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# MULTIVARIATE ANALYSIS OF VARIANCE OF DISSOLUTION DATA IN THE DEVELOPMENT OF ORAL SUSTAINED RELEASE FORMULATIONS

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## ABSTRACT

A multivariate analysis of variance applied to polinomial interpretation of growth curves in used for the interpretation of dissolution curves of four experimental, sustained release, wax type theophylline tablets.

The factors under study were glyceril palmitic stearate, carboxypolimethylene contents and compression force. The tablets were formulated according an experimental design based on 4 x 4 Hadamard matrix. The USP type I apparatus for dissolution test and CHI 0.1 N plus 0.1% polysorbate 80 as dissolution medium was used.

The statistical interpretation of results showed: first, that dissolution rates were almost constant for the four formulations during 8 h; second, the main difference between formulation dissolution rates can be inputed to fat excipient content and in much lesser extent to carboxipolymethylene content; third, the theopylline release rate was unaffected by compression force.

## INTRODUCTION

One way of avoiding interpretation of the findings of sustained-release oral formulation dissolution tests involving as yet little-understood kinetics, is to use Box' statistical analysis method of "growth curves" (1) better known as "split plot" (2,3).

The chief disadvantage of this method is the complete lack of a kinetic interpretation albeit empirical. One alternative within the empirical terrain in which controlled release oral form dissolution assays are interpreted is the use of polynomial variables. However, fitting these variables to the findings from dissolution assays must take the following facts into account: 1./ each formulation or batch is assayed more than once, usually six times; 2./ if a closed system is used, such as the USP, there will be high correlation between the successive points of the dissolution curve; 3./ if the formulations being tested have been manufactured according to some experimental design, interpretation of the results must be in accordance with the factors included in the design. Khatri's multivariant regression method (4) meets all these requirements and so would seem an alternative to the Box method.

This paper gives the results of a dissolution assay of four formulations of sustained-release theophylline tablets made in accordance with a D-optimum experimental design which enables the influence of three technological factors, on the dissolution rate to be studied.

## EXPERIMENTAL

### Manufacturing method

The formulations manufactured were of the fat matrix type based on glyceril palmitic stearate (Atomized Precirol®) and carboxypolymethylene (Carbopol® 940). Their manufacture basically consisted of: melting the glyceril palmitic steareate at 50·C, adding theophylline until evenly dispersed throughout the tablet, cooling to a semi-solid consistency, granulation and sieving. Pre-

TABLE 1

Composition of the Formulations Studied

| COMPONENTS | F-1 | F-2 | F-3 | F-4 |
|---|---|---|---|---|
| GLYCERIL PALMITIC (mg) STEARATE (Precirol®) | 458 | 458 | 258 | 258 |
| CARBOXYPOLYMETHYLENE (mg) (Carbopol® 940) | 34 | 14 | 34 | 14 |
| THEOPHYLLINE (mg) | 300 | 300 | 300 | 300 |
| TALC (mg) | 21 | 21 | 21 | 21 |
| MAGNESIUM STEARATE (mg) | 7 | 7 | 7 | 7 |
| COMPRESSION FORCE (Kgf) | 1.744 | 253 | 253 | 1.744 |

sieved carboxypolymethylene (sieve size, 0.4 mm), talcum and magnesium stearate were added to the granulometric fraction collected between the 1 and 0.4 mm mesh. The granulate thus obtained was compressed in an double punch excentric tablet press with 12 mm ø punches and a system to measure the compression force (5).

To achieve optimum composition an experimental design was used with the glyceril palmitic stearate content, P(1), the carboxypolymethylene content, P(2) and compression force, P(3) included as the variables. This design follows the Hadamard 4x4 matrix (6).

$$\begin{pmatrix} 1 & 1 & 1 & 1 \\ 1 & 1 & -1 & -1 \\ 1 & -1 & 1 & -1 \\ 1 & -1 & -1 & 1 \end{pmatrix}$$

Where 1 and -1 are the upper and lower levels of each factor under study. At the outset of the study we had no information about the effect of these variables on the release rate, so we took the values for each variable as the maximum and minimum compatible with the granulation and release processes. Table 1 shows the composition of each of the formulations studied.

### Dissolution assay

USP Apparatus I (7) was used (Turū Grau Mod. D-6) with HCl 0.1 N plus 0.1% polysorbate 80 as solvent. The samples were filtered and diluted with HCl 0.1 N and the theophylline was determined by U.V. spectrophotometry at 271 nm (Beckman, Mod. 25). The samples were taken at one-hour intervals for 10 hours and six simultaneous assays per formulation were made.

### Evaluation of the results

The statistical model for Khatri's multivariant linear regression is as follows:

$$X = B.Z + e \qquad \text{Eq.1}$$

The matrix X corresponds to the experimental observations and each row contains the observations on one dissolution assay; thus it is equal to N x p, where N = N(1) + N(2) + .. + N(r) is the total number of assays performed, r, the number of formulations assayed, and p the number of sampling times per assay. The first column of matrix B, p x q, contains only ones; the second column gives the sampling times, the third their squares, and so forth until the desired polynomial degree is reached. Matrix Z, q x r, contains the r vector columns, for each of the formulations assayed with the polynomial function coefficients. Matrix e is the error matrix.

If both the selection of sampling times and experimental design used in the manufacturing of formulations are based on orthogonal vectors which ensure the statistical independence, then several null hypothesis can be tested, on the elements of estimated coefficient matrix, $\hat{Z}$. A detailed explanation of the way in which this method is applied as well as the Heck's charts and Pillai's tables, are given by Morrison (8). The calculations use software intended for calculations with matrices (9).

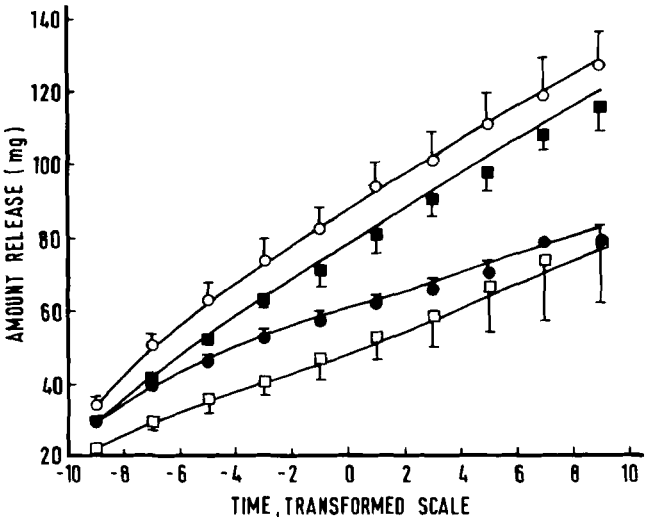


**Figure 1**: Experimental dissolution curves and fitted polinomial functions: F-1 (■), F-2 (□), F-3 (○) and F-4 (●). The bars are equals to one standard desviation.

## RESULTS AND DISCUSSION

Figure 1 gives the mean values and standard deviations from the mean dissolution curves obtained experimentally (a listing with the experimental results and instructions for estimating the model parameters is available on demand); the model originally chosen was a polynomial to the fifth power.

As the sampling times were equally spaced it was feasible to transform the time scale so that the vector columns in Matrix B were orthogonal; therefore, the statistic independence of several statistical hypotheses was thus ensured.

Estimates for matrix Z were as follows:

$$\hat{Z} = \begin{pmatrix} 77.6 & 49.5 & 86.0 & 59.6 \\ 4.94 & 2.97 & 5.11 & 2.80 \\ -0.374 & 0.105 & -0.638 & -0.461 \\ 0.0161 & 0.0110 & 0.0305 & 0.0347 \\ -0.0139 & -0.0240 & -0.0281 & -0.0206 \\ -0.0093 & 0.0033 & 0.0452 & -0.0222 \end{pmatrix} \quad \text{Eq. 2}$$

As we mentioned above, each vector of this matrix is the coefficient of the polynomial variable fitted for each

formulation, using the transformed time scale. The first element of each vector is the independent term, the second the coefficient of the linear term and so forth.

Figure 1 gives the mean curves produced by the full model calculated by the equation, $\hat{\bar{X}} = B.\hat{Z}$, where $\hat{\bar{X}}$ is the matrix of means estimated by the four formulations.

From the standpoint of the development of a new formulation, it is more interesting to check the influence of the factors included in the experimental design than merely compare pairs of formulations. Inspection of matrix $\hat{Z}$ and Figure 1 shows that the terms that control the dissolution curve are basically the linear term and to a lesser extent the quadratic; the higher order terms have a less important role. Moreover, in view of the low coefficients obtained for the higher terms we must ask if the possible differences, although statistically significant, could contribute to the optimization of the release process.

Table 2 sets outs the results obtained in the orthogonal crosschecking to identify the technological factor(s) controlling the linear or quadratic or cubic coefficient.

TABLE 2

Results of the orthogonal contrasts based on Snedecor F distribution, for each set of matrix $\hat{Z}$ coefficients.

| | | FACTOR | | |
|---|---|---|---|---|
| COEFFICIENT | | P(1) | P(2) | P(3) |
| LINEAR | Ho :<br>F = | 0.0009<br>- | 4.29<br>53.4 (*) | -0.348<br>- |
| QUADRATIC | Ho :<br>F = | 0.830<br>11.4 (*) | -0.656<br>6.20 (*) | -0.303<br>- |
| CUBIC | Ho :<br>F = | -0.0381<br>12.2 (*) | 0.0009<br>- | 0.0093<br>- |

(*), the null hypothesis, Ho, is rejected for $\alpha = 0.01$ with 6 and 18 degrees of freedom ; P(1), glyceril palmitic stearate; P(2), carboxypolymethylene; P(3), compression force.

As can be seen, the linear coefficient is determined by the carboxypolymethylene content; Figure 1 bears out this conclusion; observe how the formulations with the fastest dissolution rate, F-1 and F-3, are in fact those with the highest carboxypolymethylene content. Glyceril palmitic stearate plays a certain role, less important quantitatively at quadratic and cubic level; the compression force has no effect whatsoever within the range of the variables under study.

As mentioned at the beginning, the design variables were taken as the most extreme values consonant with the tablet manufacturing process; the findings show that increasing the carboxypolymethylene content increases the theophylline release rate. However, this excipient cannot be added in greater proportion than that employed in the glycerine palmitic stearate theophylline granulate as the mixture would not be stable.

The overall interpretation of the results must take two points into account. One, the polynomial variables are empirical approximations to dissolution kinetics with a mechanism unknown to us. Two, use of Hadamard matrixes enables D-optimum experimental designs to be constructed with the least possible number of experiments but does assume that the response is a monotonous decrease or increase of the design variables. As these conditions are very restricting, this design is useful for planning a series of stages in the optimization process, in each one of which we may hope to identify the variable(s) controlling the process, and hence, the direction in which we should modify these variables to attain the maximum response point.

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