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APPENDIX I

FACTORIAL DESIGN (60)

The factorial design is frequently employed by workers who have no interest in statistical analysis, because it provides a picture of a complete field of investigation rather than a detailed examination of a few selected paths. By combining the factorial method of design with a statistical method of analysis of the results it is possible to obtain information on the effects and interactions of the factors involved, together with unbiased significance tests for these factors. The conclusions obtained are likely to be of wider application because the tests carried out cover a greater range of experimental conditions.

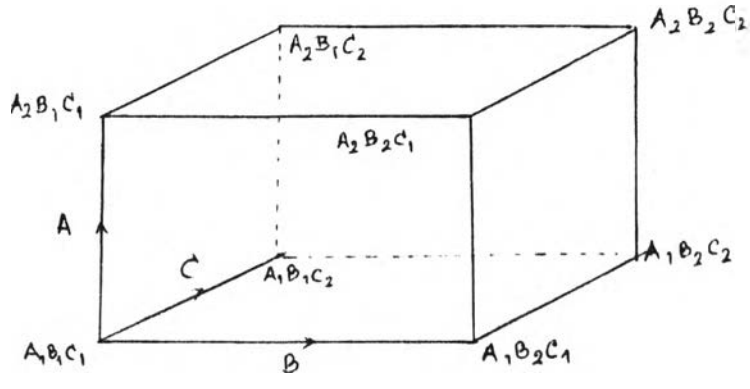
As an illustration of the application of the two methods consider an investigation of the efficiency of an extraction column. Suppose that 2 types of packing, helices and Raschig rings, were under consideration, and that 3 levels of the concentration of salting agent were to be examined. By the classical method we should carry out 4 tests, 2 to differentiate between the types of packing at a fixed concentration, and an additional 2 to examine the 2 remaining levels of salting agent for 1 type of packing. A factorial scheme would involve 6 experimental units corresponding to the 6 possible combinations of treatments. If we suppose that each type of design were replicated and that the total number of observations by the 2 methods were equal (e.g., by 3 replicates of the classical method and 2 of the factorial method), then the advantages of the factorial

design may be summarized as

(1) Greater efficiency in estimating the main effects.

(2) Greater scope, since the two-factor interaction effects may be estimated.

In order to generalize these conclusions we consider the case of an experiment involving 3 factors each at 2 levels. Denoting the levels by A_1A_2, B_1B_2, and C_1C_2, we have in all 8 experimental units. If 16 tests



are to be carried out, we could replicate the design ($A_1B_1C_1$, $A_2B_1C_1$, $A_1B_2C_1$, $A_1B_1C_2$) 4 times, which would enable us to calculate the overall mean effect, the 3 differences between the means of given treatment combinations, and leave (16 - 3 - 1)= 12 degrees of freedom available for an estimate of the residual variance. The estimates of the differences between the means would in each case relate only to the first level of the remaining factors, and the means themselves would be based on 4 observations so that the variance of the estimate of a given effect would be (estimate of residual variance)/2.

Alternatively, the full design of 8 treatment combinations could be replicated twice, the resulting observations being illustrated

schematically in Figure 1 where each vertex of the cube represents the two observations made under these conditions. The 8 observations of the upper face of the cube differ from the 8 of the lower face only in having factor A at level 2 in place of level 1. The difference between these means then provids an estimate of the effect due to increasing the level of A from 1 to 2, and all the observations are employed in this comparison. Similarly a comparison of the mean of the 8 observations constituting the right face of the cube with the corresponding mean for the left face gives the effect of change in the level of B; the effect of a change in the level of C may be determined from the front and rear face of the cube. If we suppose that there are no real interaction effects, then the analysis of variance for this experimant takes the form of the accompanying table. The estimate of the residual variance is again based on 12 degrees of freedom, but each treatment effect is computed from means based on 8 observations, so that the estimated variance of any treatment effect is given by (estimated residual variance)/4.

Source of Estimate	D.F.	Average Value of Mean Square
Between treatments	3	$\sigma^2 + 8\ ^2$
Residual	12	σ^2
Total	15	

In the event that real interactions do exist they can be estimated only from the factorial design. The existence of an interaction A x B implies that the magnitude of the effect of a change of level of A (e.g., from A_1 to A_2) depends upon the level of B at which this change is achieved. If we sum the terms in corresponding positions on the front and rear faces of the cube, we obtain

$$(A_2B_1C_1 + A_2B_2C_1) \qquad (A_2B_2C_1 + A_2B_2C_2)$$
$$(A_1B_1C_1 + A_1B_1C_2) \qquad (A_1B_2C_1 + A_1B_2C_2)$$

The mean value for each vertex of the resultant square is then based on 4 observations. The difference in A effects with B at the first level is given by

$$(A_2B_1C_1 + A_2B_1C_2 - A_1B_1C_1 - A_1B_1C_2)$$

and with B at the second level by

$$(A_2B_2C_1 + A_2B_2C_2 - A_1B_2C_1 - A_1B_2C_2)$$

and the interaction A x B which is the difference of these two is given by

$$(A_2B_2C_1 + A_2B_2C_2 + A_1B_1C_1 + A_1B_1C_2 - A_1B_2C_1 - A_1B_2C_2 - A_2B_1C_1 - A_2B_1C_2)$$

If we interchange the subscripts on A and B in each term of the above expression, the value of the expression is unchanged, indicating that the interactions A x B and B x A are identical and that we could have reversed the roles of A and B in determining the interaction. Similar geometrical derivations can be given for the remaining two-factor interactions, and for the three-factor interaction ABC, but these will be deferred until an alternative nomenclature

has been developed. If the interaction effects are real, the degrees of freedom available for the estimation of the residual variance are reduced by the number necessary to estimate these effects. The scope of the analysis is then increased, but the sensitivity is reduced.

In the analysis of the factorial design it is important to consider the type of population involved when significance tests are being made. Factorial experiments frequently involve scale factors and are carried out to investigate conditions at a number of fixed levels of these factors. Since the inference to be drawn in this case does not relate to a population of possible levels from whichthose selected represent a random sample, significant interactions should not be used for testing effects of lower order without careful consideration of the individual problem and of the average value of the mean squares involved.

A factorial design involving k factors each at 2 levels will include a total of 2^k treatment combinations. In such an experiment each main effect, and every possible interaction between factors, will have 1 degree of freedom in the analysis of variance, a total of $2^k - 1$ degrees of freedom in all. The analysis of the treatment effects in a factorial experiment can be made by breaking down the treatment sum of squares in to components each with a sigle degree of freedom, and proceeding with an analysis of variance. In the case of the 2^k design it is possible to substitute a more direct method which gives a better understanding of the results of the

experiment. We shall illustrate this method for the case of a 2^3 design, and for this purpose we adopt a notation which has been generally used in this type of experiment.

Let us designate by a, b, and c the presence of the factor A, B, and C at the second level, assigning the term arbitrarily in cases where 2 distinct categories are involved. Hence the treatment a would consist of factor A at the second level and factor B and C at the first level, and the treatment ab would consist of A and B at the second level and C at the first level. If we denote the combination in which all factors are at the first level by (1), then the possible treatment combinations is a 2^3 factorial design are given by (1), a, b, c, ab, ac, bc, and abc. We also designate the result or total for a given experimental unit by these symbols. The letters A, B, C, AB, AC, BC, ABC are used to denote the totals of the effects due to these factors, and also the experimental estimates of these totals. Thus the estimate of the total effect of A would be the difference of the sum of observations from experimental units involving treatment a and the sum of observations from units not involving this treatment :

$$A = abc + ab + ac + a - bc - b - c - (1)$$

and similarly

$$B = abc + ab + bc + b - ac - a - c - (1)$$

$$C = abc + ac + bc + c - ab - a - b - (1)$$

Although these totals are generally described as, e.g., "The effect of A ..., " they do not represent the mean effect on the

observations of moving from the lower to the upper level of A, but 4 times this effect in a 2^3 design. In the 2^k design there are 2^{k-1} experimental units at the upper level of A and a similar number at the lower level. The difference between the sums of these two groups therefore represents 2^{k-1} times the meaneffect of a change of level.

With the above notation the interaction effect AB is given by the difference between the effect of A at the upper level of B and the effect of A at the lower level of B, that is:

$$AB = (abc - bc + ab - b) - (ac - c + a - (1))$$
$$= abc + ab + c + (1) - ac - bc - a - b$$

and similarly

$$AC = abc + ac + b + (1) - ab - bc - a - c$$
$$BC = abc + bc + a + (1) - ab - ac - b - c$$

As in the previous treatment the same results are obtained if we interchange the roles of the factors involved in the interactions. To obtain the single three-factor interaction we first consider the estimate of the effect due to A for each of the 4 combinations of B and C. These are (abc - bc), (ab - b), (ac - c), and (a - (1)). The AB interaction estimates computed separately for the two levels of C are (abc - bc) - (ac - c) and (ab - b) - (a -(1)) , and the difference between these expressions represents the effect of the level of C upon the AB interaction, i.e., the ABC interaction. Thus we have

$$ABC = (abc - bc) - (ac - c) - (ab - b) + (a - (1))$$
$$= abc - ab - ac - bc + a + b + c - (1)$$

Since the interchange of the symbols a, b, and c does not affect the estimate ABC, we should obtain the same result by interchanging the roles of A, B, and C in the derivation of this effect. This is generally true for interaction of any order. As with the main effects, the estimates of the interaction effects obtained by this method represent a comparison between observation totals, and in order to estimate the mean effect we devide these totals by 4 in the case of a 2^3 design, or by 2^{k-1} in the case of a 2^k design.

Table 1 gives the signs which must be employed in combining the observations from the experimental units to obtain estimates of the main effects and interactions.

TABLE 1

Treatments / Effects	(1)	a	b	ab	c	ac	bc	abc
1	+	+	+	+	+	+	+	+
A	-	+	-	+	-	+	-	+
B	-	-	+	+	-	-	+	+
AB	+	-	-	+	+	-	-	+
C	-	-	-	-	+	+	+	+
AC	+	-	+	-	-	+	-	+
BC	+	+	-	-	-	-	+	+
ABC	-	+	+	-	+	-	-	+

The numerical coefficient is unity in each instance so that the comparisons are orthogonal, and therefore independent. The contribution of each effect to the total sum of squares may be obtained by squaring the effect estimates and dividing by the sum of squares of the coefficients involved in the comparison. In a 2^3 design this divisor is 8, since 8 experimental units are involved and each is employed in every comparison. More generally, for a 2^k design the divisor is 2^k.

If a design of this type is carried out in r replicates, the difference between replicates within experimental units provides an estimate of the inherent variability, and the effect estimates may be obtained from Table 1 by employing the sums of the observations for given experimental units as thefactors in the comparison. In this case the overall mean will be estimated as $1/2^k r$, and the remaining effects and interactions will be estimated by dividing the total effect estimates by $2^{k-1}r$. The divisor for the components of the sum of squares will be equal to the total number of observations, i.e, $2^k r$.

Example. The data of Table 2 represent results from a factorial experiment on a spinning band laboratory fractionating column. The factors involved in the design are:

A. Clearance between band and static tube (two levels).

B. Boil-up rate (two levels).

C. Rate of rotation of band (two levels).

The observations represent the number of equivalent theoretical plates as determined by computation from the refractive index of the still and condenser lipids and the known characteristics of

the binary mixture examined.

TABLE 2

A. Band clearance	0.05 inch				0.1 inch			
B. Boil-up rate	1		2		1		2	
C. Band speed (rpm)	750	1500	750	1500	750	1500	750	1500
Number of equilibrium theoretical plates	11.8	20.9	8.5	16.2	9.9	18.3	8.1	16.0
Treatment combination	(1)	c	b	bc	a	ac	ab	abc

The main effects and interactions could be computed directly as, e.g.,

$$A = abc + ab + ac + a - bc - b - c - (1)$$

$$= 16.0 + 8.1 + 18.3 + 9.9 - 16.2 - 8.5 - 20.9 - 11.8$$

$$= 5.1$$

but a systematic tabular method due to Yates 52 is more convenient. The original data are listed in column (2) of Table 3 in the order indicated in column (1). The first 4 entries in column (3) are the sums (i) + (ii) , (iii) + (iv) , (v) + (vi) , and (vii) + (viii) , and second 4 are the differences (ii) - (i) (iv) - (iii) , (vi) - (v) , and (viii) - (vii) , so that, for example, the term 16.6 represents (ab + b). Operating on column (3)

Table 3

(1) Treatment Combination	(2) Observation		(3)	(4)	(5) Effect		$(\text{Effect})^2/8$
(1)	11.8	(i)	21.7	38.3	+109.7	I	1,504.261
a	9.9	(ii)	16.6	71.4	-5.1	A	3.251
b	8.5	(iii)	39.2	-2.3	-12.1	B	18.301
ab	8.1	(iv)	32.2	-2.8	+3.9	AB	1.901
c	20.9	(v)	-1.9	-5.1	+33.1	C	136.951
ac	18.3	(vi)	-0.4	-7.0	-0.5	AC	0.031
bc	16.2	(vii)	-2.6	+1.5	-1.9	BC	0.451
abc	16.0	(viii)	-0.2	+2.4	+0.9	ABC	0.101

in the same way gives column (4), of which the first entry is (1) + a + b + ab and the secon C + ac + bc + abc . On forming column (5) from (4) in the same way we obtain for the first entry

$$c + ac + bc + abc + (1) + a + b + ab = I$$

and for the fifth

$$c + ac + bc + abc - (1) + a + b + ab = C$$

The other entries may be shown to correspond to the appropriate effects. The final column contains the contribution to the uncorrected sum of squares of each effect. Since the 8 comparisons are orthogonal, this may be obtained by squaring the effect total and dividing by the sum of squares of the coefficients. As a check on the results, the sum of squares of the original observations may be computed and compared with the total of column (7).

The formal analysis of variance for the components, each with a single degree of freedom, is given in Table 4.

Table 4

Source of Estimate	Sum of Squares	D.F.
Effect A	3.25	1
B	18.30	1
C	136.95	1
Interaction AB	1.90	1
AC	0.03	1
BC	0.45	1
ABC	0.10	1
Total (corrected for mean effect)	160.98	7

In view of the small number of tests we cannot hope to learn much about the interactions. Since $F_{1,1,\,0.05} = 164$, there is no reason to suppose that any of them are disproportionately large, and we pool them to provide an estimate of the residual based on 4 degrees of freedom. This proves to be 0.62, and testing the main effect components of the sum of squares against it we obtain variance ratios:

A : 3.25/0.62 = 5.2 ($F_{1,4,0.1} = 4.5$)

B : 18.30/0.62 = 29.5** ($F_{1,4,0.01} = 21.2$)

C : 137/0.62 = 221*** ($F_{1,4,\,0.001} = 74.1$)

The data provide significant evidence of an improvement in fractionation performance with increase in band speed and decrease in boil-up tate. They suggest that within the range examined the column may be more effecient as the clearance is reduced.

APPENDIX II

Determination of Citric acid content

$$\text{Citric acid (mg)/100 (ml)} = \frac{64}{2} \times \frac{\text{ml titre}}{\text{ml sample}} \times 0.1\ \text{NaOH} \times 100$$

Normality of NaOH used is = 0.0712

ml sample used = 2

$$\text{So Citric acid (mg)/100 (ml)} = \frac{64}{2} \times \frac{\text{ml titre}}{2} \times 0.0712 \times 100$$

$$= 113.92 \times \text{ml titre}$$

Appendix III

Hedonic scale

Lime juice study

Name .....

Date .....

Direction : You will be servedwith lime juice and will be asked to comment how much you like or dislike it. Put a check (/) at the point of scale which describe your feeling about the lime juice

	Sample No.		
	A	B	C
like extremely			
like very much			
like moderately			
like slightly			
neither like nor dislike			
dislike slightly			
dislike moderately			
dislike very much			
dislike extremely			

Comments : .....

.....

VITA

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She has been working as food and drug technologist in the Food control division, Food and Drug Administration, Ministry of Public Health.