The Need of Considering the Interactions in the Analysis of Screening Designs

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Abstract: Fractional factorial designs are widely used experimental plans for identifying important factors in screening studies where many factors are involved. Traditionally, Plackett-Burman (PB) and related designs are employed for in such studies because of their cost efficiencies. The caveat with the use of PB designs is that interactions among factors are implicitly assumed to be non-existent. However, there are many practical situations where some interactions are significant and ignoring them can result in wrong statistical inferences, including biased estimates, missing out on important factors and detection of spurious factors. We reanalyze data for three chemical experiments using the Hamada and Wu's method and show that we are able to identify significant interactions in each of these chemical experiments and improve the overall fit of the model. In addition, we analyze the data using a Bayesian approach that confirms our findings. In both approaches, graphical tools are employed along with easily available software for analysis.

Key words: Complex aliasing, fractional factorial design, Plackett-Burman design, screening experiment, variable selection

1 Introduction

In many scientific investigations, the interest is to study effects of many factors simultaneously and identify a few important effects. Design issues are important because they can drastically affect our statistical inference. In the extreme case, a poorly-designed study may not be able to answer the posed scientific hypotheses. Experiments are also increasingly complex

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and expensive to run in terms of cost and labor. However, there is considerable scope for reducing resources required in research by designing more efficient studies. Careful design considerations even with only minor variation in traditional designs can lead to a more efficient study in terms of more precise estimates or an ability of estimating more effects in the study at the same cost.

Factorial designs are popular experimental plans for studying several factors in a scientific investigation. They can be used to detect interactions between two or more factors in an experiment. Such designs were suggested by the US Environmental Protection Agency as one valuable statistical approach for risk assessment of chemical mixtures [1]. A full factorial experiment allows all factorial effects to be estimated independently and is commonly used in practice. However, it is often too costly to perform a full factorial experiment. For example, if we have 8 factors to investigate and each factor has two levels, we need to have $2^8 = 256$ runs. Instead, a fractional factorial design, which is a subset or fraction of a full factorial design, is often preferred because much fewer runs are required. When this fraction is properly selected, the resulting design has optimal properties. Basic methods for analyzing factorial and fractional factorial designs are given in a highly readable book by Montgomery [2] now in its 7th edition.

Plackett-Burman (PB) designs were proposed more than 6 decades ago [3] and they have been popular since, especially in industrial applications. PB designs are recommended by international organizations for the study of the robustness of analytical procedures. A main reason for their broad appeal is their run size economy and their capability of estimating all main effects. In particular, PB designs of 12, 20 and 24 runs, all special cases of fractional factorial designs, are useful for studying a large number of two-level factors. However, except for obvious cases where some interactions are orthogonal to main effects, analysis of such experiments has been confined to estimating main effects only. This is because of the complex aliasing pattern among main effects and the intricate interactions in these designs. For example, consider the widely used 12-run PB design with 11 factors. There are 55 two-factor interactions and the main effect of each factor X is partially aliased with the 45 two-factor interactions not involving X, thereby making it difficult to disentangle or interpret the significance of interactions. Therefore, PB designs are traditionally recommended only for screening purposes under the assumption of additivity of the factor main effects [4]. However, in many

practical situations it is often questionable whether the additivity assumption is reasonable. More background material on PB and related designs is available in [5].

Hamada and Wu [6] went beyond the traditional approach of analyzing PB designs and proposed a novel analysis strategy capable of identifying important interactions. They demonstrated that some interactions could be entertained and estimated through their complex aliasing structure. They pointed out that ignoring interactions can lead to (i) important effects being missed, (ii) spurious effects being detected, and (iii) estimated effects having reversed signs resulting in incorrectly recommended factor levels. Following their ground-breaking work, Lin [7], Wang and Wu [8] and Cheng [9, 10] studied the projection properties of PB designs and other orthogonal arrays. These studies provide an explanation for the success of the analysis strategy due to Hamada and Wu [6]. Since 1999, PB designs and more generally non-regular designs, have and continue to attract interest in the selection and construction of optimal non-regular fractional factorial designs; see for example [11, 14, 12, 13, 15]. Xu, Phoa and Wong [16] reviewed recent developments in non-regular designs.

Despite recent developments in design theory and data analysis, many data from PB designs are still analyzed in the traditional way where interactions among factors are conveniently ignored. The purpose of this paper is to demonstrate the shortcomings of the traditional analysis and to discuss analytical methods capable of identifying more effects more accurately without additional cost. We demonstrate our claims by reanalyzing data from three recent chemical experiments in the literature and show our conclusions offer new insights and findings. In Section 2, we introduce two simple yet reliable analysis strategies: a frequentist approach [6] and a Bayesian approach [17]. For other analysis strategies, see the textbooks [5, 18]. In Section 3, we describe three chemical experiments and reanalyze them without having to assume that all interactions are non-existent. In each experiment, we discover important but missed interactions in the traditional analysis. The new models all have improved fit to the data and the Bayesian approach confirms their superiority over the models found from the traditional analysis. Section 4 offers summary and conclusions.

2 Analysis Strategies

2.1 A Frequentist Approach

Hamada and Wu [6] proposed an interesting method to identify significant interactions beyond main effects in PB and related designs. The gist of the idea was given in their paper with examples and they may be described in three key steps as follows:

Step 1. Entertain all the main effects. Use standard analysis methods such as ANOVA and half-normal plots to select significant effects.

Step 2. Entertain the significant effects identified in the previous step and all the two-factor interactions that consist of at least one significant main effect. Identify significant effects using a forward selection regression procedure.

Step 3. Entertain the significant effects identified in the previous step and all the main effects. Identify significant effects using a forward selection regression procedure.

Iterate between steps 2 and 3 until the selected model stops changing.

This analysis strategy is based on two assumptions. The first assumption is the validity of the *factor sparsity* principle [19], that is, the number of truly important factors in an experiment is small. The second assumption is the validity of the *effect heredity* principle [6], that is, when a two-factor interaction is significant, at least one of the corresponding factor main effects is also significant. This precept is needed because it is often difficult to provide a good physical interpretation for a significant interaction XY with neither X nor Y being significant.

Note that the traditional approach of analysis using PB or other non-regular designs ends at step 1. If the effect heredity principle is not assumed, it is possible to obtain, in step 2, a statistically good fitting model with only interactions and no main effects, which is difficult to be interpreted in physical sense. The addition of step 3 avoids the possibility of missing main effects in step 1 because of the existence of interactions.

2.2 A Bayesian Approach

The Bayesian approach suggested by Box and Meyer [17] considers all the possible explanations (models including interactions) of the data from a screening experiment and identifies those that fit the data well.

The prior assumptions are as follows:

1. Effects calculated for inactive factors may be represented approximately as items from a normal distribution with mean zero and standard deviation σ.

2. For a proportion π of active factors the resulting effects are represented as item from a normal distribution with mean zero and a larger standard deviation $\gamma\sigma$.

The prior information is represented by two parameters: γ, the ratio of the standard deviation of the active to the inactive effects, and π, the percentage of active factors. Box, Hunter and Hunter [18] suggested to choose γ between 2 and 3 and $\pi = 0.25$, based on a survey of a number of published analyses of factorial designs. A recent study has confirmed that the results are not very sensitive to moderate changes in γ and π when active factors are present.

A Bayesian framework is used to assign posterior probabilities to all the models considered. These posterior probabilities are then accumulated to marginal posterior probabilities for each factor. The technical details of the Bayesian analysis are complicated and we refer the reader to [17, 18]. In practice, one can use the BsProb function in the R library BsMD to calculate these probabilities. R is a free software environment for statistical computing and graphics and can be downloaded from the R project home page [20]. The output result of interest to us from the BsProb function is the marginal posterior probabilities for factor activity. A factor X which has a relatively high posterior probability implies that either the main effect of X or an interaction involving X or both are important.

3 Results from Three Real Experiments

In this section, we reanalyze data from three real chemical experiments using the analysis strategies introduced in the previous section. These examples illustrate the opportunity for identifying additional important effects that the traditional approach misses, as well as correcting the biased estimates of the main effects from significant interactions. We use the

frequentist approach proposed by Hamada and Wu first and use the Bayesian approach to confirm the results. For the latter approach, we use $\gamma = 2$ and $\pi = 0.25$ as recommended by Box, Hunter and Hunter [18]. In practice, we advocate that both approaches be used in any order to confirm findings.

3.1 Example 1: High-Performance Liquid Chromatography (HPLC) Experiment

Vander Heyden et al. [21] used the high-performance liquid chromatography (HPLC) method to study the assay of ridogrel and its related compounds in ridogrel oral film-coated tablet simulations. They chose to use a 12-run PB design to identify the importance of 8 factors on several responses. There were several responses in their study but to fix ideas, we consider only one specific response, which is the percentage recovery of ridogrel. Table 1 gives the factors, levels, design matrix and the observed responses. Their analyses showed there was no significant relationship between any of the factors and this response.

Figure 1 (left) shows the half-normal plot for all factor main effects. A half-normal plot is a graphical tool that uses the ordered estimated absolute effects to help assess the importance of factors. This method is popular among practitioners because it is easy to use and results are easy to interpret. The basic idea is that the larger an estimate the more important an effect. The half-normal plot in Figure 1 identifies two important effects E and F, the percentages of organic solvent $\%B$ in the mobile phase at the start and the end of the gradient. The traditional main effect analysis confirms that these factors are the two most important factors. However, the p-values of E and F are 0.16 and 0.24, respectively. This shows important factors are not necessarily significant factors. The model that consists of only the main effects of E and F has $R^2 = 0.41$.

Using the analysis strategy in Section 2.1, we find a significant EF interaction in step 2. Adding EF to E and F increases R^2 from 0.41 to 0.89. In step 3, we further identify factor H (flow of the mobile phase), which is missed in the traditional approach, as significant at the 5% level. We repeat steps 2 and 3 iteratively until no more new significant effects are identified and the model does not change anymore. When this happens, we stop the procedure

and report the final model, which is

$$\hat{Y} = 101.04 - 0.56E + 0.44F - 0.30H + 0.88EF \tag{1}$$

Here E, F and H take on the values either 1 or -1 corresponding to the $+$ and $-$ factor levels respectively. This model has $R^2 = 0.96$, indicating a good fit. In the model (1), H is significant at the 5% level (p-value=0.012) and E, F and EF are significant at the 1% level.

Figure 1 (right) is the EF interaction plot, which shows the average responses at the level combinations of E and F. The solid (dashed) line represents the change of the average value of the response at the high (low) level of E when F changes from its low level to its high level. Note that if E is at its high level, the response increases rapidly when F changes from its low level to its high level. On the other hand, if E is at its low level, the response decreases slowly when F changes from its low level to its high level. In other words, F has a large positive effect when E is at its low level whereas F has a small negative effect when E is at its high level. Because the effect of F depends on the level of E, the EF interaction is significant. (If the EF interaction was not significant, the two lines in the interaction plot would be nearly parallel.)

In the main effect model, the estimate of factor H is -0.01 and is not significant at all. This can be explained by the aliasing structure of the PB design. When two-factor interactions are present, the expected value of the estimate of the main effect of H is

$$\begin{aligned} &H + \frac{1}{3}(AB - AD - AE - AF + AI - AJ - BD - BE + BF - BI \\ &+BJ + DE - DF + DI + DJ + EF - EI - EJ - FI - FJ - IJ). \end{aligned} \tag{2}$$

If only EF is significant and all other interactions are null, the estimate of H in the main effect model actually estimates $H + \frac{1}{3}EF$, which is $-0.30 + \frac{1}{3}(0.88) \approx -0.01$ according to the model (1). The main effect of H is not significant in the main effect model because it is partially canceled by the EF interaction.

We next analyze the data via the Bayesian approach. The posterior probability plot in Figure 2 (top) shows the marginal posterior probabilities for each factor. For some models, the posterior probability is high for factors E and F, moderate for factor H and small for other factors. This suggests that factors E, F and H are active. However, the marginal posterior probabilities do not tell which two-factor interactions are significant. Since the frequentist

approach identifies the EF interaction as significant, we perform a second Bayesian analysis by treating the EF interaction as a new factor. The resulting posterior probability plot in Figure 2 (bottom) shows that EF is as significant as factors E and F. Factor H is also significant, but not as significant as E, F and EF. The finding is consistent with the frequentist approach.

The posterior probability plot in Figure 2 (top) is produced by the free and popular statistical software R using the commands

```
library(BsMD)
bs=BsProb(X, y, mInt=2, p=0.25, g=2)
plot(bs, code=F)
```

The first command loads the BsMD package into R and the second and third commands perform the Bayes computation and produce the posterior probability plot. Here X is the 12×8 design matrix and y is the 12×1 response vector given in Table 1(b). The option `mInt=2` means that the Bayesian approach evaluates models with two-factor interactions besides the main effects. The options `p=0.25` and `g=2` specify $\pi = 0.25$ and $\gamma = 2$. Our experience is that the choices of π and γ do not seem to be critical. Figure 2 (bottom) is produced with the same commands except that X includes an extra column representing the EF interaction.

3.2 Example 2: Pressurized Liquid Extraction (PLE) Experiment

Moreda-Pineiro et al. [22] developed the acetic acid-pressurized liquid extraction (PLE) for the simultaneous extraction of major and trace elements from edible seaweeds. They used a 12-run PB design with two replicates to study the importance of 8 factors on the mean recovery percentage of released elements, which is defined as:

$$\text{mean recovery}(\%) = \frac{1}{N}\sum((C_{PLE}/C_{digested}) \times 100) \tag{3}$$

where C_{PLE} and $C_{digested}$ are the element concentrations obtained after the PLE procedure and the acid digestion procedure respectively, and N is the number of elements studied. In their study, they used $N = 10$ in (3). Note that Moreda-Pineiro et al. [22] defined their eighth variable as an imaginary dummy variable for evaluating the possible systematic error and/or the existence of important variables that have not been considered. Our analysis simply treats

this dummy variable the same as other 7 variables. Table 2 gives the factors, levels, design matrix and the responses.

The three most significant factors identified by the traditional approach are r (mass/sample ratio), s (particle size) and T (extraction temperature), although s and T have much smaller effects; see the half-normal plot in Figure 3 (left). The p-value of r, s and T are 0.01, 0.07 and 0.12 respectively. Using the conventional 5% level, Moreda-Pineiro et al. [22] concluded that only r is significant. The model that consists of only the main effect of r has $R^2 = 0.27$.

Using the analysis strategy in Section 2.1, we find a significant rs interaction in step 2. In step 3, we identify factor s as significant at the 1% level. When steps 2 and 3 are repeated, no new significant effects are identified and the model obtained is:

$$\hat{Y} = 83.42 + 2.83r + 1.92s - 2.67rs, \tag{4}$$

which has $R^2 = 0.63$. For comparison, the model that consists of the three most significant main effects (r, s and T) has $R^2 = 0.48$. In the model (4) r and rs are significant at the 1% level and s is significant at the 5% level (p-value=0.02).

The rs interaction plot in Figure 3 (right) reveals that s has a large positive effect when r is at its low level but s has a small negative effect when r is at its high level. It also reveals that a large positive effect of r is observed at the low level of s.

The main effect Pareto chart in [22] and the half-normal plot in Figure 3 (left) arrange the factors in the order from the most significant to the least significant as follows: $r > s > T > t > P > A > S > D$. This order of significance to the response is based on the main effect model as follows.

$$\hat{Y} = 83.42 - 0.25A - 1.58T + 1.00t - 0.17S + 0.58P + 1.92s + 2.83r + 0.08D. \tag{5}$$

This main effect model does not include the significant rs interaction, and the inclusion leads to a dramatic change in the significance order. The model consisting of all main effects and the rs interaction is as follows.

$$\hat{Y} = 83.42 - 1.97A + 0.14T - 0.72t - 1.89S - 1.14P + 1.92s + 2.83r - 1.64D - 5.17rs. \tag{6}$$

The new descending order of factor significance, based on their p-values, becomes $rs > r > s > A > S > D > P > t > T$.

The new order features some major position shifts like factors A and T, and the change is due to the bias from the partial aliasing pattern between the main effects and the significant rs interaction. For example, when two-factor interactions are present, the expected value of the estimate of the main effect of T is

$$T + \frac{1}{3}(-At + AS + AP - As - Ar + AD - tS + tP + ts - tr \\ -tD - SP - Ss + Sr - SD - Ps - Pr - PD + rs + sD - rD). \quad (7)$$

If only rs is significant and all other interactions are null, the estimate of T, $\hat{T} = -1.58$, in the main effect model (5) actually estimates $T + \frac{1}{3}rs$. Since $\hat{rs} = -5.17$ is found in the model (6), one needs to correct the estimate of T by $\hat{T} - \frac{1}{3}\hat{rs} = -1.58 - \frac{1}{3}(-5.17) = 0.14$, which is the coefficient of T in (6). Note that this bias correction changes the sign of T from negative to positive, which means that the effect of changing T from high level to low level is actually opposite to what the main effect model suggested. In addition, the bias correction leads to a change of p-value of T from 0.12 in the main effect model (5) to 0.85 in the model (6). As a consequence, T changes from the third most significant factor in the main effect model (5) to the least significant factor in the model (6). This explains why our final model (4) does not include factor T.

We further analyze the data via the Bayesian approach. The posterior probability plot in Figure 4 (top) shows that for some models, the posterior probability is high for factors r and s and small for other factors. Factors r and s are identified as active. Figure 4 (bottom) displays the posterior probability plot when we add a new factor representing the rs interaction. Factors r, s and rs are identified as significant. Moreover, the posterior probability of factor T is negligibly small, suggesting that factor T is not important. In conclusion, Figure 4 supports the model (4) well.

3.3 Example 3: Compound Extraction Experiment

Dopico-Garcia et al. [23] developed a two-step analytical methodology that allowed the chemical characterization of white grapes by simultaneously determining their most important phenolic compounds and organic acids. Among all 11 phenolic compounds and organic acids tested in the original experiment, only one phenolic compound, kaempferol-3-O-rutinoside + isorhamnetin-3-O glucoside, will be considered in this example. They used a 12-run PB

design to select 8 variables that have influence in both two steps on the system. Note that their ninth variable is an imaginary dummy variable for error evaluation. Table 3 gives the factors, levels, design matrix and the responses.

The traditional approach identifies factors D (temperature) and F (sorbent type) as the most important; see the half-normal plot in Figure 5 (left). The p-value of D and F are 0.24 and 0.31 respectively. The model that consists of the main effects of D and F only has $R^2 = 0.41$.

Using the analysis strategy in Section 2.1, we find a significant AD interaction in step 2 and further identify factor C (extraction time) as significant at the 1% level in step 3. However, factor F is not significant even at the 10% level. We obtain the following model on the mean recovery percentage

$$\hat{Y} = 5.51 + 1.11C - 1.03D + 1.73AD. \tag{8}$$

This model has $R^2 = 0.93$, a significant improvement of the model of D and F only. All effects in the model (8) are significant at the 1% level.

The AD interaction plot in Figure 5 (right) reveals some interesting findings regarding extraction solvent (factor A) and temperature (factor D) on the response. When methanol (MeOH) is used as extraction solvent (A at its high level), the temperature only has a small effect on the response. However, when acid water is used as extraction solvent (A at its low level), the response decreases rapidly when temperature increases from 40^oC to 50^oC.

The posterior probability plot in Figure 6 (top) shows that the marginal posterior probabilities of factors A, C and D are around 0.4–0.5 while that of other factors are much smaller. Box and Meyer [17] pointed out that "the absolute magnitudes of the probabilities are less important than the pattern of probabilities and their relative magnitudes." Indeed, if we use $\pi = 0.35$ (instead of $\pi = 0.25$), the marginal posterior probabilities of factors A, C and D are around 0.6–0.7 while all other factors have negligible posterior probabilities. Therefore, it seems reasonable to conclude that factors A, C and D are active. The posterior probability plot in Figure 6 (bottom) includes the AD interaction as a new factor. The AD interaction appears to be as significant as factors C and D. It is interesting to observe that the marginal posterior probability increases for factors C and D and decreases for factor A. This suggests that the main effect of A is no longer significant when the AD interaction is included in the

model. Moreover, the marginal posterior probability of factor F is negligibly small, suggesting that factor F is not significant. In conclusion, the posterior probability plots in Figure 6 support the model (8).

The main effect model, used in [23], misidentified the sorbent type (factor F) as significant because of its partial aliasing pattern with the truly significant AD interaction. Factor F is found to be insignificant after being disentangled from the AD interaction term. In contrast, the main effect model did not identify the extraction time (factor C) as significant because of the effect cancelation with AD. The disentangelement reveals the significant relationship of the factor C to the response.

The misidentification of significant factors may lead to inefficient experiment setting. Assume that the purpose of this experiment is to maximize the response. According to the main effect model $\hat{Y} = 5.51 - 1.03D - 0.84F$, the maximum response is $5.51 - 1.03(-1) - 0.84(-1) = 7.38$ units, which is obtained by setting both factors D and F at their low levels. According to our model (8), the maximum response is $5.51 + 1.11(+1) - 1.03(-1) + 1.73(-1)(-1) = 9.38$ units, which is obtained by setting factors A and D at their low levels and factor C at its high level. Our model increases the response by 2.0 units or 27%, which should be confirmed in practice though.

4 Summary and Conclusions

The traditional practice among practitioners is to use PB and related designs to analyze factor main effects only. In this paper, we discussed two analysis plans that are capable of estimating some two-factor interactions in addition to main effects in PB and related designs. We reanalyzed three real-life chemical experiments and demonstrated that the traditional analysis of ignoring possible interactions can be misleading. In each of our examples, we found a significant interaction and its inclusion resulted in a substantially improved model. In the first example, we identified a new significant factor after disentangling the partial aliasing from the significant interaction. In the second example, we pointed out that the estimates from the main effect model could have an opposite sign, and this in turn could lead to recommending the wrong factor levels for application. In the last example, we identified two new important factors and detected a spurious factor, which could lead to a potential

27% increase in the response maximization process.

We remark that the three main steps outlined in Section 2.1 are not cast in stone and there is flexibility. For example, in step 3, one may use a stepwise selection procedure rather than a forward procedure. Another alternative is to use all subset regressions if possible. Wu and Hamada [5] (Section 8.4) suggested several alternative analysis strategies for designs with complex aliasing. Procedures such as stepwise and all subset regressions are described in many monographs on multiple linear regression analysis such as [24, 25, 26, 27]. Clearly, there is no one right way for fitting a model and the reader should always consult experts' opinion and make informed judgement at each stage of the model selection process.

While two-level PB designs are cost-effective in screening variables, they cannot identify nonlinear relationship between the response and factors. One way to cope with this concern is to add a few (3–5) runs at the center. Adding center points has two advantages: (i) it provides a check on a curvature effect and (ii) it provides an unbiased estimate of the error variance. If a curvature effect is present, the researchers should conduct further experiments to investigate the nonlinear relationship.

In conclusion, ignoring the existence of interactions in the analysis of PB and related designs can lead to at least three pitfalls: (i) important factors may be missed, (ii) spurious factors may be detected, and (iii) the factor level assignments may be opposite to what they should be. Our recommendation is that the traditional analysis of PB and related designs must be used with caution and some skepticism, and should always be accompanied by other methods for confirmation. We use two methods here and show that when we entertain interactions in our analysis, we can avoid the above pitfalls. The upshot is that with the same design, we can estimate all main effects and some interactions more accurately with no additional cost required. As always, confirmation experiments should be conducted to verify the results whenever possible.

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References

[1] Svensgaard DJ, Hertzberg RC. Statistical methods for the toxicological evaluation of the additivity assumption as used in the environmental protection agency chemical mixture risk assessment guideline. *Toxicology of Chemical Mixtures*. Academic Press, San Diego, CA, 1994, 599-640.

[2] Montgomery DC. *Design and analysis of experiments*, (7th edn). John Wiley, 2009.

[3] Plackett RL, Burman JP. The design of optimum multifactorial experiments. *Biometrika* 1946; **33**: 305-325.

[4] Daniel C. *Applications of Statistics to Industrial Experimentation*, Wiley: New York, 1976.

[5] Wu CFJ, Hamada M. *Experiments: Planning, Analysis and Parameter Design Optimization*, Wiley: New York, 2000.

[6] Hamada M, Wu CFJ. Analysis of designed experiments with complex aliasing. *Journal of Quality Technology* 1992; **24**: 130-137.

[7] Lin DKJ, Draper NR. Projection properties of Plackett and Burman designs. *Technometrics* 1992; **34**: 423-428.

[8] Wang JC, Wu CFJ. A hidden projection property of Plackett-Burman and related designs. *Statistica Sinica* 1995; **5**: 235-250.

[9] Cheng CS. Some projection properties of orthogonal arrays. *Annals of Statistics* 1995; **23**: 1223-1233.

[10] Cheng CS. Some hidden projection properties of orthogonal arrays with strength three. *Biometrika* 1998; **85**: 491-495.

[11] Deng LY, Tang B. Generalized resolution and minimum aberration criteria for Plackett-Burman and other nonregular factorial designs. *Statistica Sinica* 1999; **9**: 1071-1082.

[12] Tang B, Deng LY. Minimum G_2-aberration for non-regular fractional factorial designs. *Annals of Statistics* 1999; **27**: 1914-1926.

[13] Xu H, Wu CFJ. Generalized minimum aberration for asymmetrical fractional factorial designs. *Annals of Statistics* 2001; **29**: 1066-1077.

[14] Deng LY, Tang B. Design selection and classification for Hadamard matrices using generalized minimum aberration criteria. *Technometrics* 2002; **44**: 173-184.

[15] Xu H, Cheng SW, Wu CFJ. Optimal projective three-level designs for factor screening and interaction detection. *Technometrics* 2004; **46**: 180-292.

[16] Xu H, Phoa FKH, Wong WK. Recent developments in nonregular fractional factorial designs. *Statistics Surveys* 2009, in press.

[17] Box GEP, Meyer RD. Finding the active factors in fractionated screening experiments. *Journal of Quality Technology* 1993; **25**: 94-105.

[18] Box GEP, Hunter WG, Hunter JS. *Statistics for Experimenters: Design, Innovation, and Discovery* (2nd edn). Wiley: New York, 2005.

[19] Box GEP, Meyer RD. An analysis for unreplicated fractional factorials. *Technometrics* 1986; **28**: 11-18.

[20] The R Project for Statistical Computing. http://www.R-project.org/ [12 May 2009].

[21] Vander Heyden Y, Jimidar M, Hund E, Niemeijer N, Peeters R, Smeyers-Verbeke J, Massart DL, Hoogmartens J. Determination of system suitability limits with a robustness test. *Journal of Chromatography A* 1999; **845**: 145-154.

[22] Moreda-Pineiro J, Alonso-Rodriguez E, Lopex-Mahia P, Muniategui-Lorenzo S, Prada-Rodriguez D, Moreda-Pineiro A, Bermejo-Barrera P. Development of a new sample pretreatment procedure based on pressurized liquid extraction for the determination of metals in edible seaweed. *Analytica Chimica Acta* 2007; **598**: 95-102.

[23] Dopico-Garcia MS, Valentao P, Guerra L, Andrade PB, Seabra RM. Experimental design for extraction and quantification of phenolic compounds and organic acids in white Vinho Verde grapes. *Analytica Chimica Acta* 2007; **583**: 15-22.

[24] Draper NR, Smith H. *Applied Regression Analysis*, (3rd edn). Wiley: New York, 1998.

[25] Kutner MH, Nachtsheim CJ, Neter J, Li W. *Applied Linear Statistical Models*, (5th edn), Irwin, 2005.

[26] Montgomery DC, Peck EA, Vining GG. *Introduction to Linear Regression Analysis*, (4th edn). Wiley: New York, 2006.

[27] Kleinbaum DG, Kupper LL, Nizam A, Muller KE. *Applied Regression Analysis and Other Multivariable Methods* (4th edn). Duxbury: Belmont, CA, 2008.

Table 1: High-Performance Liquid Chromatography (HPLC) Experiment

(a) Factors and Levels

Symbol	Factor	Unit	Factor Level	
			Low (−)	High (+)
A	pH of the buffer		6.5	7.1
B	Column manufacturer		Alltech	Prodigy
D	Column temperature	^{o}C	23	33
E	$\%B$ in the mobile phase at the start of the gradient	%	24	26
F	$\%B$ in the mobile phase at the end of the gradient	%	41	45
H	Flow of the mobile phase	ml/min	1.4	1.6
I	Detection wavelength	nm	260	270
J	Concentration of the buffer	%, m/v	0.225	0.275

(b) Design Matrix and Responses

	Design								Response
Run	A	B	D	E	F	H	I	J	$\%MC$
1	+1	+1	−1	+1	+1	+1	−1	−1	101.6
2	+1	+1	+1	−1	−1	+1	+1	+1	101.7
3	+1	−1	+1	−1	+1	−1	−1	+1	101.6
4	+1	−1	−1	+1	+1	−1	+1	+1	101.9
5	+1	−1	−1	−1	−1	+1	+1	−1	101.8
6	−1	+1	+1	−1	+1	−1	+1	−1	101.1
7	−1	+1	−1	−1	+1	+1	−1	+1	101.1
8	−1	−1	+1	+1	+1	+1	+1	−1	101.6
9	−1	−1	+1	+1	−1	+1	−1	+1	98.4
10	−1	+1	−1	+1	−1	−1	+1	+1	99.7
11	+1	+1	+1	+1	−1	−1	−1	−1	99.7
12	−1	−1	−1	−1	−1	−1	−1	−1	102.3

Table 2: Pressurized Liquid Extraction (PLE) Experiment

(a) Factors and Levels

Symbol	Factor	Unit	Factor Level	
			Low (−)	High (+)
A	Acetic acid concentration	M	0.75	1.5
T	Extraction temperature	^{o}C	25	100
t	Extraction time	min	5	10
S	Extraction step		1	3
P	Pressure	MPa	10.3	17.2
s	Particle size	μm	125	200
r	Mass/sample ratio		1	4
D	Dummy factor		−1	+1

(b) Design Matrix and Responses

	Design								Response
Run	A	T	t	S	P	s	r	D	% Recovery
1	+1	−1	+1	−1	−1	−1	+1	+1	88
2	+1	+1	−1	+1	−1	−1	−1	+1	69
3	−1	+1	+1	−1	+1	−1	−1	−1	73
4	+1	−1	+1	+1	−1	+1	−1	−1	81
5	+1	+1	−1	+1	+1	−1	+1	−1	86
6	+1	+1	+1	−1	+1	+1	−1	+1	88
7	−1	+1	+1	+1	−1	+1	+1	−1	84
8	−1	−1	+1	+1	+1	−1	+1	+1	83
9	−1	−1	−1	+1	+1	+1	−1	+1	88
10	+1	−1	−1	−1	+1	+1	+1	−1	85
11	−1	+1	−1	−1	−1	+1	+1	+1	85
12	−1	−1	−1	−1	−1	−1	−1	−1	81
13	+1	−1	+1	−1	−1	−1	+1	+1	88
14	+1	+1	−1	+1	−1	−1	−1	+1	71
15	−1	+1	+1	−1	+1	−1	−1	−1	79
16	+1	−1	+1	+1	−1	+1	−1	−1	88
17	+1	+1	−1	+1	+1	−1	+1	−1	88
18	+1	+1	+1	−1	+1	+1	−1	+1	83
19	−1	+1	+1	+1	−1	+1	+1	−1	89
20	−1	−1	+1	+1	+1	−1	+1	+1	89
21	−1	−1	−1	+1	+1	+1	−1	+1	83
22	+1	−1	−1	−1	+1	+1	+1	−1	83
23	−1	+1	−1	−1	−1	+1	+1	+1	87
24	−1	−1	−1	−1	−1	−1	−1	−1	83

Table 3: Compound Extraction Experiment

(a) Factors and Levels

Symbol	Factor	Unit	Factor Level	
			Low (−)	High (+)
A	Extraction solvent		Acid Water	MeOH
B	Extraction volume	mL	50	250
C	Extraction time	min	5	20
D	Temperature	^{o}C	40	50
E	Extraction type		Ultrasonic	Stirring
F	Sorbent type		EC	NEC
G	Elution solvent		EtOH	MeOH
H	Elution volume	mL	20	150
I	Dummy factor		−1	+1

(b) Design Matrix and Responses

	Design									Response
Run	A	B	C	D	E	F	G	H	I	Y
1	+1	−1	+1	−1	−1	−1	+1	+1	+1	6.98
2	+1	+1	−1	+1	−1	−1	−1	+1	+1	5.31
3	−1	+1	+1	−1	+1	−1	−1	−1	+1	9.67
4	+1	−1	+1	+1	−1	+1	−1	−1	−1	6.45
5	+1	+1	−1	+1	+1	−1	+1	−1	−1	5.23
6	+1	+1	+1	−1	+1	+1	−1	+1	−1	5.34
7	−1	+1	+1	+1	−1	+1	+1	−1	+1	4.03
8	−1	−1	+1	+1	+1	−1	+1	+1	−1	3.76
9	−1	−1	−1	+1	+1	+1	−1	+1	+1	2.10
10	+1	−1	−1	−1	+1	+1	+1	−1	+1	2.65
11	−1	+1	−1	−1	−1	+1	+1	+1	−1	7.40
12	−1	−1	−1	−1	−1	−1	−1	−1	−1	7.14

Figure 1: The HPLC Experiment: (Left) Half-normal plot and (Right) Interaction plot of EF.

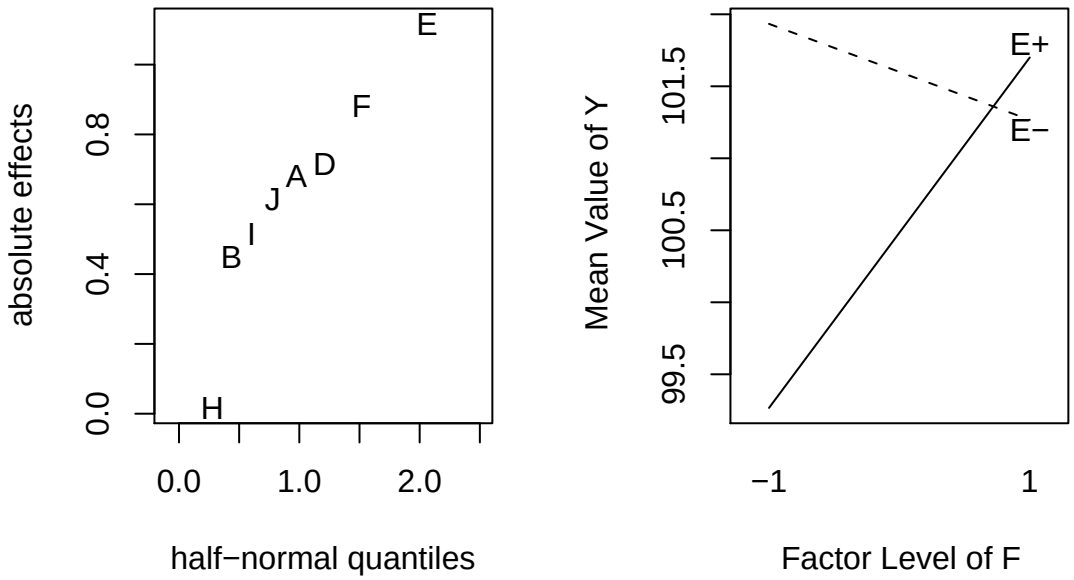


Figure 2: The HPLC Experiment: (Top) The posterior probability plot with the original factors and (Bottom) The posterior probability plot with the EF interaction.

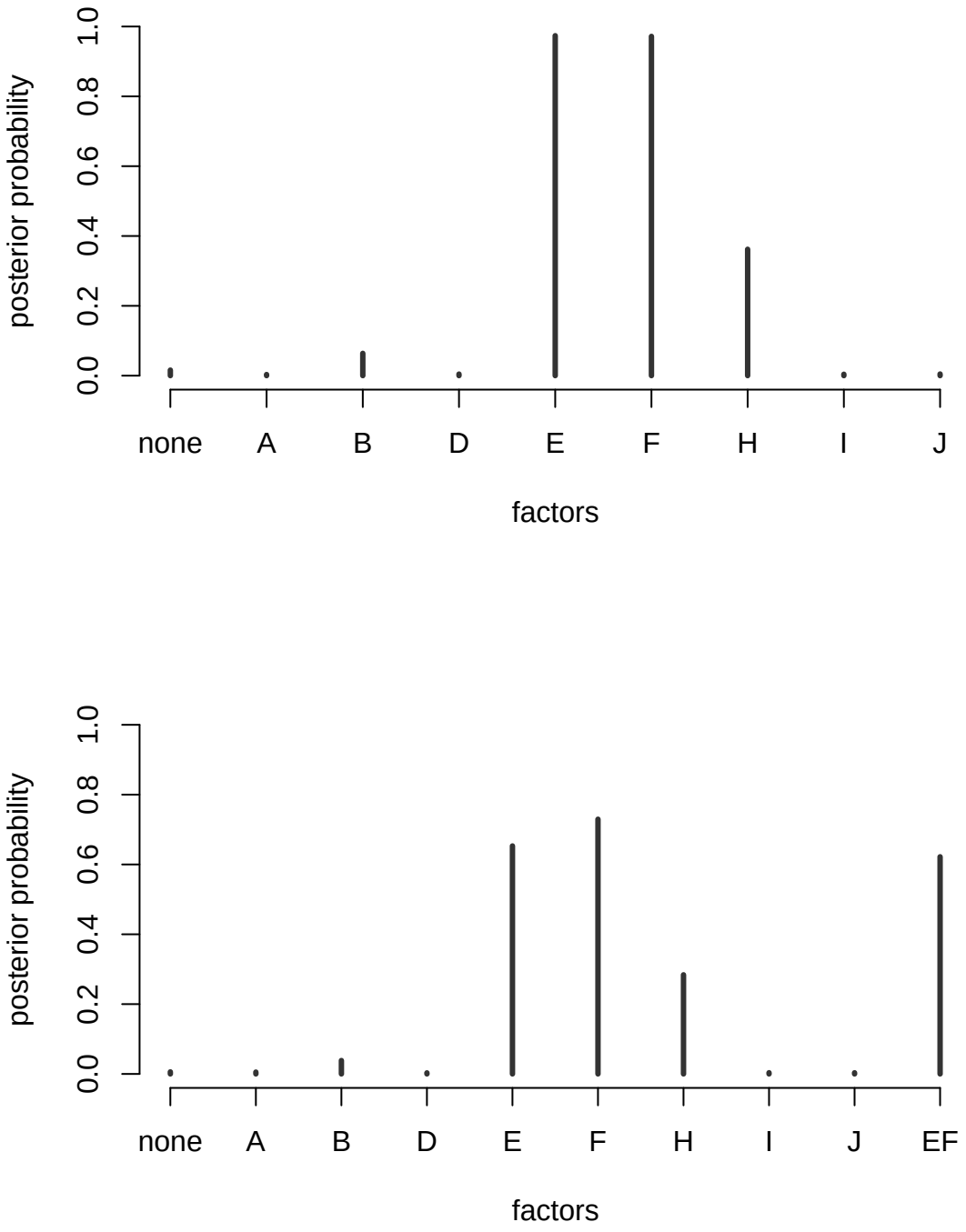


Figure 3: The PLE Experiment: (Left) Half-normal plot and (Right) Interaction plot of rs.

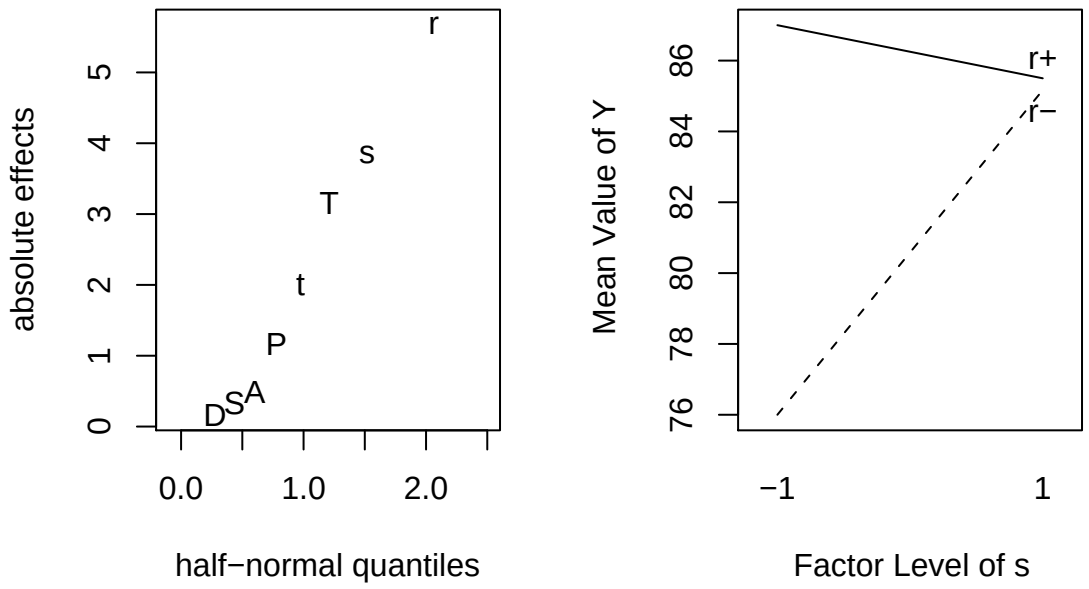
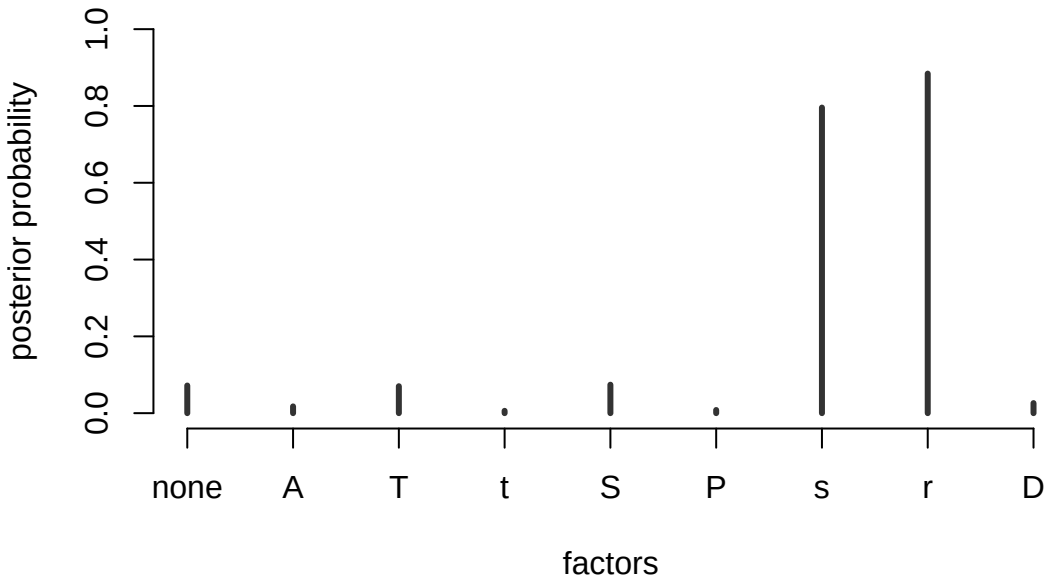


Figure 4: The PLE Experiment: (Top) The posterior probability plot with the original factorsand (Bottom) The posterior probability plot with the rs interaction.



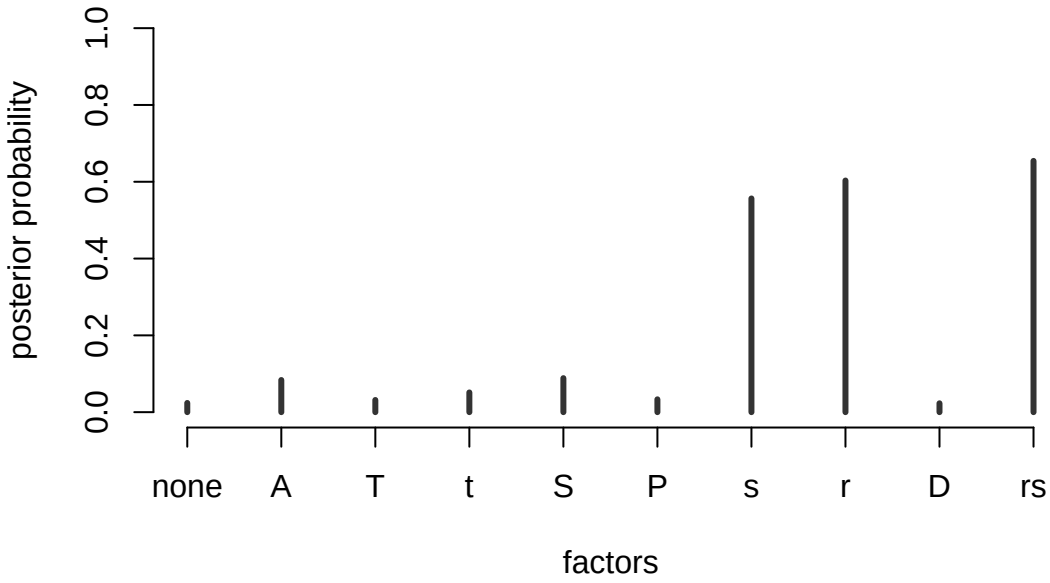


Figure 5: The Compound Extraction Experiment: (Left) Half-normal plot and (Right) Interaction plot of AD.

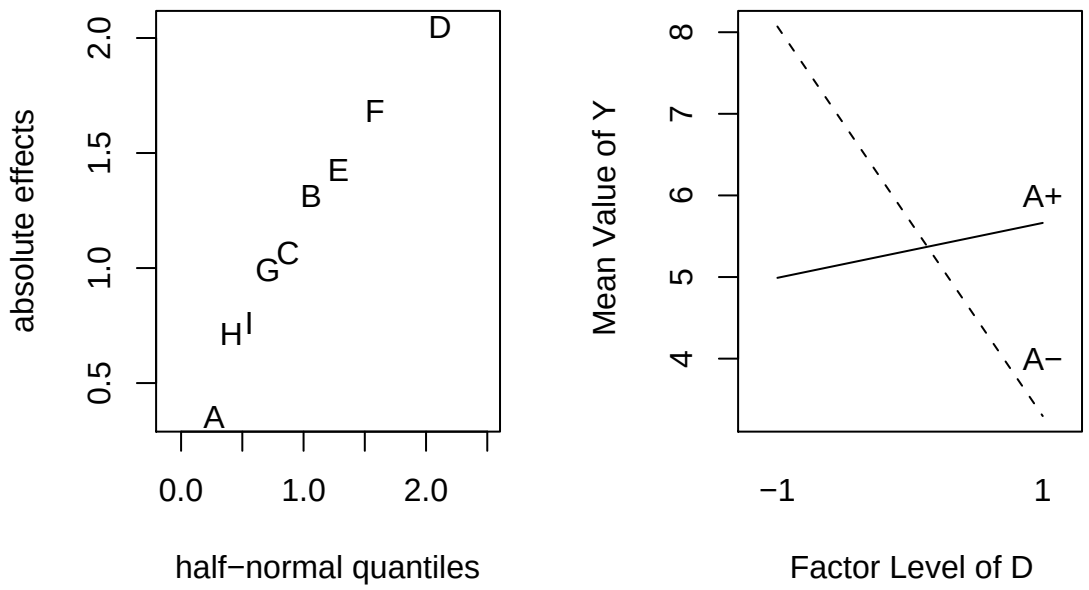


Figure 6: The Compound Extraction Experiment: (Top) The posterior probability plot with the original factors and (Bottom) The posterior probability plot with the AD interaction.