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Construction of Latin Square Design from Confounded 3^3 Factorial Experimental Designs

Akong, Custic Obim

Department of Statistics, Faculty of Physical Science, Cross River State University of Technology, (CRUTECH) Calabar, Cross River State, Nigeria
Author E-mail: custicakong@gmail.com

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ABSTRACT: For practical experimental work, it becomes impossible to perform all experiments of a multi-factor experiment in a complete factor structure due to the complexity of the problem and the fact that the number of possible factor combinations in a multi-factor experiment is a product of the levels of the single factors. This result in waste of time, uses large amount of test materials, uses large number of experimental animals, and involves many people, which tend to increase experimental uncertainty. This work introduces a fundamental method to reduce the experimental work very considerably in relation to the complete factorial experiment, group such experiments into small blocks. A 3^3, factorial experimental design was considered which effects were confounded into 9 (nine blocks) of 27 (twenty-seven) effects at three levels (0, 1, 2) each. 10 (Ten) generalized interaction terms were obtained and three of the terms were selected and used to construct a Latin square design of order 3. Also show how ANOVA can be constructed for each Latin square formed.

Keywords: Confounded 3^3 factorial, experimental designs, Latin Square Design

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INTRODUCTION

Latin square design is a commonly used experimental design in various fields of research, including agriculture, biology, and engineering. It is particularly useful when there are multiple factors to be tested and the researchers want to control for potential confounding variables. Latin square design is a type of experimental design that allows researchers to control for the potential confounding effects of row and column variables. It is called a Latin square because it resembles a square grid, with each treatment combination appearing only once in each row and column. This design ensures that each treatment is tested an equal number of times and that the potential confounding effects of row and column variables are minimized. The Latin square design is particularly useful when there are three or more factors to be tested. By using this design, researchers can control for the potential confounding effects of two factors, while still being able to test the main effects and interactions of all factors. This design is especially valuable when the factors being tested have a natural order or sequence, such as time or dosage levels. To illustrate the use of Latin square design, let's consider an example in agriculture. Suppose a researcher wants to test the effects of three different fertilizers (A, B, and C) on the growth of a specific crop. Additionally, the researcher wants to control for the potential confounding effects of different soil types and watering frequencies. By using a Latin square design, the researcher can assign each fertilizer to a specific row and column combination, ensuring that each fertilizer is tested once in each soil type and watering frequency combination. However, Latin square design has its limitations. It assumes that the potential confounding variables are evenly distributed across the rows and columns, which may not always be the case. Additionally, this design may not be suitable for experiments with a large number of factors, as it becomes increasingly difficult to construct a Latin square with a high number of treatments. In some cases, researchers may encounter situations where they want to test the main effects and interactions of three factors, but they also suspect that there may be confounding effects between two of these factors. In such cases, a confounded 3^3 factorial experimental design can be used. A confounded 3^3 factorial experimental design is an extension of the Latin square design that allows researchers to test the main effects and interactions of three factors, while also accounting for potential confounding effects between two of these factors. This design involves constructing three Latin squares, with each square representing one factor. The treatment combinations are then assigned in a way that ensures that each treatment appears once in each row and column of each Latin square. By using a confounded 3^3 factorial experimental design, researchers can control for potential confounding effects between two factors while still being able to test the main effects and interactions of all three factors. This design is particularly useful when there are strong suspicions of confounding effects between two factors but still a desire to investigate the main effects and interactions of all three factors.

Factorial designs were used in the 19th century by John Bennet Lawes and Joseph Henry Gilbert of the Rothamsted Experimental Station. Ronald Fisher argued in 1926 that "complex" designs (such as factorial designs) were more efficient than studying one factor at a time.

Objectives of the study

i. To confounding a 3^3 factorial design using the main effects $A, B, and\ C$ and obtaining generalized interactions.
ii. To construct Latin Square design of order three (3) from the generalized interactions ABC, AB^2C and ABC^2 obtained from the confounded design.
iii. To fit ANOVA for the constructed Latin squares designs.

LITERATURE REVIEW

Factorial Design

Factorial design is used for the study of the effects of two or more factors simultaneously. It has distinct advantages over a series of simple experiments, each designed to test a single factor. A factorial experiment can be regarded as a number of simple experiments superimposed on each other where every observation supplies information on each of the factors studied. One factorial experiment serves the purpose of a number of simple experiments of the same size. But a series of simple experiments will throw no light whatever on the interaction of the different factors, and this will be shown in a factorial experiment. Rodriguez et al. (2005) developed an experimental factorial design to study the influence of seed cell density, agitation rate and culture condition (open or closed system) in order to maximize the expression of recombinant protein transfixed in CHO cell line. A 3^3 experimental design included those independent variables was running and total protein expression and specific protein expression was measure as dependent variables. A low and high level was established for each variable assuming working experience and literature review. There were running 8 experiments keeping similar culture conditions except variables under study.

Result show that 3 independent variables have statistic significance in the expression of target protein but not interaction between them. These results are very useful in order to establish culture condition at large scale production of biopharmaceuticals and for validation purposes.

Ayamah and Darkwah, (2011) used factorial design to model the effect of National Road Safety Strategy (NRSS). In the reduction of road traffic accident in the Northern and Ashanti region of Ghana, two level factorial designs with mixed factors was use for the presentation and analysis of the data. The result revealed that the campaign on the NRSS helped in the reduction of the seriousness of traffic road accident.

Khattak et al. (2012) used factorial design approach to investigate the effect of different factors on the resilient modulus of Bituminous paving mixes. The research study investigates the effect of four factors namely bitumen content, specimen diameter, test temperature and load duration on resilient modulus of bituminous paving mixes. The result from the study suggest that in measuring the resilient modulus, an appropriate temperature and load duration should be selected to quantify the representative resilient modulus for in-situ condition.

Gunter, (2018) in his work used two simple examples to show how and why well known methods of factorial experimentation in which multiple potential sources are simultaneously varied provide a better alternative, can be understood as a straightforward extension of standard practice, and could be embedded into the quality control procedures of routine experimental practice.

Mapfumo (2016) applied two level full factorial designs and response surface methodologies in the optimization of variables associated with the determination of platinum using inductivity coupled Plasma-Atomic Emission Spectrometry (ICP-AES) using four *variables* carrier gas flow rate, pump speed, plasma observation height and plasma power as factors in the optimization process.

Oprime et al. (2017) studied the use of systematic methods to generate experimental designs with good statistical properties and low costs. The research focused on the sequences of experiments and analysis the results of three different approaches used to build (orthogonal or non-orthogonal) two-level factorial designs, wherein sequencing is randomly or systematically performed. The result indicates that in comparison to the random order, systematic sequences may lead to fewer factor level changes and to linear trend effects. Therefore, may attach design cost and quality.

Factors, Effects, and Contrasts

Experiments performed by analysts are usually for the purpose of determining the effects of a factor(s) on the response variable of interest. Response variables include product yield, quality, performance, etc.

The analyst is able to gain an advantage if the experiment is designed in such a way that the effect of changing any one variable can be analyzed independently of the other variables. One way of obtaining this goal is to determine the factors of interest for the response variable being studied, select the values (levels) that the factors will assume, and then conduct the experiment. This type of experimentation is known as a factorial experiment.

The factors are features of the experiment that may be deliberately altered between trials. Thus, the factors are variables that the analyst has some control over.

Examples of factors include temperature, time, pressure, concentration, etc. There may be two different types of factors in an experiment. A qualitative factor is a variable that cannot have its levels arranged by order of magnitude. For instance, different pieces of material produced in different factories would be qualitative since we cannot place them in a specific, meaningful, numerical order. Quantitative factors, on the other hand, can be arranged based on their numerical values. Examples of quantitative factors would be temperature, pressure, concentration.

The values that a factor assumes during an experiment are known as factor levels. This terminology was first used to describe quantitative factors, where its meaning is easier to understand, but it has also been applied to qualitative factors. The particular combination of levels for the factors used in a single trial (run) of the experiment is known as the treatment or treatment combination. The effect for a given factor is the change in the response variable caused by a change in the level of the factor. For a two-level experiment, the main effects are defined as the difference between the average response at the two levels of a factor.

The two-factor interactions are defined as half the difference between the main effects of one factor at the two levels of a second factor Gunst and McDonald, (1989).

Randomization

Randomization is an important feature that is useful in all types of experimental designs. In a completely randomized design all of the combinations for the factor levels in the experiment are randomly assigned to experimental units or to the sequence of test runs. This is done in such a way that each factor level combination has an equal chance of being assigned to any test sequence (Gunst and McDonald, 1989). The importance of randomization is due to the fact that the analyst cannot always be certain that all of the major influences on the response have been considered. Cochran and Cox, (1957) compares randomization to a form of insurance. There may be unanticipated events that may or may not occur and these may or may not be of a serious nature and randomization in all instances, including those in which no major problems are anticipated even without randomizing, is there to protect against unforeseen events that can disrupt the analysis. Examples of these unforeseen events include drifting instrumentation, malfunctioning equipment, operator errors, etc. More specifically, if an analyst wants to compare two different types of computers, with respect to average current flow, and has five of each type available for his use, in what order should the 10 computers be tested? If the testing order used is to examine all five units of one type followed by the five units of the other type, then problems of accountability may arise. Suppose that the line voltage drifts during the testing procedure. The analyst may ascertain that there is a significant difference between the two types of computers, when what actually accounts for the major portion of the difference is the drifting line voltage. By randomizing the order in which the experiment is performed the effect due to the drifting line voltage will tend to average out over the varying experimental conditions.

This relates directly to factorial experiments where the analyst cannot prevent unforeseen events, line voltage drifts, but can spread the problem over all of the factor levels, computer types.

Confounding in experimental designs

Confounding is a distortion (inaccuracy) in the estimated measure of association that occurs when the primary exposure of interest is mixed up with some other factors that is associated with the outcome. In other for confounding to occur, the extraneous factor must be associated with both the primary exposure of interest and the disease outcome of interest.

Raja, (1974) proposed an alternative method for construction of confounded effects of a confounded design. His method was based on the theory of groups which makes the construction of P.B. for, any number of factors in 2^n factorial experiment. Mann and Wood, (2012) discussed the concept of confounding by way of examples and offers a simple guide for learners of evidence-based medicine. Cornelius et al., (2015) in their work assessed risk of confounding by indication and healthy vaccines bias for each study and calculated ratios of odds ratios (rude /adjusted) to quantify the effect of confounder adjustment. Vaccine effectiveness (VE) during and outside influenza seasons were compared to assess residual confounding by healthy vaccine effects. They identified 23 studies reporting 11 outcomes. Of these 19 (83%) showed high risk of bias: fourteen due to confounding by indication, two for healthy vaccine bias, and three studies showed both forms of confounding/bias.

Dafoe et al. (2015) in their work show how to forecast bias and how to use placebo test as diagnostics. They illustrate with several examples, including a study of the effect of democracy on support for force: describing a country as a “democracy” make respondents more likely to think the country is wealthy, European, majority Christian and white, and interdependent and allied with the US. They also evaluated two strategies for reducing the risk of confounding: controlling for other factors in the vignette and embedding a hypothetical natural experiment in the scenario. They discovered that controlling reduces the risk of confounding from controlled characteristic, but not other characteristics; the embedded natural experiment reduces the risk from all characteristics.

Generalized effect in confounded Factorial experimental design

Khan and Jalil, (2015) proposed in their work titled “A general method of constructing layout with single factorial effect” a linear equation method of designing simultaneous confounding of one or more factorial effects in n p-factorial experiment. It is demonstrated that it becomes easier to construct the design of simultaneous confounding of one or more factors in a n p-factorial experiment especially when the number of factors as well as the number of levels becomes larger. It is also discussed that such approach is very much convenient from the viewpoint of computation.

Zhou and Zhang, (2014) proposed the generalized general minimum order confounding criterion for non-regular designs and extend the work of Zhang et al. [Statistica Sinica 18, 1689–1705] for non-regular designs and propose two new concepts, i.e., the generalized aliasing effect-number pattern (G2-AENP) and the generalized general minimum lower order confounding (G2-GMC). It proves that (i) isomorphic designs have the identical G2-AENP and (ii) the generalized minimum aberration (GMA) and minimum moment aberration (MMA) can both be treated as ones that optimize functions over the G2-AENP. That is, the G2-GMC criterion is more sensitive in the identification and classification of designs

Cheng and Makerjee (2002), in their work Generalized General Minimum Lower Order Confounding Criterion for General Orthogonal Designs. extend the AENP and the GMC criterion proposed by Cheng and Zhang, (2014) to the case of non-regular orthogonal designs. A G-AENP and correspondingly a G-GMC criterion are proposed. The confounding frequency vector (CFV) and the generalized word-length pattern (GWLP), as the base of MGA and GMA criteria, are shown to be functions of the G-AENP. Some optimal properties of G-GMC designs are obtained. At the last, we give an efficient algorithm for finding optimal designs and tabulate some G-GMC designs with 16- and 18-run for application and comparison with GMA and MGA designs.

Blocking in confounded factorial design

Ke et al. (2007). In their work titled “Selection of blocked two-level fractional factorial design for agricultural experiment” shows how blocked two-level fractional factorial designs are a very useful tool for efficient data collection in agricultural and other scientific research. In most experiments, in addition to the main effects, some two-factor interactions are also meaningful and need to be estimated. We propose a method for efficiently selecting blocked two-level fractional factorial designs when some of the two-factor interactions are non-negligible. We then present some results for a design with only 8 or 16 runs to illustrate how to use this method.
Wei et al. (2014) in their paper, considered the problem of constructing optimal blocked regular fractional factorial designs. The concept of minimum aberration due to Fries and Hunter is a well-accepted criterion for selecting good unblocked fractional factorial designs. Cheng, Steinberg and Sun showed that a minimum aberration design of resolution three or higher maximizes the number of two-factor interactions which are not aliases of main effects and also tends to distribute these interactions over the alias sets very uniformly. We extend this to construct block designs in which (i) no main effect is aliased with any other main effect not confounded with blocks, (ii) the number of two-factor interactions that are neither aliased with main effects nor confounded with blocks is as large as possible and (iii) these interactions are distributed over the alias sets as uniformly as possible. Such designs perform well under the criterion of maximum estimation capacity, a criterion of model robustness which has a direct statistical meaning. Some general results on the construction of blocked regular fractional factorial designs with maximum estimation capacity are obtained by using a finite projective geometric approach.

Constructing Latin Squares Designs

Dukes et al (2014) proposed two ways of constructing maximal sets of mutually orthogonal Latin squares are presented. The first construction uses maximal partial spreads in PG (3, 4) PG (3, 2) with r lines, where r ∈ {6, 7}, to construct transversal-free translation nets of order 16 and degree r + 3 and hence maximal sets of r + 1 mutually orthogonal Latin squares of order 16. Thus sets of t MAXMOLS (16) are obtained for two previously open cases, namely for t = 7 and t = 8. The second one uses the (non)existence of spreads and ovoid of hyperbolic quadrics Q+ (2m + 1, q), and yields infinite classes of q^{2m-1}– 1 MAXMOLS (qq^{2m}), for n ≥ 2 and q a power of two, and for n = 2 and q a power of three.
Jamiu and Agrawal, (1999) presented a general method

of constructing a complete set of Mutually Orthogonal Latin Squares (MOLS) of the order of any prime, via the use of generating functions denned on the neofields of this order. Apart from using the generating function to get a complete set of Mutually Orthogonal Latin Squares, the studies of the generating functions open up the possibility of getting at the deep structural properties of MOLS. Copious examples were given for detailed illustrations.

Peter and Alan, (2014) their work showed that, under mild conditions, “triple products” of MOLS can result in a gain of one square. In terms of transversal designs, the technique is to use a construction of Rolf Rees twice: once to obtain a coarse resolution of the blocks after one product, and next to reorganize classes and resolve the blocks of the second product. As consequences, we report a few improvements to the MOLS table and obtain a slight strengthening of the famous theorem of MacNeish.

Bedford, (1993) in his work “construction of orthogonal Latin Squares using left neofields” describes a general method of construction for sets of mutually orthogonal Latin squares (MOLS) from left neofields. We give detailed information for all isomorphically distinct left neofields of order less 10 and summarized information for orders up to 14. We show that a number of recent constructions of MOLS implicitly employ the construction which we describe, in particular the recent constructions of three MOLS of order 14 and four of order 20.

METHODOLOGY

This section presents method of constructing Latin square designs from confounded 3^3 factorial experimental design using linear combination approach of treatments combination by combining three different treatments (main effects A, B, and C). The method of calculating the sum of squares, mean squares and the F-statistics for the ANOVA from the different combined Latin Squares design is given here.

Complete P^k factorial experiments

We now consider experiments with k factors each on p levels, where p is everywhere assumed to be a prime number. In addition, complete randomization is generally assumed. In cases where experiments are discussed in which blocking is used, complete randomization is assumed within blocks. The factors are called $A, B, C,$ etc. Factor A is the first factor, B the second factor etc. In addition, (to the highest possible extent) we use the indices $i, j, k,$ etc. for the factors $A, B, C,$ etc., respectively.

The experiment is generally called a P^k factorial experiment, and the number of possible different factor combinations is Precisely $P \times P \times \ldots \times P = P^k$. For an experiment with 3 factors, A, B, and C, the standard mathematical model is:

$$Y_{ijkv} = \mu + A_i + B_j + AB_{ij} + C_k + AC_{ik} + (BC)_{jk} + (ABC)_{ijk} + E_{ijkv}$$

Where $i, j, k = (0,1)\ and\ v = (1, \ldots, r)$

The index $v = (1,2, \ldots, r)$ gives the number of repetitions of each single experiment in the experiment. The other indices assume the values $(0, 1, 2, \ldots, p-1)$. It should be noted that the index always runs from 0 up to and including $P-1$. In the case where $P = 3\ and\ k = 3$ we have the following (Table 1), which shows all the single experiments in the complete 3^3 factorial experiment.

Factorial experiment

The procedures in the indices for all effect, for **A** the indices table are form from L_A values as follows, the first index table is formed at K=0,while i and j varies from 0 to 2.

Where $0 = \alpha$, 1= $\beta\ and\ 2 = \gamma$

The first element α in the first row $j = 0$ of the first column $i = 0$ is from L_A column corresponding to the treatment combinations (000) indicating $i = 0,\ j = 0,\ k = 0$. The second element β in the first row $j = 0$ of the second column $i = 1$ is obtained from L_A column corresponding to the treatment combination (100) indicating $i = 1, j = 0, k = 0$. The third element γ in the first row $j = 0$ of the third column $i = 2$ is obtained from L_A column corresponding to treatment value (200) indicating $i = 2, j = 0\ and\ k = 0$. Similar approaches is being applied in obtaining other elements in the indices table 1.

Linear combination

The Linear combination model gives a systematic modular combination of the factors at their respective levels according to the treatment combination. In this research modulo 3 is used for the linear combination model, where the linear model is given as:

Where, $A = X_1$, $B = X_2 C = X_3$ and the coefficient of A $= 1,\ B = 1,\ C = 1$

For $L_A = X_1 + 0X_2 + 0X_3$ coefficient of $A = 1,\ B = 0,\ C = 0$

For $L_{AB^2C} = X_1 + 2X_2 + X_3$ coefficient of $A = 1,\ B = 2,\ C = 1$

$L_{AB} = X_1 + X_2 + 0X_3$ coefficient of $A = 1,\ B = 1,\ C = 0$

$L_{BC} = 0X_1 + X_2 + X_3$ coefficient of $A = 0,\ B = 1,\ C = 1$

$L_{BC^2} = 0X_1 + X_2 + 2X_3$ coefficient of $A = 0,\ B = 1,\ C = 2$

$L_{ABC^2} = X_1 + X_2 + 2X_3$ coefficient of $A = 1,\ B = 1,\ C = 2$

$L_{AC} = X_1 + 0X_2 + X_3$ coefficient of $A = 1,\ B = 0,\ C = 1$

$L_{AC^2} = X_1 + 0X_2 + 2X_3$ coefficient of $A = 1,\ B = 0,\ C = 2$

$L_{AB^2} = X_1 + 2X_2 + 0X_3$ coefficient of $A = 1,\ B = 2,\ C = 0$

$L_B = 0X_1 + X_2 + 0X_3$ coefficient of $A = 0,\ B = 1,\ C = 0$

Table 1: Single experiment in a complete 3^3

	0	1	2
$B=0$	(1)	a	a^2
$B=1$	b	ab	a^2b
$B=2$	b^2	ab^2	a^2b^2
$k=0$			

0	1	2
c	ac	a^2c
bc	abc	a^2bc
b^2c	ab^2c	a^2b^2c
$k=1$		

0	1	2
c^2	ac^2	a^2c^2
bc^2	abc	a^2bc^2
b^2c^2	ab^2c^2	$a^2b^2c^2$
$k=2$		

	$i=0$	$i=1$	$i=2$
$j=0$	0	1	2
$j=1$	0	1	2
$j=2$	0	1	2
$k=0$			

⇨

	$i=0$	$i=1$	$i=2$
$j=0$	α	β	γ
$j=1$	α	β	γ
$j=2$	α	β	γ
$k=0$			

Table 2: Latin Square Design ANOVA Table

$Source\ of\ variation$	df	SSQ	MS	F
$AB^2C\ (row)$	$p-1$	SSQ_{AB^2C}	$MS(AB^2C)$	F_{AB^2C}
$ABC^2\ (col)$	$p-1$	SSQ_{ABC^2}	$MS(ABC^2)$	F_{ABC^2}
$ABC(treatment)$	$p-1$	SSQ_{ABC}	$MS(ABC)$	F_{ABC}
$Error$	$(p-1)(p-2)$	SSQ_E	MSE	
$Total$	$N-1$	SSQ_T		

Treatment Combinations

Treatment combinations shows different level of combination of the effects of each factor in a given relation. For instance, treatment combination 000, tells us that A is at level 0 (low), B is at level 0 (low) and C is at level 0 (low). Treatment combination 100 indicates that A is at level 1 (intermediate), B and C are at level 0 (low). Treatment combination 012 indicates that A is at level 0 (low), B is at level 1 (intermediate) while C is at level 2 (high). Other treatment combinations are interpreted in similar manner.

Calculation of Sum of Squares for the Latin square formed

In this section we describe the procedures used to obtain the sum of squares, mean squares, degrees of freedom and the F-values for the ANOVA. The design of analysis of variance (Table 2) for the Latin Square design to be formed is also given in this section.

Sum of Squares Treatment

$$T_{ABC_0} = 000 + 210 + 120 + 201 + 021 + 111 + 102 + 012 + 222$$
$$T_{ABC_1} = 100 + 010 + 220 + 001 + 211 + 121 + 202 + 112 + 022$$
$$T_{ABC_2} = 200 + 110 + 020 + 101 + 011 + 221 + 002 + 212 + 122$$
$$SSQ_{ABC} = \frac{T_{ABC_0}{}^2 + T_{ABC_1}{}^2 + T_{ABC_2}{}^2}{p} - \frac{(T_{ABC_0} + T_{ABC_1} + T_{ABC_2})^2}{p^2}$$

Sum of Squares Row

$$T_{AB^2C_0} = 000 + 110 + 220 + 201 + 011 + 121 + 102 + 212 + 022$$
$$T_{AB^2C_1} = 100 + 210 + 020 + 001 + 111 + 221 + 202 + 012 + 122$$
$$T_{AB^2C_2} = 200 + 010 + 120 + 101 + 211 + 021 + 002 + 112 + 222$$
$$SSQ_{AB^2C} = \frac{T_{AB^2C_0}{}^2 + T_{AB^2C_1}{}^2 + T_{AB^2C_2}{}^2}{p} - \frac{(T_{AB^2C_0} + T_{AB^2C_1} + T_{AB^2C_2})^2}{p^2}$$

Sum of Squares Column

$$T_{ABC_0^2} = 000 + 110 + 220 + 201 + 011 + 121 + 102 + 212 + 022$$
$$T_{ABC_1^2} = 100 + 010 + 220 + 201 + 111 + 021 + 002 + 212 + 122$$
$$T_{ABC_2^2} = 200 + 110 + 020 + 001 + 211 + 121 + 102 + 012 + 222$$
$$SSQ_{ABC^2} = \frac{{T_{ABC_0^2}}^2 + {T_{ABC_1^2}}^2 + {T_{ABC_2^2}}^2}{p} - \frac{(T_{ABC_0^2} + T_{ABC_1^2} + T_{ABC_2^2})^2}{p^2}$$

Sum of Squares Total

$$ST = {T_{ABC_0}}^2 + {T_{ABC_1}}^2 + {T_{ABC_2}}^2 + {T_{AB^2C_0}}^2 + {T_{AB^2C_1}}^2 + {T_{AB^2C_2}}^2 + {T_{ABC_0^2}}^2 + {T_{ABC_1^2}}^2 + {T_{ABC_2^2}}^2$$
$$CF = \frac{(T_{AB^2C_0} + T_{AB^2C_1} + T_{AB^2C_2} + T_{ABC_0^2} + T_{ABC_1^2} + T_{ABC_2^2} + T_{ABC_0} + T_{ABC_1} + T_{ABC_2})^2}{p^2}$$
$$SSQ_T = ST - CF$$

Sum of Squares Error

$$SSQ_E = SSQ_T - (SSQ_{ABC^2} + SSQ_{AB^2C} + SSQ_{ABC})$$
$$MS(ABC^2) = \frac{SSQ_{ABC^2}}{df} = \frac{SSQ_{ABC^2}}{n-1} = \frac{SSQ_{ABC^2}}{3-1} = \frac{SSQ_{ABC^2}}{2}$$
$$MS(AB^2C) = \frac{SSQ_{AB^2C}}{df} = \frac{SSQ_{AB^2C}}{n-1} = \frac{SSQ_{AB^2C}}{3-1} = \frac{SSQ_{AB^2C}}{2}$$
$$MS(ABC) = \frac{SSQ_{ABC}}{df} = \frac{SSQ_{ABC}}{n-1} = \frac{SSQ_{ABC}}{3-1} = \frac{SSQ_{ABC}}{2}$$
$$MSE = \frac{SSE}{df} = \frac{SSE}{(n-1)(n-2)} = \frac{SSE}{(3-1)(3-2)} = \frac{SSE}{2}$$
$$F_{AB^2C} = \frac{MS(AB^2C)}{MSE}$$
$$F_{ABC^2} = \frac{MS(ABC^2)}{MSE}$$

PARAMETER ESTIMATION

$$\mu = \frac{\sum_{i=0}^{2}(ABC_i) + \sum_{i=0}^{2}(AB^2C_i) + \sum_{i=0}^{2}(ABC_i^2)}{p^2}$$
$$\hat{\alpha}_i = \frac{\sum_{i=0}^{2}(AB^2C_i)}{p} - \mu$$
$$\hat{\beta}_j = \frac{\sum_{i=0}^{2}(ABC_i^2)}{p} - \mu$$
$$\hat{\gamma}_k = \frac{\sum_{i=0}^{2}(ABC_i)}{p} - \mu$$

RESULTS AND DISCUSSION

Data Presentation

The data used in this work was obtained from Armitage and Berry (2012, p. 345). The data show the effect of three diets on lives cholesterol in ratio (A= control, B=body weight + vegetable fat, C=control + Animal fat). Body weight of rat was classification as (H=high, M=Medium, and L=Low) and the three different litters from which they came were used to form a balanced set of Latin Square. The litters were in squares (i.e. different litter were used in each squares), where rows (weight classification) and column represents litter.

Linear Combination Approach of treatment combinations in mod 3

In this section we present some interactions$(ABC, AB^2C, and\ ABC^2)$obtained by combining the three main effects A, B, and C using the linear combination approach as earlier presented in 3.2. Table 3 show these defining relations formed by linear combination approach of treatment combinations in modular 3.

Interaction effects of $ABC, AB^2C, and\ ABC^2$showing 3 blocks of 9runs and 27 effect

In this section we present (Tables 4, 5 and 6) showing the interaction effects in three blocks of 9 runs at different levels of the main effects A, B, and C. Each table shows a total of 27 treatments grouped in blocks 1, 2, and 3 according to their levels.

Linear Combinations for generalized interactions

In this section we present a (Table 7) showing ten (10) generalized interactions obtained by combining the main effects A, B, and C using linear combination approach in modulo 3 and for treatment level 0, 1, and 2as already described in 4.2. The total number of effects obtained is 27.

Forming indices for each defining relation

Below are the indices for individual defining relations: for the three main effects and the ten (10) interaction effects at treatment levels 0, 1, and 2. Where $i,\ j,\ and\ k$represent the column, row and treatments level respectively (Table 8).

Result of analysis of Variance of Latin Square Design

In this section we present the result for Latin Square design analysis of the data in (Table 1) and treatment respectively in the analysis of variance (Table 9).

Parameters value

$Alpha(\alpha_j) = -0.19,\quad Beta(\beta_j) = 0.31,\quad Gamma(\gamma_k) = -0.12$

Table 3: linear treatment combination for interaction effects $ABC, AB^2C, and\ ABC^2$.

Treatment	$ABC = x_1 + x_2 + x_3$	L_1	$AB^2C = x_1 + 2x_2 + x_3$	L_2	$ABC^2 = x_1 + x_2 + 2x_3$	L_3
0 0 0	$0+0+0$	0	$0+2(0)+0$	0	$0+0+2(0)$	0
1 0 0	$1+0+0$	1	$1+2(0)+0$	1	$1+0+2(0)$	1
2 0 0	$2+0+0$	2	$2+2(0)+0$	2	$2+0+2(0)$	2
0 1 0	$0+1+0$	1	$0+2(1)+0$	2	$0+1+2(0)$	1
1 1 0	$1+1+0$	2	$1+2(1)+0$	0	$1+1+2(0)$	2
2 1 0	$2+1+0$	0	$2+2(1)+0$	1	$2+1+2(0)$	0
0 2 0	$0+2+0$	2	$0+2(2)+0$	1	$0+2+2(0)$	2
1 2 0	$1+2+0$	0	$1+2(2)+0$	2	$1+2+2(0)$	0
2 2 0	$2+2+0$	1	$2+2(2)+0$	0	$2+2+2(0)$	1
0 0 1	$0+0+1$	1	$0+2(0)+1$	1	$0+0+2(1)$	2
1 0 1	$1+0+1$	2	$1+2(0)+1$	2	$1+0+2(1)$	0
2 0 1	$2+0+1$	0	$2+2(0)+1$	0	$2+0+2(1)$	1
0 1 1	$0+1+1$	2	$0+2(1)+1$	0	$0+1+2(1)$	0
1 1 1	$1+1+1$	0	$1+2(1)+1$	1	$1+1+2(1)$	1
2 1 1	$2+1+1$	1	$2+2(1)+1$	2	$2+1+2(1)$	2
0 2 1	$0+2+1$	0	$0+2(2)+1$	2	$0+2+2(1)$	1
1 2 1	$1+2+1$	1	$1+2(2)+1$	0	$1+2+2(1)$	2
2 2 1	$2+2+1$	2	$2+2(2)+1$	1	$2+2+2(1)$	0
0 0 2	$0+0+2$	2	$0+2(0)+2$	2	$0+0+2(2)$	1
1 0 2	$1+0+2$	0	$1+2(0)+2$	0	$1+0+2(2)$	2
2 0 2	$2+0+2$	1	$2+2(0)+2$	1	$2+0+2(2)$	0
0 1 2	$0+1+2$	0	$0+2(1)+2$	1	$0+1+2(2)$	2
1 1 2	$1+1+2$	1	$1+2(1)+2$	2	$1+1+2(2)$	0
2 1 2	$2+1+2$	2	$2+2(1)+2$	0	$2+1+2(2)$	1
0 2 2	$0+2+2$	1	$0+2(2)+2$	0	$0+2+2(2)$	0
1 2 2	$1+2+2$	2	$1+2(2)+2$	1	$1+2+2(2)$	1
2 2 2	$2+2+2$	0	$2+2(2)+2$	2	$2+2+2(2)$	2

The Model

$$Y_{ijk} = 16.18222 - 0.1922(AB^2C)_i + 0.31(ABC^2)_j - 0.12(ABC)_k + \varepsilon_{ijk}$$

DISCUSSION OF FINDINGS

We presented in (Table 3), the treatment effects formed using linear combination approach by combining results obtained form 3 linear equations L_1, L_2, and L_3 for the interactions $ABC, AB^2C, and\ ABC^2$ respectively. The first treatment 000(in row 1, column 1 of table 3)is obtained by combining $L_1 = 0$, $L_2 = 0$, and $L_3 = 0$ resulting from the linear equations $ABC, AB^2C, and\ ABC^2$. The second treatment 100(in row 2, column 1 of table 3) is obtained by combining $L_1 = 1$, $L_2 = 0$, and $L_3 = 0$, which is also combination of the linear equations for $ABC, AB^2C, and\ ABC^2$. Subsequent treatments are obtained using similar method. From the table it is observed that each interaction effect ($ABC, AB^2C, and\ ABC^2$) forms 27 treatment effects that can be grouped into 3 blocks of 9 runs. This corresponds with the work of Henrik, S. (2002) on design and analysis of experiments with K factors having p levels.

Table 4: Interaction effects $ABC, AB^2C, and\ ABC^2$ at different levels of A

Block 1	Block 2	Block 3
A at level 0	A at level 1	A at level 2
000	100	200
010	110	210
020	120	220
001	101	201
011	111	211
021	121	221
002	102	202
012	112	212
022	122	222

Table 5: Interaction effects $ABC, AB^2C, and\ ABC^2$ at different levels of B

Block 1	Block 2	Block 3
B at level 0	B at level 1	B at level 2
000	010	020
100	110	120
200	210	220
001	011	021
001	111	121
201	211	221
002	012	022
102	112	122
202	212	222

Tables 5 to 9 present interaction effects ($ABC, AB^2C, and\ ABC^2$) each having 3 blocks of 9 runs and 27 effects at level 0, 1, and 2. Table 4 shows the effects $ABC, AB^2C, and\ ABC^2$ for different levels of A (at 1, 0, and 2) in 3 blocks (block1, block 2, and block 3) with each block containing 9 runs to form 27 treatments effects. When A is at level 0 (in block 1), all treatments start with 0. for A at level 1 (in block 2), all treatments start with 1, and for A at level 2 (in block 3), all treatments start with 2. Table 5 shows the effects $ABC, AB^2C,$ and $ABC^2$for different levels of B (at 0, 1, and 2) in 3 blocks (block1, block 2, and block 3) each block containing 9 runs to form 27 treatments. When B is at level 0 (in block 1), the second digits of all the treatments is 0. When B is at level 1 (in block 2), the second digits of all the treatment is 1, and when B is at level 2 (in block 3), the second digits of all treatments is 2.

Table 6 shows the effects $ABC, AB^2C,$ and ABC^2 for different levels of C (at 0, 1, and 2) in 3 blocks (block1, block 2, and block 3) each block containing 9 runs to form 27 treatments. For C, at level 0 (in block 1), all last digit of the treatments is 0. When C is at level 1 (in block 2), the last digits of all 9 runs is 1, and for C,at level 2 (in block 3), all last digits of the treatments is 2. The formation of the effects shown in (Tables 4, 5, and 6) corresponds with the work of Oehlert, G. W. (2010) on ‘A first Course in design and analysis of experiments’.

Table 7 is an extension of table 3 to include the main effects A, B, and C and all general interactions formed by combining the main effects A, B, and C.

Table 6: Interaction effects $ABC, AB^2C, and\ ABC^2$ at different levels of C

Block 1	Block 2	Block 3
C at level 0	C at level 1	C at level 2
000	001	002
100	101	102
200	201	202
010	011	012
110	111	112
210	211	212
020	021	022
120	221	122
220	001	222

Table 7: Linear combination for generalized interactions (at levels 0, 1, 2 and 27 effect).

Treatment	L_A	L_B	L_C	L_{AB}	L_{AB^2}	L_{AC}	L_{AC^2}	L_{BC}	L_{BC^2}	L_{ABC}	L_{ABC^2}	L_{AB^2C}	$L_{AB^2C^2}$
0 0 0	0	0	0	0	0	0	0	0	0	0	0	0	0
1 0 0	1	0	0	1	1	1	1	0	0	1	1	1	1
2 0 0	2	0	0	2	2	2	2	0	0	2	2	2	2
0 1 0	0	1	0	1	2	0	0	1	1	1	1	2	2
1 1 0	1	1	0	2	0	1	1	1	1	2	2	0	0
2 1 0	2	1	0	0	1	2	2	1	1	0	0	1	1
0 2 0	0	2	0	2	1	0	0	2	2	2	2	1	1
1 2 0	1	2	0	0	2	1	1	2	2	0	0	2	2
2 2 0	2	2	0	1	0	2	2	2	2	1	1	0	0
0 0 1	0	0	1	0	0	1	2	1	2	1	2	1	2
1 0 1	1	0	1	1	1	2	0	1	2	2	0	2	0
2 0 1	2	0	1	2	2	0	1	1	2	0	1	0	1
0 1 1	0	1	1	1	2	1	2	2	0	2	0	0	1
1 1 1	1	1	1	2	0	2	0	2	0	0	1	1	2
2 1 1	2	1	1	0	1	0	1	2	0	1	2	2	0
0 2 1	0	2	1	2	1	1	2	0	1	0	1	2	0
1 2 1	1	2	1	0	2	2	0	0	1	1	2	0	1
2 2 1	2	2	1	1	0	0	1	0	1	2	0	1	2
0 0 2	0	0	2	0	0	2	1	2	1	2	1	2	1
1 0 2	1	0	2	1	1	0	2	2	1	0	2	0	2
2 0 2	2	0	2	2	2	1	0	2	1	1	0	1	0
0 1 2	0	1	2	1	2	2	1	0	2	0	2	1	0
1 1 2	1	1	2	2	0	0	2	0	2	1	0	2	1
2 1 2	2	1	2	0	1	1	0	0	2	2	1	0	2

Table 7 continued

0 2 2	0	2	2	2	1	2	1	1	0	1	0	0	2
1 2 2	1	2	2	0	2	0	2	1	0	2	1	1	0
2 2 2	2	2	2	1	0	1	0	1	0	0	2	2	1

Index for A_i	$k=0$			$k=1$			$k=2$		
	$i=0$	$i=1$	$i=2$	$i=0$	$i=1$	$i=2$	$i=0$	$i=1$	$i=2$
$j=0$	0	1	2	0	1	2	0	1	2
$j=1$	0	1	2	0	1	2	0	1	2
$j=2$	0	1	2	0	1	2	0	1	2
Index for B_j									
$j=0$	0	0	0	0	0	0	0	0	0
$j=1$	1	1	1	1	1	1	1	1	1
$j=2$	2	2	2	2	2	2	2	2	2
Index for C_k									
$j=0$	0	0	0	1	1	1	2	2	2
$j=1$	0	0	0	1	1	1	2	2	2
$j=2$	0	0	0	1	1	1	2	2	2
Index for AB_{i+j}									
$j=0$	0	1	2	0	1	2	0	1	2
$j=1$	1	2	0	1	2	0	1	2	0
$j=2$	2	0	1	2	0	1	2	0	1
Index for AB^2_{i+2j}									
$j=0$	0	1	2	0	1	2	0	1	2
$j=1$	2	0	1	2	0	1	2	0	1
$j=2$	1	2	0	1	2	0	1	2	0
Index for AC_{i+k}									
$j=0$	0	1	2	1	2	0	2	0	1
$j=1$	0	1	2	1	2	0	2	0	1
$j=2$	0	1	2	1	2	0	2	0	1
Index for AC^2_{i+k}									
$j=0$	0	1	2	2	0	1	1	2	0
$j=1$	0	1	2	2	0	1	1	2	0
$j=2$	0	1	2	2	0	1	1	2	0
Index for BC_{j+k}									
$j=0$	0	0	0	1	1	1	2	2	2
$j=1$	1	1	1	2	2	2	0	0	0
$j=2$	2	2	2	2	2	2	1	1	1

Table 8 is the index table formed from (Table 7) by grouping 9 treatments into 3 by 3 tables at levels 0, 1, and 2. In table 7, the first digits are A levels denoted by i used to show column effects. The second digits are B

Table 8 continued

Index for BC^2_{j+k}									
$j=0$	0	0	0	2	2	2	1	1	1
$j=1$	1	1	1	0	0	0	2	2	2
$j=2$	2	2	2	1	1	1	0	0	0
Index for ABC									
$j=0$	0	1	2	1	2	0	2	0	1
$j=1$	1	2	0	2	0	1	0	1	2
$j=2$	2	0	1	0	1	2	1	2	0
Index for AB^2C_{i+2j+k}									
$j=0$	0	1	2	1	2	0	2	0	1
$j=1$	2	0	1	0	1	2	1	2	0
$j=2$	1	2	0	2	0	1	0	1	2

Table 8 continued

Index for ABC^2_{i+j+2k}	$k=0$			$k=1$			$k=2$		
	$i=0$	$i=1$	$i=2$	$i=0$	$i=1$	$i=2$	$i=0$	$i=1$	$i=2$
$j=0$	0	1	2	2	0	1	0	1	2
$j=1$	1	2	0	0	1	2	2	0	1
$j=2$	2	0	1	1	2	0	1	2	0
Index for $AB^2C^2_{i+2j+2k}$									
$j=0$	0	1	2	2	0	1	1	2	0
$j=1$	2	0	1	1	2	0	0	1	2
$j=2$	1	2	0	0	1	2	2	0	1

Table 9: Latin Square Design ANOVA

$Source\ of\ Variation$	$d.f$	SSQ	MSE	F
$AB^2C(ROW)$	2	0.0705	0.9	0.02882
$ABC^2(COL)$	2	0.2689	0.44	0.1100
$ABC(Latin\ letter)$	2	0.6609	0.33	0.2704
$Error$	2	2.4444	1.21	
$Total$	8	3.4447		

levels denoted by j used to show row effects, and the third digits are C levels denoted by k used to show third factor (treatment effect). From table 8, the index for A_i is formed by taking $i, j, and\ k$ at their different levels of 0, 1, and 2. When $k=0$, $i=0$, and $j=0$, we have 0. For $k=0$, $i=1$, $j=0$, we have 1 and at $k=0$, $i=2$, and $j=0$, we have 2.

In the index for ABC, when $k=0$, $i=0$, $j=0$ we have 0. When $k=0$, $i=1$, $j=0$, we have 1 and for $k=0$, $i=2$, $j=0$, we have 2. Other values are obtained in similar manner. Clearly it is obvious that the indices for $ABC$are characteristically Latin Squares. Similarly, the indices for AB^2C, and ABC^2 form Latin Squares. This corresponds with the work of Henrik, S. (2002) on design and analysis of experiments with K factors having p levels.

Consequently, the Latin square formed in this work was obtained from combining three interaction effectsABC, AB^2C, and ABC^2 at their different levels of 0, 1, and 2. Where AB^2C is considered the column effect, ABC^2 is the row effect while ABC is the treatment effect. The result for the analysis of variance, parameter estimation and model for the Latin Squares formed was computed using the R-software.

Summary

The main objective of this research was to construct a Latin square design using defining splits from a complete confounded 3^3 factorial design. The defining split ABC, AB^2C and ABC^2 where confounded into 9 blocks of 27 effects as shown in (Table 2). The factorial experimental design with three main effects A, B, C were confounded to obtain 10 different defining splits with 27 effects. Each effect can be grouped into 9 blocks at levels 0, 1, and 2 respectively, which would be used subsequently to represent low medium and high in our proposed Latin squares design. A linear combination approach was used to show the combination of effects at different treatment levels, and the exponents of effects are defined to $modulo\ 3$. Consequently, ten generalized interactions are obtained and presented in (Table 3). From these ten generalized interactions five of them $(AB^2C, ABC, AB^2, ABC^2, AB)$ can be used to construct Latin squares design of order 3, while the other five $(AC, BC, AC^2, BC^2, AB^2C^2)$ cannot be used to construct Latin Square design.
In this research three of the generalized effects (AB^2C, ABC, ABC^2) were chosen to fit Latin squares design of order 3, with AB^2C representing the row, ABC^2 representing the column and ABC representing the treatment (Latin letters) as shown in the Analysis of variance table. The regression model is obtained and given in equation 4.1.

Conclusion

From the result of the analysis, the following conclusion can be drawn;

- A 3^3 factorial design with main effect A, B and C can be confounded into 9 blocks of 27 runs.
- 10 generalized interactions can be obtained from a defining relation ABC, AB^2C & ABC^2.
- From the ten generalized interaction four (4) ABC, AB^2C, ABC^2 and AB^2C^2 can be used to construct Latin square of order 3 while 6 cannot.

Recommendation

This work recommends the use of Latin squares design constructed from confounding a 3^3 factorial experimental design in analysis of experiments by field Statistician.

- Researchers interested in further studies in this area may wish to consider Construction of Graeco-Latin square design from confounded 3^3 factorial design.
- Also higher order design (i.e. order 4, 5, etc.) may be considered as well.

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