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Design and Modeling for Order of Addition Experiments with Amount Effects

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Abstract

The order of addition experiment is increasingly used in many scientific fields, such as chemistry， biology and medicine. The existing literature considers order of addition rather than amount combination. In this paper, we consider order of addition experiments with amount effects. A new proposed model incorporates both order effects and amount effects, which is called the order of addition-amount combination model. In addition, we propose an experimental design that can ensure the estimations of order of addition effects, but the experimental design are uncorrelated with the estimations of amount combination effects. In this paper, we give an illustrative example to explain this design. In terms of MSE, residual and fitting the real model, the proposed design is more appropriate than the random design.

Keywords: Statistical model; Amount combination; Orthogonal design; MSE; Residual graph

1. Introduction

In many chemical and biological experiments, the order of addition reactants and reagents often has a significant impact on the results of the reaction. We call these experiments the

order of addition experiments. A famous example is the tea tasting experiment for women Fisher, (1971)[1]. The woman claimed that she could distinguish whether tea or milk was first added to her cup. Fisher experimented with four replicates of two sequences: tea before milk or milk before tea. There are many order of addition experiments in applications, involving two or three components. Black et al. (2001)[2] conducted an order of addition experiment that involved a standard export reaction, a wash step, and an additional 30 minute incubation with the indicated factors in a nuclear export assay. Preuss et al. (2009)[3] performed order-of-addition experiments to determine the potential order of action of two components in the analysis of protein function in an in vitro transport system. Ding et al. (2015)[4] reported the use of a combination of three drugs (bortezomib, camptothecin, and doxorubicin) in the treatment of oral cancer and showed that the order of drug addition played an vital role in ensuring that the combination was effective. In applications, the order-of-addition experiments have been popularly involved in many areas such as pharmaceutical science Rajaonarivony et al. (1993)[5], bio-chemistry Shinohara and Ogawa, (1998)[6], nutritional science Karim et al.(2000)[7] and food science Jourdain et al. (2009)[8]. However, most of the existing papers on the order of addition experiments do not take the different amount combinations into account. In the actual various chemical and biological experiments, these two aspects should be jointly considered. In this paper we propose a model, which combining the order of addition and the amount combination. The model is called the order of addition-amount combination model. Under such a model, a general product design combining order of addition and amount combination is constructed, which shows that the order effects and amount effects which have estimated not relevant on each other, and this property is proved. An illustrative example is provided to demonstrate that the order of addition-amount combination design is more advantageous in MSE, residuals and the fitting effect of real model.

The remainder of this paper is organized as follows. In Section 2, we present the OAAC(order of addition-amount combination) model. In Section 3, we propose a new design table for this model and investigate its statistical properties. In Section 4, an illustrative example is given to demonstrate the effectiveness of this new design table. Finally, a summary of key points and conclusion are given in Section 5.

2. Model Formulation

In this section, we propose a new statistical model which is called the order of addition experiments with amount effects. Suppose that the components are denoted by 1,2,.... m, and the permutations of these numbers indicate the feasible orders for the experiments. For any pair of components i and j, let $x_{i,j}$ be the pair wise ordering (PWO) factors, where $x_{i,j}=1$ means that component *i* is added before *j*, otherwise $x_{i,j}=-1$.

By using the PWO factors, Van[9](1985) proposed the following model for order effects:

$$Y=\beta_0+\sum_{i=1}^{m-1}\sum_{j=i+1}^{m}\beta_{i,j}X_{i,j}+\varepsilon \tag{2.1}$$

For simplicity, we call this model a pair-wise order of addition model. On this basis, we put forward the order of addition-amount combination model, which is as follows:

$$Y=\eta_0+\sum_{i=1}^{m-1}\sum_{j=i+1}^{m}\eta_{i,j}X_{i,j}+\sum_{i}^{m}\eta_{ij}X_iX_j+\sum_{i}^{m}\eta_iX_i+\varepsilon \tag{2.2}$$

where $X_{i,j}$ is about the component order of addition reactions, X_i is the main effect of components, X_iX_j is the first-order interaction between reaction terms, η_0 is the intercept reaction item, ε is a random error assumed to be independent and have a normal distribution $N(0,\sigma^2)$. It is easy to see that the above model not only considers the order of addition but also includes the amount combination.

3. Design construction

In this section, According to the model (2.2), we constructed the design table, and then we study the statistical properties of the model. Suppose that the amount of each component is experimented at two levels, denoted by -1 and +1, where -1(+1) represents the low (high) amount for the component. As stated before, each PWO factor $X_{i,j}$ also has two levels marked as + 1 and - 1, where + 1 means *i* was added before *j*, and - 1 means *i* was added after *j*. Let us introduce the product design.

Definition 1. With design matrix *A*, *B*, where *A***B*=*C*, the combination design of *A* and *B* is

called product design.

Let *F* be the design matrix of the order of addition and *O* be the design matrix of the components amount combination. Let us introduce the combination orthogonal.

Definition 2. Suppose *O* is the full factor design matrix, *C* is zero matrix, and *F*O=C The* columns of *F* are orthogonal to the columns of *O*. The combination design of *F* and *O* is called combination orthogonal.

For comprehensive discussion on the theory of factorial designs, we refer to Dey and Mukerjee (1999)[9], Mukerjee and Wu (2006)[10] and Wu and Hamada (2009)[11].

Theorem 1: The product design of a design table with an orthogonal design table is still an orthogonal design table. This kind of product design is called combination orthogonal.

Proof: The full order of addition design matrix F and full amount combination design matrix O are as follows:

$$F = \begin{bmatrix} X_{11} & \cdots & X_{1m} \\ X_{n1} & \cdots & X_{nm} \end{bmatrix}; \qquad \mathrm{O} = \begin{bmatrix} y_{11} & \cdots & y_{1M} \\ y_{N1} & \cdots & y_{NM} \end{bmatrix}$$

Where m is the number of component, $n = \mathrm{C}_m^2$, $\mathrm{N} = 2^m$, $\mathrm{M} = m + C_m^2$

Let F*O=C. Take the first N lines of product design C as C_1, So

$$\mathrm{C}_1 = \mathrm{F}_1 * O_1 = \begin{bmatrix} \mathrm{X}_{11} & \cdots & X_{1m} & y_{11} & \cdots & y_{1M} \\ & & & & & \\ X_{11} & \cdots & X_{1m} & y_{N1} & \cdots & y_{NM} \end{bmatrix}$$

Then:

$$\begin{aligned} &\mathrm{X}_{11} y_{11} + ... + X_{11} y_{N1} = 0 \\ &..... \\ &X_{1m} y_{11} + ... + X_{1m} y_{N1} = 0 \end{aligned}$$

In a combination orthogonal design table, all levels combination occur the same number, and the inner product of any two columns is 0. For example, we can found that any columns

of F_1 multiplied by any columns of O_1 is 0, and each level combination occur same number. F_1 and O_1 are combination orthogonal. Similarly, the whole F and O are also combination orthogonal. □

From theorem 1, we can get the estimator of order of addition effect and amount combination effect is uncorrelated. Actually, we can select a fraction of the order of addition design and amount combination design, when both of them are balance designs, we can combine them by product design. In summary, this section proposed a new design about the order of addition and the amount combination.

4. An illustrative example

In this section, an illustrative example is given. Considering an order of addition experiment with three components,we suppose that each component are two levels. First of all, we present the order of addition design. There are six cases denoted by: 123,132,213,231,321 and 312. Its design matrix is given as follows:

Table 4.1: Order of addition design table

Ordering	$x_{1,2}$	$x_{1,3}$	$x_{2,3}$
123	1	1	1
132	1	1	-1
213	-1	1	1
231	-1	-1	1
321	1	-1	-1
312	-1	-1	-1

Next, considering the amount combination design. There are eight cases. its design matrix can be written as follows:

Table 4.2: Amount combination design table

Amount-combination	x_1	x_2	x_3	x_1x_2	x_1x_3	x_2x_3
	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

Then, the product of the above two designs can be shown as follows:

Table 4.3: Order of addition-amount combination design table

Addition-amount	$x_{1,2}$	$x_{1,3}$	$x_{2,3}$	x_1	x_2	x_3	x_{12}	x_{13}	x_{23}
	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
	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
	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
	-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
	-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
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
-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

For simplicity, setting the above matrix as $\mathrm{x}_1, \mathrm{x}_1$ is referred to as the experimental group for this example. On the other hand, the control group adopts the random design method. Different from the experimental group, the values of amount combinations of the random design are randomly selected between -1 and 1. The purpose of this example is to compare the estimation and prediction performance of the competing designs. Assuming that the true model is as follow:

$$\mathrm{Y} = \eta_0 + \sum_{i=1}^{m-1}\sum_{j=i+1}^{m}\eta_{i,j}X_{i,j} + \sum_{i}^{m}\eta_{ij}X_iX_j + \sum_{i}^{m}\eta_iX_i + \varepsilon \qquad (4.1)$$

where:

$$\eta_{1,2} = 1,; \quad \eta_{1,3} = 2; \quad \eta_{2,3} = \text{-}3; \quad \eta_1 = 5; \quad \eta_2 = 1; \quad \eta_3 = 0;$$
$$\eta_{12} = 8; \quad \eta_{13} = 2, \quad \eta_{23} = \text{-}2; \quad \eta_0 = 3; \quad \varepsilon \text{ - } \mathrm{N}(0,10^2)$$

We set the experimental group model as E_1, the control group model as E_2. The design matrix of the experimental group x_1, which is also called the training set. The training set substitute into model (4.1), and it can produce the response values y_1 and y_2. By fitting y_1 and using OLS, a multivariate linear model, which is called the E_1 is obtained. Similarly E_2 also is obtained. We design a testing set matrix with 4800 rows and 9 columns, it keeps the order of addition part unchanged, the amount combination part of the matrix takes values between -1 and 1. We can get the response value y_1 and y_2, when we put the testing set matrix into E_1 and E_2 respectively. Drawing the scatter diagram of $y - y_1$ as shown in Figure 4.1

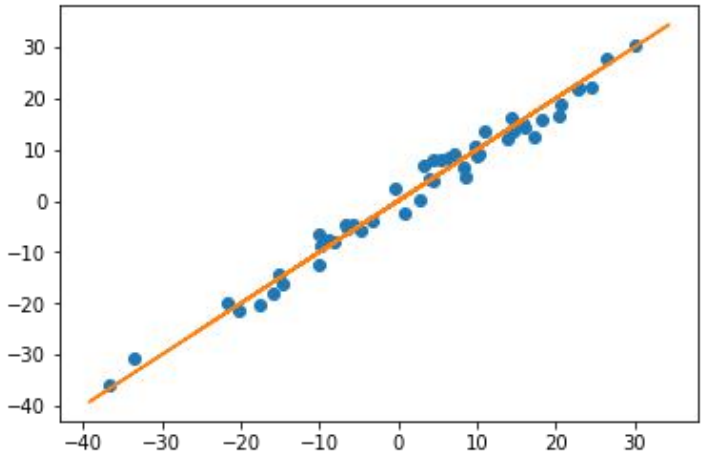

Figure 4.1: blue dot is the value of the $y - y_1$, yellow line is diagonal line

Repeating the above data generation and fitting model 1000 times. *MSPE* is mean squared prediction error .

$$\text{MSPE} = \frac{1}{N}\sum_{i=1}^{N}(y - y_i)^2 \quad \text{(i=1,2)}$$

The *MSPE* values are visualized in the box-plot below:

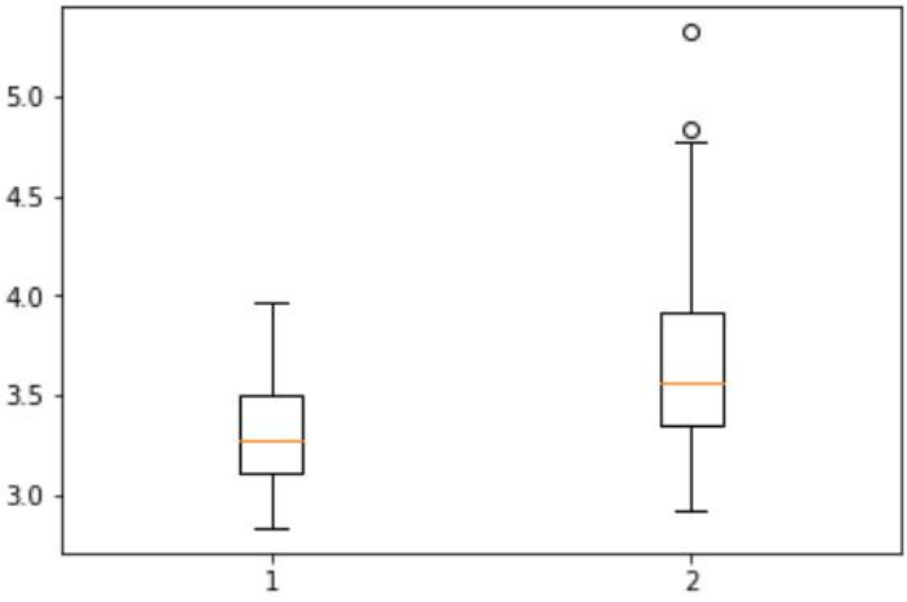

Figure4.2 1: *MSPE* of experimental group 2: *MSPE* of control group

One of the simulation results is listed below. This table shows the OLS Regression Results of the experimental and control group.

Table 4.3: experimental group(control group) OLS Regression Results table

	coef	stderr	t
$\eta_{1,2}$	0.76(0.0744)	1.92(2.29)	0.39(-0.032)
$\eta_{1,3}$	1.73(2.36)	1.92(2.29)	0.9(1.02)
$\eta_{2,3}$	-3.55(-4.03)	1.92(2.29)	-1.84(-1.75)
η_1	3.69(-0.79)	1.57(1.87)	2.35(-0.42)
η_2	1.62(1.95)	1.57(1.87)	1.03(1.04)
η_3	-0.12(-1.36)	1.57(1.87)	-0.08(-0.72)
η_{12}	7.56(-1.53)	1.57(1.87)	4.82(-0.81)
η_{13}	1.41(-1.63)	1.57(1.87)	0.90(-0.87)
η_{23}	-5.3(-2.89)	1.57(1.87)	-3.43(-1.54)

From the simulation study, we can include that the E_1 is more desirable than E_2 in prediction accuracy, by comparing their *MSPE* values. According to the above table, it demonstrates that the proposed design compares very favorably with the random design in estimation efficiency.

5. Conclusion

Order of addition experiments are important in many chemical and biological studies. In this paper, we proposed a new model that incorporates both order effects and amount effects, which is called the order of addition-amount combination model. We also propose an experimental design that can ensure the estimations of the order of addition effects are uncorrelated with the amount combination effects. An illustrative example is provided to explain this model. Furthermore, it demonstrates that the proposed design is more desirable than the random design with respect to prediction accuracy and estimation efficiency.

The primary aim of this article is to incorporates both order effects and amount effects. The future work can take into account the high-order interaction between various factors. We can select a part from both order of addition design matrix and amount combination design matrix by binding two of them in a spliced way.

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