra-laboratory variations are in fact a very important part of the ruggedness test. Nevertheless, it is also important to know the significance of the flow and temperature accuracy, whether or not the dissolution medium, i.e., the buffer solution, needs degassing, and if minor deviations in the conductivity of the buffer solution affect the results. As regards conductivity, such deviations may appear if the concentration of the salts ($KH_2PO_4$ and NaOH) is not correct. The deviation will not necessarily cause measurable deviations in the pH.

## Materials and Methods

*The Weibull transformation procedure*

The Weibull transformation of dissolution profiles offers great advantages for fitting and comparing dissolution profiles. The basic principle is that the dissolution profiles are transformed to straight lines described by the slope and intercept. For the transformation procedure observed values of percentages of dissolved substance and corresponding sampling times are converted to values that provide the basis for linear regression.

The mathematical equation used is basically an expression for a cumulative probability distribution but has proved applicable for fitting in vitro dissolution profiles in general. The equation is

$$\%R(t) = \left(1 - \exp(-t/\tau)^{\beta}\right)100 \qquad (1)$$

where $\%R(t)$ is the estimated percentage of dissolved substance at time $t$, $\tau$ denotes a scale parameter representing the time when 63.2% has been dissolved and $\beta$ is a shape parameter.

For the linear regression that determines $\beta$ and $\tau$, the logarithms of the sampling times are calculated, $\ln(t_i)$ and the observed percentages of dissolved substance, $\%R(t_i)$ are converted to Weibull values, $W(\%R(t_i))$ calculated as

$$W(\%R(t_i)) = \ln\left(\ln\left(1/(1 - \%R(t_i)/100)\right)\right) \qquad (2)$$

The linear regression is then performed on $W(\%R(t_i))$ as a function of $\ln(t_i)$. The slope of the regression line is $\beta$ and $\tau$ is calculated as

$\exp(-\alpha/\beta)$, where $\alpha$ is the $y$-axis intercept ($\ln(\tau)$ is the $x$-axis intercept).

The Weibull function is very sensitive to minor deviations when $\%R(t_i)$ is close to 0 and 100. It is therefore recommended to base the regression analysis on $\%R(t_i)$ values between 10 and 90 (otherwise in order to compensate for the great sensitivity, a weighting procedure can be employed (Langenbucher, 1976) using $(-\ln(1-\%R(t_i)/100)\,(1-\%R(t_i)/100))^2$ as weights).

The meaning of $\tau$ as the scale parameter is that $\tau$ reflects the degree of retardation of the drug release, i.e., the higher the $\tau$ value the slower the release. However, $\tau$ does not provide any information about the retardation mechanism. For that purpose the $\beta$ value is valuable. This is illustrated by the equation for the rate function (first derivative of Eqn 1):

$$\mathrm{d}(\%R(t))/\mathrm{d}t = \beta\tau^{-\beta}t^{\beta-1}\exp(-t/\tau)^{\beta}100 \quad (3)$$

which reaches a maximum at

$$t = 0 \quad \text{for } \beta \leq 1 \quad (4)$$

and at

$$t = \tau((\beta-1)/\beta)^{1/\beta} \quad \text{for } \beta > 1 \quad (5)$$

This means that for $\beta \leq 1$, the curvature (second derivative of Eqn 1) is negative for all time values as the rate decreases during time. For $\beta$ greater than unity, the dissolution curve becomes S-shaped as the maximum rate occurs after some time. Further, a high $\beta$ will reduce the release phase and consequently lead to its abrupt termination.

Different $\tau$s and equal $\beta$s are often obtained for formulations with varying degrees of retardation but developed on the same mechanism. An example is different quantities of coating on reservoir systems.

When $\beta$ and $\tau$ are calculated they can be used in Eqn 1 for non-linear curve fitting. For visual comparison of profiles it is usually advantageous to use the linearised profiles in a Weibull plot. For the experiment in this paper, the $\beta$s and $\tau$s are used as statistical parameters in the ANOVA.

*Experimental design for ruggedness testing*

The ruggedness testing will usually include several factors which, if tested separately, would involve a great deal of experimental work and important information about possible interactions between the factors would not be recovered. These problems are resolved by using $2^k$ factorial designs (Brøndum and Monrad, 1988), meaning that $k$ factors are tested at two levels. In this paper a $2^4$ factorial design has been used and a full structure without replicants has been chosen. The main effects and two-factor interactions are estimated, while the values for three- and four-factor interactions are pooled to estimate the random error. A total of 16 calibrator tablets are therefore required for this test.

Full and fractional designs as well as the ANOVA for the statistical evaluation can be performed by several computer programs. In this study Statgrafics version 5.0 was used.

*Test formulation*

USP dissolution calibrator tablets (lot J, non-disintegration type, containing 300 mg of salicylic acid) were used as test formulation (USP-NF Reference Standards, Rockville, U.S.A.).

*In vitro dissolution tests*

A flow through apparatus (Sotax AG, Switzerland) with cells of 12 mm inner diameter supplied with dissolution medium by a piston pump were used in all experiments. The apparatus has been described thoroughly by Möller (1983).

As already mentioned, the flow rate in the original method was 16 ml/min. Rates of 14 and 18 ml/min were used to examine the flow ruggedness.

The temperature should be set at 37°C, but in order to test temperature ruggedness, settings of 36 and 38°C were chosen.

It was indicated in the first of the above-mentioned collaborative studies using salicylic acid calibrator tablets (Nicklasson et al., 1987) that degassing of the dissolution medium affects the results significantly. Buffer solutions with and without previous degassing were therefore tested. If degassing proves effective, it can be recommended in order to obtain optimum reproducibil-

ity. Furthermore, this parameter indicates whether particular attention should be paid to the tightness of the pumping system, since air might be absorbed in the medium through any leaks.

The pH of the dissolution medium was maintained at $7.40 \pm 0.05$ but the conductivity was varied as the volumes of water for dissolving the buffer salts were 97.5 and 102.5% of the prescribed volume. These deviations do not result in measurable pH deviations, therefore conductivity measurement is needed for this control.

The experiment was performed in totally randomized fashion and each test run was carried out separately, i.e., repeated use of only one of the six cells. The sampling times were 0.5, 1, 2 and 3 h.

## Results and Discussion

Table 1 shows an example of the Weibull transformation of dissolution data – Expt 1 in Table 2 was used. The $W(\%(R(t_i))$ was calculated as shown in Eqn 2.

TABLE 1

*Example (Expt 1 in Table 2) of Weibull transformation of observed dissolution values for linear regression*

| Sampling no. ($i$) | Sampling time (h) ($t_i$) | Dissolved salicylic acid [$\%R(t_i)$] | $\ln(t_i)$ | $W$ [$\%R(t_i)$] |
|---|---|---|---|---|
| 1 | 0.5 | 10.9 | −0.693 | −2.159 |
| 2 | 1.0 | 27 7 | 0 | −1 126 |
| 3 | 2.0 | 49.0 | 0.693 | −0 395 |
| 4 | 3.0 | 65.4 | 1.099 | 0.0595 |

Slope = $\beta = 1.22$; intercept = $\alpha = -1\ 24$; $\tau = \exp(-\alpha/\beta) =$ 2 76

Table 2 shows the experimental design used for the ruggedness testing and the values of $\beta$ and $\tau$ obtained. The levels of the tested factors were chosen in order to delimit realistic deviations from the prescribed method. The pH of the media was measured before each test and all

TABLE 2

*Experimental design for ruggedness testing and the values of $\beta$ and $\tau$ obtained by Weibull transformation of the dissolution results*

| Code for experiment | Factors | | | | Results | |
|---|---|---|---|---|---|---|
| | A Flow rate (ml/min) | B Temperature (°C) | C Volume of buffer solution in % | D Degassing | $\beta$ | $\tau$ |
| (1) | 14 | 36 | 97.5 | − | 1 22 | 2 76 |
| a | 18 | 36 | 97 5 | − | 1.17 | 2.00 |
| b | 14 | 38 | 97.5 | − | 1.13 | 2.12 |
| ab | 18 | 38 | 97 5 | − | 1.02 | 2.10 |
| c | 14 | 36 | 102 5 | − | 1.00 | 2.92 |
| ac | 18 | 36 | 102.5 | − | 1.06 | 2.26 |
| bc | 14 | 38 | 102 5 | − | 0 89 | 3 54 |
| abc | 18 | 38 | 102.5 | − | 1 15 | 1.92 |
| d | 14 | 36 | 97.5 | + | 1.21 | 2.27 |
| ad | 18 | 36 | 97 5 | + | 1 28 | 1 72 |
| bd | 14 | 38 | 97 5 | + | 1.19 | 2.33 |
| abd | 18 | 38 | 97.5 | + | 1.32 | 1.69 |
| cd | 14 | 36 | 102.5 | + | 1.17 | 2.32 |
| acd | 18 | 36 | 102.5 | + | 1.25 | 1.79 |
| bcd | 14 | 38 | 102.5 | + | 1.27 | 1.82 |
| abcd | 18 | 38 | 102 5 | + | 1.34 | 1.63 |
| | | | | means: | 1.17 | 2.20 |

values were between 7.39 and 7.43, showing that a deviation of ±2.5% in the prescribed volume for the buffer solution does not affect the pH at all. The conductivity measurements fell within the range of 6.60–6.93 mS/cm and were strongly correlated to the volume of buffer solution.

The results show $\beta$ values within the range of 0.89–1.34 and a total mean of 1.17. Values within this range are normal for most retarded formulations. If $\beta = 1$ the response of release corresponds to first-order kinetics, meaning that the release rate is constant relative to the unreleased part of the drug. For $\beta > 1$ this rate will increase with time and vice versa for $\beta < 1$. In this experiment $\tau$ is regarded as a technical parameter, and the results show that a mean of 2.20 h of dissolution is required for 63.2% release. As regards $\tau$ values in general, no typical range can be stated as these are dependent on the biological absorption area. Furthermore, an in vivo-in vitro correlation is necessary in order to evaluate $\tau$ values from a biological point of view.

Table 3 lists the results of the statistical evaluation. The interpretation of the data is as follows. The $p$ values are the probability of the two tested levels giving identical results. If a $p$ value is less than 0.05 the effect of the actual factor is defined as statistically significant. The 'total error I' is used for testing the interactions (AB, AC, etc.) and as none of these were statistically significant

TABLE 3

*Statistical evaluation of $\beta$ and $\tau$ obtained from the Weibull transformation of the dissolution profiles*

| Effect | Sum of squares | DF | Mean square | $F$ ratio | $p$ values |
|---|---|---|---|---|---|
| Scale parameter $\beta$ | | | | | |
| A flow | 0.0169 | 1 | 0.0169 | 2.301 | 0.157 |
| B· temperature | 0.0001 | 1 | 0 0001 | 0.014 | 0.910 |
| C: buffer volume | 0 0110 | 1 | 0 0110 | 1 501 | 0 246 |
| D: degassing | 0.1225 | 1 | 0.1225 | 16 682 | 0.001 [b] |
| AB | 0.0025 | 1 | 0 0025 | 0 445 | 0.541 |
| AC | 0 0110 | 1 | 0.0110 | 1.962 | 0.220 |
| AD | 0.0025 | 1 | 0 0025 | 0 445 | 0 541 |
| BC | 0.0090 | 1 | 0 0090 | 1 606 | 0 260 |
| BD | 0.0144 | 1 | 0.0144 | 2.562 | 0 170 |
| CD | 0.0132 | 1 | 0.0132 | 2.353 | 0 185 |
| Total error I | 0.0281 | 5 | 0.0056 | | |
| Total error II | 0.0808 | 11 | 0 0073 | | |
| Mean $\beta$: | without degassing, 1 08; with degassing, 1.26 | | | | |
| Scale parameter $\tau$· | | | | | |
| A flow | 1.5500 | 1 | 1.5500 | 13.994 | 0 003 [b] |
| B: temperature | 0.0506 | 1 | 0.0506 | 0.457 | 0 520 |
| C buffer volume | 0.0930 | 1 | 0.0930 | 0.840 | 0.388 |
| D. degassing | 1.0201 | 1 | 1.0201 | 9.210 | 0.011 [a] |
| AB | < 0 0001 | 1 | < 0.0001 | < 0.001 | 0.990 |
| AC | 0 0650 | 1 | 0.0650 | 0 417 | 0.553 |
| AD | 0 0841 | 1 | 0.0841 | 0.540 | 0.503 |
| BC | 0.0012 | 1 | 0.0012 | 0.008 | 0 933 |
| BD | 0.0081 | 1 | 0.0081 | 0.052 | 0.831 |
| CD | 0.2809 | 1 | 0 2809 | 1.803 | 0.237 |
| Total error I | 0 7790 | 5 | 0.1558 | | |
| Total error II | 1.2184 | 11 | 0 1108 | | |
| Mean $\tau$. | flow = 14 ml/min, 2 51; flow = 18 ml/min, 1.89; without degassing, 2.45; with degassing, 1.95 | | | | |

[a,b] Statistical significant effect ([a] $p < 0.05$, [b] $p < 0.01$).

the sum of squares from these effects was pooled with the total error I giving the 'total error II'. The main effects are evaluated by means of total error II.

It appears that degassing of the dissolution medium affects the shape ($\beta$) of the release profiles. The probable explanation is that the introduction of gas bubbles on the tablet surface decreases the release rate (and therefore $\beta$) as the contact area between tablet and medium is reduced. During dissolution testing gas bubbles were in fact observed on the tablets when using dissolution medium without previous degassing, but not when degassed medium was employed. None of the other factors affect $\beta$ significantly, indicating that the mechanism of release is rugged to flow, temperature and buffer volume (conductivity). The $\tau$ values were also unaffected by changes in temperature and buffer volume, therefore it can be concluded that the method is rugged to these two factors. Degassing and flow variations have a significant effect on $\tau$. Such effects are logical as both a high flow and elimination of gas bubbles improve solubility conditions.

Figs 1 and 2 illustrate the Weibull plot and the non-linear curve fit. Both figures show the effect of degassing the dissolution medium. The testing

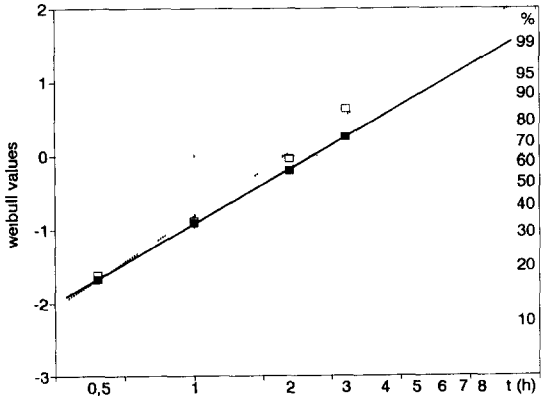


Fig. 1. Weibull plot corresponding to the linear regression performed on data for untreated/degassed dissolution medium, respectively. The markers show the observed average values transformed to Weibull values calculated according to Eqn 2. (■ ——— ■) Untreated dissolution medium, (□ · · · · · □) degassed dissolution medium.

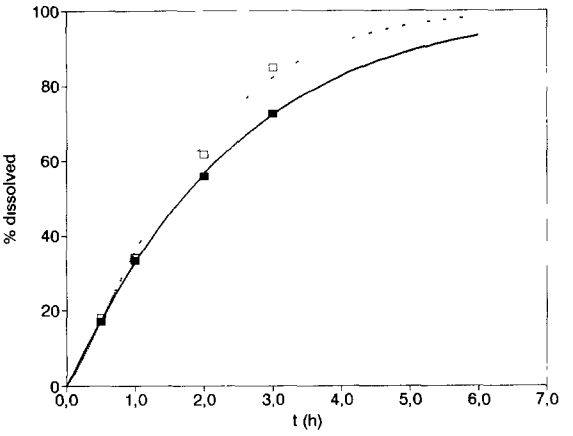


Fig. 2 Estimated dissolution profiles based on the $\beta$s and $\tau$s for untreated/degassed dissolution medium, respectively The markers show the observed average values. (■ ——— ■) Untreated dissolution medium, (□ · · · · · □) degassed dissolution medium

included eight experiments carried out using degassed medium (Expt 1, a, b, ab, c, ac, bc, abc) compared to the eight with untreated medium (d, ad, bd, abd, cd, acd, bcd, abcd). The average values of $\%R(t_i)$ for each of the four sampling times (0.5, 1, 2 and 3 h) are calculated for the two groups of data, and $\beta$ and $\tau$ for the two average profiles are used for the curve fitting. Figs 1 and 2 show that the effect of degassing is most apparent towards the end of the profile, which clarifies the fact that $\beta$ is higher for the profiles obtained with degassed dissolution medium. This result confirms the assumption stated by Nicklasson et al. (1987). Apparently, the best curve fit is achieved by the use of untreated medium. However, the correlation coefficient obtained in the linear regression for degassed medium is better than 0.99.

## Conclusion

It can be concluded that ruggedness testing of dissolution methods may be performed systematically by means of factorial experiments using Weibull transformed release data as the basis for the statistical evaluation.

The design for this experiment, i.e., a full structure $2^k$ experiment without replicants, appears to be suitable for testing four factors. Testing of higher numbers of factors of fractional designs should be considered. However, the number of experiments should be sufficient to test all two-factor interactions, as this information can be very valuable and is relatively easy to achieve in factor experiments.

The described test showed that for flow through cell dissolution of salicylic acid calibrator tablets, degassing of the medium is essential for the observed degree of retardation ($\tau$) as well as for the shape ($\beta$) of the release profile. The flow of the dissolution medium affects $\tau$ but not $\beta$. Neither temperature nor conductivity of the medium showed significant effect on the dissolution profiles. The statistical evaluation showed no interactions between the four tested factors.

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