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Experimental design, an efficient tool for studying the stability of parenteral nutrition

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Summary

Stability of nutrient mixtures is studied here with regard to its pH measurement. It is shown that the pH may be described as a linear function of the period of storage and of the parameters corresponding to the operative conditions. Then the Plackett and Burman designing method appears to be an efficient tool in order to reduce the number of experiments for determining this first-degree model with a good degree of accuracy.

Introduction

The stability of nutrient mixtures containing all the essential nutrients in a single bag is an important characteristic of good quality. If it is not well-controlled, a decrease of pH values and an increase of coalescence phenomenon can occur which may be extremely hazardous for the safety of patients. Stability depends on the length of storage and preparative conditions; in previous papers we showed that modelling allows one to quantify precisely the influence of each parameter (Antonelli et al. 1986; Rochat et al. 1987).

The purpose of this paper is not to provide more results about the stability of all-in-one nutrient mixtures, but to present the model-building used in our different studies and to demonstrate the great interest of experimental design for data analysis (Bayne and Rubin, 1986; Box et al., 1978; Stetsko, 1986).

Experimental

The methodology of modelling will be detailed here through a specific example which shows that 360 experiments could have been reduced to 32 experiments without any loss of precision. The parameter measured in this study is the pH of a ternary mixture containing amino acids, dextrose and lipids to which subsequently KCl is added.

Then the four following parameters have been considered:

(1) Parameter P_1. Reduced concentration of added electrolyte (K^+) with levels 1, 2, 6 corre-

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sponding, respectively, to 13.4, 26.8 and 80.4 mmol $\cdot l^{-1}$.

(2) Parameter P_2. Storage temperature: +4°C and +20°C.

(3) Parameter P_3. Storage period after addition of K^+: 24, 48 and 72 h.

(4) Parameter P_4. Storage period of ternary mixture (after addition of electrolyte into the mixture): 1, 2, 4 weeks.

Ninety experiments have been performed for each bag and one sample consists of 4 bags.

Theory

When studying an experimental response depending on N factors and for which a linear model is assumed, the statistical theory of experimental design provides optimal experimental strategies (orthogonal designs) leading to the best accuracy on the A_i values from a minimal experiments number $N+1$ (Plackett and Burman, 1946). However, such strategies are not available for any parameter number and, for instance, up to 20 parameters solutions may be obtained only for 3, 7, 11, 15 and 19 variables. We shall see later how the other cases can be treated by including dummy variables.

The determination of an optimal strategy requires the introduction of the notion of a matrix of experiments.

Let us associate to each parameter P_i a reduced variable X_i defined as:

$$X_i = 2\frac{P_i - \bar{P}}{P_{max} - P_{min}} \qquad (1)$$

$\bar{P}$, P_{min} and P_{max} being respectively the mean, minima and maxima values of P_i. One interest of such variables is a single variation range between the levels -1 and $+1$ for any parameter.

Then the matrix of experiments is a matrix containing $N+1$ rows and N columns, each row corresponding to an experiment and each column to a variable. The term located in row i and column j has as a value for the reduced variable X_i during experiment j.

From this matrix, one can define the matrix of the system by adding a column containing ($N+1$ values + 1). It has been demonstrated that an optimal strategy is obtained when this latter matrix is a Hadamard matrix $H(N)$.

Such matrix may be built easily from the method presented by Plackett and Burman (1946). We shall use here an alternative recurrent method which is valid when $N+1$ is a power of 2. Only two formulae are required:

$$H(1) = [1] \quad H(2n) = \begin{matrix} H(n) & H(n) \\ H(n) & -H(n) \end{matrix}$$

from which one obtained successively:

$$H(2) = \begin{matrix} H(1) & H(1) \\ H(1) & -H(1) \end{matrix} = \begin{bmatrix} 1 & 1 \\ 1 & -1 \end{bmatrix}$$

$$H(4) = \begin{matrix} H(2) & H(2) \\ H(2) & -H(2) \end{matrix} = \begin{bmatrix} 1 & 1 & 1 & 1 \\ 1 & -1 & 1 & -1 \\ 1 & 1 & -1 & -1 \\ 1 & -1 & -1 & 1 \end{bmatrix}$$

and so on.

Application

When using the reduced variables X_i as defined in Table 1, the linear model (1) becomes:

$$pH = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + \text{Residue} \qquad (2)$$

and its determination implies the evaluation of 5 coefficients, i.e. 5 experiments at least.

TABLE 1

Definition of parameter levels

X_i level	-1	+1
P1	1	6
P2 (°C)	4	20
P3 (h)	24	72
P4 (weeks)	0	4

As said above, there is no optimal strategy for studying from 5 experiments a response depending linearly on 5 parameters. Nevertheless if we add 3 dummy variables X_5, X_6 and X_7, it is easy to build the matrix of a system with 7 parameters as an Hadamard matrix $H(8)$:

$$H(8) = \begin{matrix} H(4) & H(4) \\ H(4) & -H(4) \end{matrix}$$

$$= \begin{array}{c} \begin{matrix} 1 & 2 & 3 & 4 & 5 & 6 & 7 \end{matrix} \\ \begin{bmatrix} 1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 1 & -1 & 1 & -1 & 1 & -1 & 1 & -1 \\ 1 & 1 & -1 & -1 & 1 & 1 & -1 & -1 \\ 1 & -1 & -1 & 1 & 1 & -1 & -1 & 1 \\ 1 & 1 & 1 & 1 & -1 & -1 & -1 & -1 \\ 1 & -1 & 1 & -1 & -1 & 1 & -1 & 1 \\ 1 & 1 & -1 & -1 & -1 & -1 & 1 & 1 \\ 1 & -1 & -1 & 1 & -1 & 1 & 1 & -1 \end{bmatrix} \end{array}$$

The suppression of the first column and of the three ones corresponding to the dummy variables leads to the matrix of experiments:

$$D = \begin{array}{c} \begin{matrix} 1 & 2 & 3 & 4 \end{matrix} \\ \begin{bmatrix} 1 & 1 & 1 & 1 \\ -1 & 1 & -1 & 1 \\ 1 & -1 & -1 & 1 \\ -1 & -1 & 1 & 1 \\ 1 & 1 & 1 & -1 \\ -1 & 1 & -1 & -1 \\ 1 & -1 & -1 & -1 \\ -1 & -1 & 1 & -1 \end{bmatrix} \end{array}$$

When coming back to the original units of variables, we obtain easily the experiments to be performed (Table 2).

Such a design allows one to optimize the data exploitation according to the model:

$$\text{pH} = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_4 X_4 + b_5 X_5 + b_6 X_6 + b_7 X_7 \qquad (3)$$

Evidently each individual term appearing in the sum: $b_5 X_5 + b_6 X_6 + b_7 X_7$ has no physical sense but this sum replaces the experimental error E and so should be negligible if the proposed model is valid. Further it is possible to estimate the standard deviation of this error by:

$$\hat{\sigma} = \sqrt{\tfrac{1}{7} \sum_{i=1}^{8} (b_5 X_5 + b_6 X_6 + b_7 X_7)_i^2} \qquad (4)$$

TABLE 2

Experiments and results

Run	P_1	P_2 (°C)	P_3 (h)	P_4 (week)	pH
1	6	20	72	4	5.30
2	1	20	24	4	6.10
3	6	4	24	4	6.00
4	1	4	72	4	5.90
5	6	20	72	0	6.50
6	1	20	24	0	6.70
7	6	4	24	0	6.70
8	1	4	72	0	6.60

Calculations of effects from experimental results shown in Table 2 lead to:

$b_0 = 6.23 \quad b_1 = -0.10 \quad b_2 = -0.075$

$b_3 = -0.15 \quad b_4 = -0.40 \quad b_5 = -0.075$

$b_6 = -0.050 \quad b_7 = -0.075$

the three last values having to be considered only for the evaluation of experimental error.

Taking into account the definition of the coded variables, we derive from the five first b_i coefficients the a_i values which are obtained from 8 runs. They are very close to those deduced from the 90 experiments (Rochat et al., 1987), as shown in Table 3.

TABLE 3

Comparison of model coefficients calculated from 8 and 90 runs

	from 8 runs	from 90 runs
a_0	7.24	7.24
a_1	−0.040	−0.043
a_2	−0.00937	−0.00579
a_3	−0.00646	−0.00639
a_4	−0.200	−0.210

It is important to point out that the differences between the experimental pH and the one predicted by the linear model, i.e. the term $b_5X_5 + b_6X_6 + b_7X_7$, never exceeded 4% of the response. Moreover the standard deviation of the experimental error is evaluated here to be 0.13 while its estimate from 90 experiments is 0.14.

Conclusion

This typical example shows the efficiency of an experimental design for studying the behaviour of nutritional solutions.

In this case an orthogonal design has been built a posteriori by using only some experiments among the performed ones. Evidently, the aim of such an experimental methodology is to build a priori an optimal strategy before running the assays.

The mathematical procedure is easy enough to work out and leads to a reduction of experiments number (from 90 to 8 for each bag, in our example) with the same accuracy on the model determination and consequently on the conclusions to be deduced.

The practical consequences of such a methodology are a gain in time and money in conducting an experimental program whatever the application field may be. Special experimental design and modelling is truly a helpful tool for pharmaceutical scientists when searching for formulations (Billardon et al., 1987) or developing processes (Jimenez et al., 1986), (Paschos et al., 1987).

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