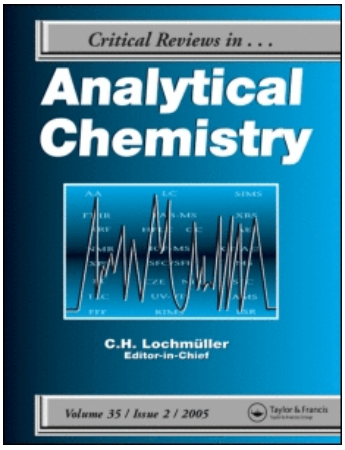

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Application of Factorial and Response Surface Methodology in Modern Experimental Design and Optimization

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Application of Factorial and Response Surface Methodology in Modern Experimental Design and Optimization

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This article critically examines the use of factorial and response surface methodology in modern experimental design and optimization. A survey of important screening and optimization techniques in the literature since 2000 are presented. Current applications in biological, environmental and pharmaceutical analysis, food technology and industrial-related processes are examined.

Keywords experimental design, factorial designs, optimization, response surface methodology, screening

INTRODUCTION

The importance of, and theoretical concepts behind, experimental design and optimization methodology in research and development efforts has been thoroughly discussed in a number of informative publications (1–17). The two main applications of experimental design are screening, in which the factors that influence the experiment are identified, and optimization, in which the optimal settings or conditions for an experiment are found. The usual approach is to start with a screening design including all controllable factors that may possibly influence the experiment, identify the most important ones, and proceed with an experimental optimization design. Models generated can be evaluated by the analysis of variance (ANOVA). The one-way ANOVA allows experimenters to compare several groups of observations. A two-way ANOVA allows one to study the effects of two factors separately as well as their interactive effects.

Although the choice of an experimental design ultimately depends on the objectives of the experiment and the number of factors to be investigated, initial experimental planning (as shown in Figure 1) is paramount. Screening techniques such as factorial designs allow the experimenter to select which factors are significant and at what levels. The most general (two-level design) is a full factorial design and described as 2^k-designs where the base 2 stands for the number of factor levels and k the number of factors each with a high and low value (3, 8, 11, 17). The lower level is usually indicated with a '−' sign; the higher level with a '+' sign. With two factors, this defines a square in the factor space, and with three factors this defines a cube (17). Fractional factorial designs are good alternatives to a full factorial design, especially in the initial stage of a project, and considered a carefully prescribed and representative subset of a full factorial design (11, 17). In fractional factorial designs, the number of experiments is reduced by a number p according to a 2^{k-p} design.

Response surface methodologies are multivariate techniques that mathematically fit the experimental domain studied in the theoretical design through a response function (1, 2). The two most common designs generally used in response surface modeling are central composite and Box–Behnken designs. In these designs the inputs take on three or five distinct levels, but not all combinations of these values appear in the design. Central composite designs contain imbedded factorial or fractional factorial designs with center points that are augmented with a group of axial (star) points that allow estimation of curvature (17). A central composite design always contains twice as many star points as there are factors in the design (11). The star points represent new extreme values (low and high) for each factor in the design.

The Box–Behnken design is considered an efficient option in response surface methodology and an ideal alternative to central composite designs (11, 17). It has three levels per factor, but avoids the corners of the space, and fills in the combinations of center and extreme levels. Overall, it combines a fractional factorial with incomplete block designs in such a way as to avoid the extreme vertices and to present an approximately rotatable design with only three levels per factor. As a result, this design is confined to situations where the experimenter is not interested in predicting response at extremes (corners of the cube). A less

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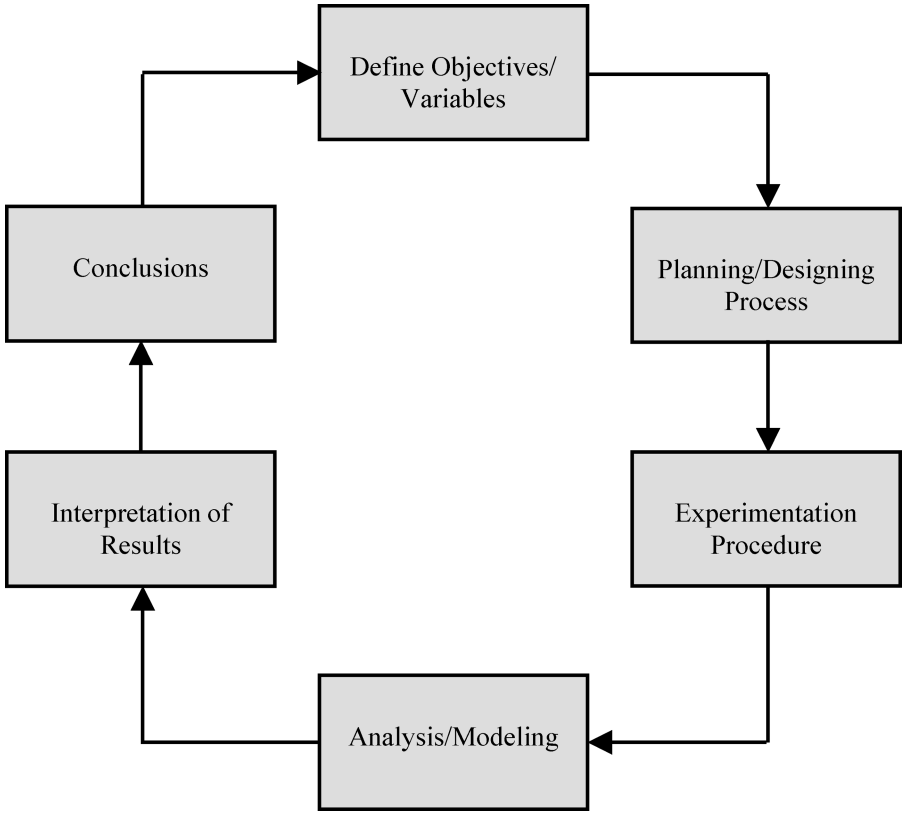

FIG. 1. Criteria for early experimental planning [modified from Hanrahan et al. (16) with permission from Elsevier].

common, but effective method is the Doehlert design. Like the Box Behnken design, Doehlert designs require lower numbers of experiments than the central composite design. Another advantage of the Doehlert design over the central composite approach is its higher efficiency value, ultimately determined by dividing the coefficient number of the quadratic equation by the number of experiments required for the design.

This paper critically examines the role that factorial and response surface methodology has played in modern (2000–Present) experimental design and optimization applications. Section one presents the theory and principles of factorial designs and response surface optimization methodology. This is followed by a detailed survey of recent applications including biological, environmental and pharmaceutical analysis, food technology and industrial-related processes. The last section summarizes the overall importance of experimental design and optimization techniques in modern research and development.

TECHNIQUES AND APPLICATIONS

Table 1 lists examples of factorial and response surface methodology applications in modern experimental design and optimization. This list has been selected to provide a representative coverage of such techniques in biological, environmental and pharmaceutical analysis, food technology and industrial-related processes. More in depth discussions in selected studies in each area are discussed below.

Biological Applications

Novotná et al. (18) used full and fractional factorial designs in combination with artificial neural networks (ANN) in the optimization of high performance liquid chromatography (HPLC) separation of neuroprotective peptides. The ultimate goal of this research was to separate as many peptides as possible, before mass spectrometric analysis. This combination of fractional designs and ANN was novel in that it allowed the optimization of the separation conditions regardless of the limited structural and physico-chemical properties of analyzed peptides. The fractional factorial design proved successful in simple matrix separations and allowed ample input data for ANN approximation. After optimization, fractions with peptides were collected and analyzed using off-line matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS).

Hanrahan et al. (19) used a full factorial design with simplex optimization in the design and development of a flow injection-capillary electrophoresis (FI-CE) analyzer. Here, the binding of D-Ala-D-Ala terminus peptides to the antibiotic vancomycin (Van) was examined. A 2^3 full factorial design was employed to asses the impact of flow rate, injection time and voltage on instrument response (absorbance). Factors that had the greatest

TABLE 1
Selected factorial and response surface methodology applications in modern experimental design and optimization

Area of concentration	Experimental design and optimization techniques	Application	Reference
Biological	Full factorial, fractional factorial, artificial neural networks	Optimization of high performance liquid chromatography separation of neuroprotective peptides.	18
Biological	Full factorial, simplex	Development of flow injection-capillary electrophoresis (FI-CE) to examine the binding of D-Ala-D-Ala terminus peptides to the antibiotic vancomycin.	19
Biological	Fractional factorial	The selection of main variables for improving *Tetrahymena thermophila* growth and enzyme production.	20
Biological	Full factorial	Determination of experimental factors influencing the silanization of glass substrates in DNA chip technology.	21
Biological	Full factorial	Designing a gas sensor based on extracts of *Pleurotus ostreatus* mycelium mushroom.	22
Biological	Full factorial	Glyphosate determination using by square wave voltammetry.	23
Biological	Full factorial	Production of antifungal antibiotic by *Thermomonospora* sp MTCC 3340.	24
Biological	Mechanistic, fuzzy and neural network components	Fed-batch fermentation optimization.	25
Biological	Central composite	Optimization of micellar electrokinetic chromatography separations.	26
Biological	Full factorial	Optimization of a plasmid DNA purification process.	27
Biological	Central composite	High-density cDNA-microarray protocol optimization.	28
Biological	Full factorial, central composite	Optimization of the production of poly(γ-glutamic acid) by *Bacillus licheniformis* CCRC 12826.	29
Biological	Central composite design	Optimization of medium constituents for griseofulvin production.	30
Biological	Central composite design	Fe^{2+} and $S_2O_3^{2-}$ determination by an optimized amperometric bacterial sensor.	31
Environmental	Full factorial, central composite	Optimization of a fluorimetric method for the speciation of Cr(VI)/Cr(III).	32
Environmental	Full factorial, simplex	Analysis of mercury species by commercially available instrumentation.	33
Environmental	Full factorial	Determination of contaminants in Duero River by solid-phase microextraction.	34
Environmental	Fractional factorial, central composite	Optimization of a sequential extraction method for metal partitioning in soils and sediments.	35
Environmental	Fractional factorial	Investigation of the influence of heavy metals and anions on cement hydration.	36
Environmental	Full factorial	Voltammetric determination of lead in water samples.	37
Environmental	Full factorial	Optimization of solid phase microextraction (SPME) conditions for butylated hydroxytoluene analysis.	38
Environmental	Full factorial, central composite	Flow-injection-hydride generation procedure for selenium determination.	39
Environmental	Full factorial, Doehlert	On-line preconcentration and determination of zinc by flow injection inductively coupled plasma atomic emission spectrometry.	40
Environmental	Full factorial	Electrochemical treatment of methyl parathion.	41

(Continued on next page)

TABLE 1
Selected factorial and response surface methodology applications in modern experimental design and optimization *(Continued)*

Area of concentration	Experimental design and optimization techniques	Application	Reference
Environmental	Full factorial, Doehlert	Determination of copper using thermospray flame furnace atomic absorption spectrometry coupled to flow injection.	42
Environmental	Full factorial	The analysis of zinc, sodium, calcium and magnesium in water samples by capillary electrophoresis.	43
Environmental	Box–Behnken	Determination of metallothionein by square wave cathodic stripping voltammetry.	44
Environmental	Full factorial, central composite	Determination of chromium with ammonium pyrrolidine dithiocarbamate using adsorptive stripping voltammetry.	45
Environmental	Fractional factorial	Urinary benzene determination by SPME/GC-MS.	46
Pharmaceutical	Fractional factorial, central composite	Screening of diuretics and ACE inhibitors using capillary zone electrophoresis.	47
Pharmaceutical	Fractional factorial, central composite	Spectrofluorimetric determination of Losartan and Valsartan in human urine.	48
Pharmaceutical	Fractional factorial	Robustness testing of a flow-through dissolution method for atovaquone.	49
Pharmaceutical	Plackett–Burman, Doehlert	Capillary electrophoretic enantioresolution of salbutamol.	50
Pharmaceutical	Fractional factorial, central composite	Development of spray-dried acetaminophen microparticles.	51
Pharmaceutical	Full factorial	Determination of optimal amounts of water in hard gelatin capsules.	52
Pharmaceutical	Full factorial	Production of diacetylmorphine/caffeine sachets.	53
Pharmaceutical	Full factorial	Increasing bioavailability of silymarin using a buccal liposomal delivery system.	54
Pharmaceutical	Full factorial	Development of an in vitro method for prediction of human drug absorption.	55
Pharmaceutical	Plackett–Burman, central composite	Investigation of pharmaceutical high-performance liquid chromatography assay bias.	56
Pharmaceutical	Full factorial	Formation variables influencing the drug release rate from matrix tablets.	57
Pharmaceutical	Full factorial	Design of experimental methods in modern pharmaceutical processes.	58
Pharmaceutical	Full factorial, fractional factorial	Optimization of process parameters in small-scale fluidized bed granulation.	59
Food/Industrial Processes	Full factorial, fractional factorial	Experimental design methodology applied to optimize an organic synthesis.	60
Food/Industrial Processes	Full factorial	Analysis of the performance of a Proton Exchange Membrane Fuel Cell (PEMFC) stack.	61
Food/Industrial Processes	Full factorial	Analysis of absorption-dehumidification processes.	62
Food/Industrial Processes	Fractional factorial	Investigation of the properties of Ti/IrO_2-Nb_2O_5 electrodes.	63
Food/Industrial Processes	Central composite, Plackett–Burman	Optimization of a flow injection system for the determination of hydroquinone in cosmetics.	65

(Continued on next page)

TABLE 1
Selected factorial and response surface methodology applications in modern experimental design and optimization *(Continued)*

Area of concentration	Experimental design and optimization techniques	Application	Reference
Food/Industrial Processes	Doehlert	Optimization of digestion procedures for the determination of Mn, Zn and Fe in food samples by FAAS.	66
Food/Industrial Processes	Central composite	Treatment of plasticized PVC to reduce plasticizer/solvent migration.	67
Food/Industrial Processes	Box–Behnken	Determination of niacin in fresh and dry cured pork products by ion chromatography.	68
Food/Industrial Processes	Central composite	Fast GC analysis of major volatile compounds in distilled alcoholic beverages.	69
Food/Industrial Processes	Full factorial	Optimization of coal flotation.	70
Food/Industrial Processes	Full factorial	Optimization of mechanical properties of polymer concrete and mix design.	71
Food/Industrial Processes	Central composite	Characterization of maize products based on their chromatographic profile.	72
Food/Industrial Processes	Central composite	Liquefaction of pine barks.	73

influence were flow rate and injection time. The simplex optimization method was used to further confirm the influencing factors and their optimum values. Optimization studies concluded the following optimum conditions: flow rate = 0.0625 mL min^{-1}; injection time = 5 sec; voltage = 10,000 kV.

Figure 2 shows a representative series of electropherograms from the FI-CE analyzer for three injections of Van and NAD as standard in a capillary filled with increased concentrations of *N*-acetyl-D-Ala-D-Ala in the running buffer. The peaks for Van and the standard are baseline resolved and can easily be differentiated from each other at all concentrations and all injections. This paper details, for the first time, the use of FI-CE for the estimation of binding constants between a receptor and ligand utilizing affinity capillary electrophoresis (ACE). Moreover, it reports on the first known referenced application of experimental design methodology for FI-CE development.

Environmental Applications

Massumi et al. (32) developed a fluorometric technique using Rhodamine-6G in the presence of H_2SO_4 to study Cr(VI)/Cr(III) speciation in wastewater using chemometric experimental design and optimization methodology. A full factorial design was first implemented to evaluate the following factors: concentration of Rhodamine-6G (R), concentration of H_2SO_4 (A), time of reaction (t) and temperature of reaction (T), on experimental response (intensity of fluorescence). Measurements were carried out for both low and high values and each effect was estimated by the Yates method. A central composite design was subsequently performed and coefficients calculated using the regression method.

The response surface plots generated are shown in Figures 3a and 3b, respectively. From the response surface, the optimum conditions were found to be at T = 109.15°C, t = 3 minutes, A = 0.415 M and R = 0.494 M. Cr(VI) and Cr(III) were measured in wastewater samples using the optimized technique. Overall, linear calibrations in the range of 8–50 ng mL^{-1} Cr(VI) with a detection limit of 0.51 ng mL^{-1} were shown at the optimum conditions from the central composite design results.

Prado et al. (46) used experimental design and response surface methodology in estimating the influence of experimental variables for biomonitoring benzene in exposed individuals. In this study, solid phase microextraction (SPME) and gas chromatography (GC) analysis was utilized in urinary benzene determination. In the screening portion, a half-fractional factorial design (five factors at two levels) was applied to ascertain the individual effects of sample temperature, incubation time, extraction time, sample volume and ionic strength on SPME extraction. The estimated effects of the factors and their interactions were calculated, with sample temperature and volume, incubation and extraction times significant. The study also showed significant interactions between these factors.

The most significant factors from the half-fractional factorial design were used to generate a response surface at three levels with an ANOVA performed to assess the significance of the model. Sample temperature and volume and their interactions had the largest effect on the response. Figure 4 shows the benzene area peak as a function of sample volume and sample temperature. As shown, increasing the sample temperature decreases the amount extracted. Low temperature had an obvious positive effect on the benzene area peak. The model predicted the following optimum factor conditions: 2.5 mL sample volume,

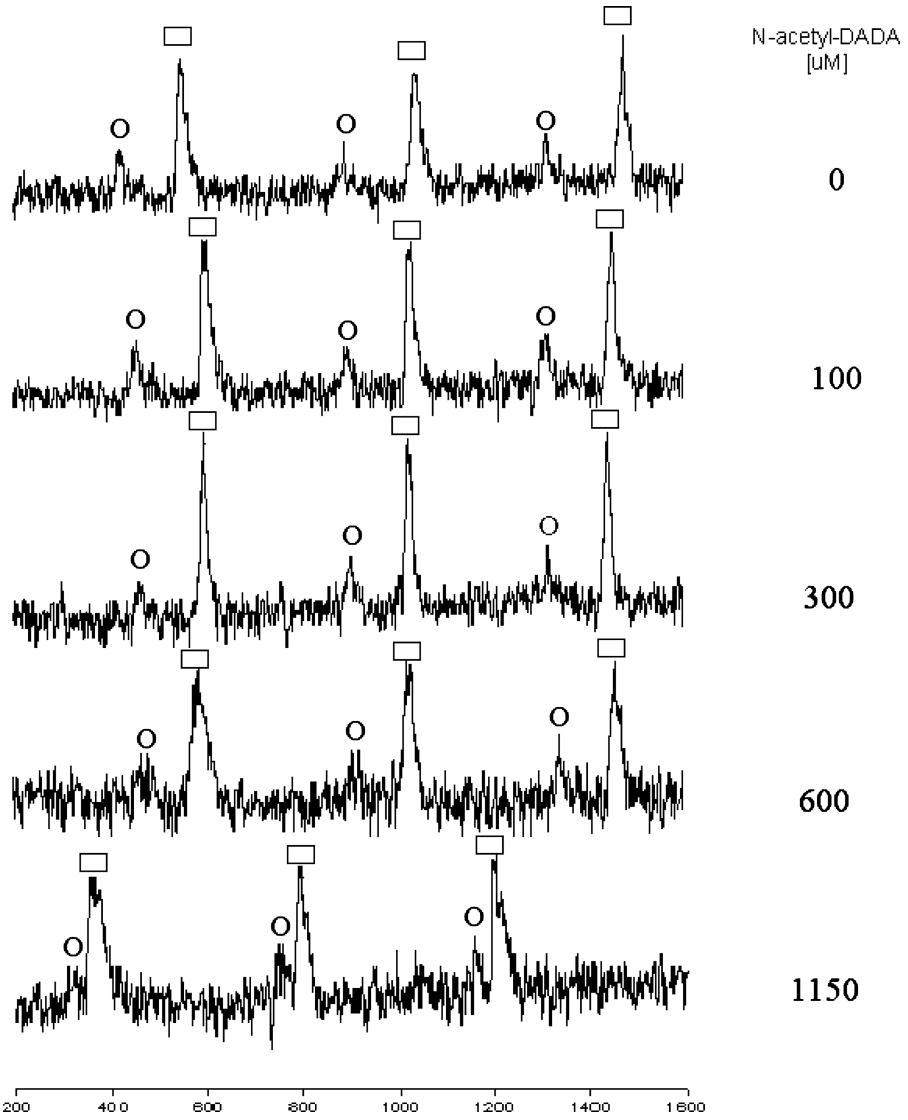

FIG. 2. A representative series of electropherograms of vancomycin (open circles) in 192 mM glycine–25 mM Tris buffer (pH 8.3) containing increasing concentrations of *N*-acetyl-D-Ala-D-Ala using the FI-CE instrument detailed herein. The total analysis time was 27 min at 10 kV (current 200 μA) using a 60-cm (inlet to outlet), 74-μm I.D. open, uncoated quartz capillary; flow rate = 0.0625 mL min^{-1}, 5-second injection time with the detector was set at 220 nm. NAD (open rectangles) was used as an internal standard [from Hanrahan et al. (19) with permission from ISC Technical Publications].

15°C temperature, 1 minute extraction time and 1 minute incubation time. Overall, the optimized method showed good calibration linearity (typical $r^2 = 0.999$) and sample reproducibility (typically <10% R.S.D.) with a detection limit of 0.043 ng mL^{-1}.

Pharmaceutical Applications

A novel spectrofluorimetric method for the determination of Losartan and Valsartan in human urine was developed with the aid of experimental design methodologies by Gagigal et al. (48). In this study, the influence of pH, buffer concentration, percentage of acetonitrile, temperature and slit width on the intrinsic fluorescence needs of Losartan and Valsartan were examined to obtain maximum sensitivity for their determination. A cleanup procedure employing solid-phase extraction using C8 cartridges was used for all urine samples.

Fractional factorial (2^{6-3}) results showed that the relative fluorescent response for both Losartan and Valsartan were negatively affected by increase pH values and positively affected by the increase of emission slit width. For Valsartan only, an increase in buffer concentration had a negative effect on the relative fluorescence response. In addition, the Valsartan relative fluorescence signal decreased as the temperature values increased.

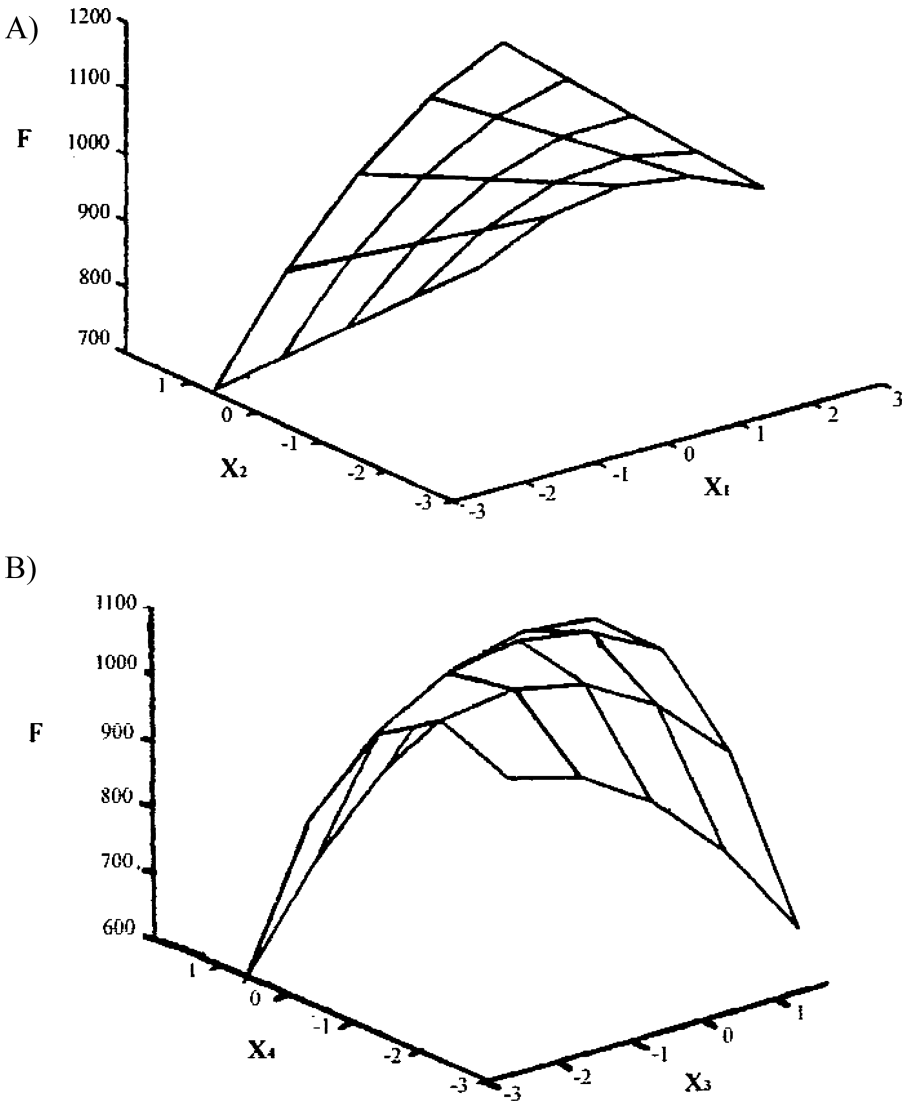

FIG. 3. (a) Plot of response function (ΔF) vs. temperature (X_1) and time (X_2) and (b) plot of response function (ΔF) vs. sulfuric acid concentration (X_3) and R-6G concentration (X_4) [from Massumi et al. (32) with permission from Elsevier].

Central composite designs employing two-level factorial designs plus star orthogonal composite designs involving 14 and 24 runs (plus three central points) were built based on the results of the fractional factorial designs for both Losartan and Valsartan, respectively. For Losartan, the relative fluorescence intensity decreased as the temperature increased. The emission slit width had a significant positive effect on the fluorescent response. In addition, interaction parameters for pH and temperature and pH and emission slit width were pronounced. For Valsartan, emission slit width had a significant positive effect over fluorescence response, the quadratic term and interactions with pH and temperature. However, buffer concentration had no significant interaction. Thus, it was determined that pH, temperature and emission slit width were the most important factors in both Losartan and Valsartan determination.

Overall, the use of experimental design methodology proved effective in optimizing the conditions for accurate (RE, 8%) and sensitive (LOQ c.a. 0.5 μg mL^{-1}) determination of Losartan and Valsartan in human urine. Total analysis time was less than 30 minutes, including the solid-phase extraction step and all results were corroborated by a complementary HPLC method.

Rambali et al. (59) used experimental design methodology to optimize the process parameters in fluidized bed granulation, the size enlargement step in the production of tablets in the pharmaceutical industries. Initially, a Plackett–Burman design was applied to screen the following parameters: spray rate (58.0–135.6 g min^{-1}), inlet air temperature (50–70°C), inlet flow rate (140–286 m^3 h^{-1}), nozzle air pressure (1.5–2.5 bar), nozzle spray diameter (1.2–2.2 mm) and nozzle position (1.0–3.0).

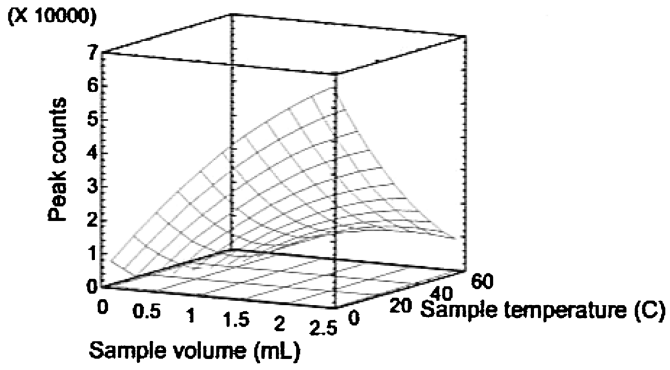

FIG. 4. Benzene area peak as a function of sample volume and sample temperature [from Prado et al. (46) with permission from Elsevier].

The significance of these factors on the percentage yield (w/w) of granules between 75 and 500 μm and the geometric mean granule size (d50) were determined. The overall intent was to obtain granule yields of >90% (between 75 and 500 μm) and a d50 between 300 and 500 μm.

Results of the ANOVA for the Plackett–Burman design for the yield of granules showed that inlet air flow and spray rate had p values <0.05, indicating that they had a significant effect on the percentage yield and d50 value. A fractional factorial design (2^{5-2}) was then employed to screen the remaining factors with the addition of nozzle aircap position and the spraying time interval. ANOVA results from the 2^{5-2} fractional factorial confirmed the significance of spray rate and inlet air flow on both percentage yield and the d50 value. It also confirmed that nozzle air pressure had a significant effect ($p < 0.05$) on the d50 value. Overall, optimum factor settings were: inlet air temperature = 55°C, spray rate = 68.0 g min^{-1}, nozzle air pressure = 2.5 bar, and inlet air flow rate = 213 m^3 h^{-1}. As the target values were achieved, no further optimization techniques were employed.

Food and Industrial Process Applications

Terezo and Pereira (63) used a 2^{6-3} fractional factorial design in determining the effects of preparation factors on the electrochemical (anodic charge) and morphological properties of Ti/IrO_2-Nb_2O_5 electrodes. The investigation of such electrodic materials are important for the oxygen evolving reaction (OER), which occurs in such electrochemical processes as metal electrowinning, water electrolysis, cathodic protection and electroorganic reduction (64).

The preparation factors studied in this investigation included temperature, time and atmosphere of calcination, heating rate, molar ratio between citric acid/ethylene glycol (CA/EG) and citric acid/precursor salt (CA/PS). It was found that calcination temperature and composition of the precursor solution were the most significant factors affecting the anodic charge, most likely due to the changes in coating morphology. More specifically, an average decrease of 26.9 (mC cm^{-2} mg^{-1}) was observed as the calcination temperature was changed from 400 to 500°C. The average anodic charge also decreased as the molar ratios between CA/EG (1:6 to 1:12) and CA/PS (6:1 to 12:1) were changed. Subsequent studies concentrated on the effects of calcination and heating rate in more detail. Here, a maximum anodic charge was observed at 300°C, while the electrode calcinated at 600°C.

Santelli et al. (66) used response surface methodology in the optimization of a focused microwave digestion procedure for the determination of Mn, Zn and Fe in food samples using flame atomic absorption spectrometry (FAAS). Here a Doehlert design involving three factors [irradiation power, irradiation time and percentage of oxidant solution—HNO_3/H_2O_2 (% v/v H_2O_2)] was developed through 13 experiments (including duplicate analysis at the central point to estimate experimental variance). In this design, irradiation time (T) was studied at seven levels (2, 4, 6, 8, 10, 12, 14 minutes), irradiation power (P) at five levels (60, 120, 180, 240, 300 W) and % H_2O_2 at three levels (10, 30, 50).

In this study, the recovery (yield of focused microwave treatment) was calculated and used as the experimental response obtained from the analysis of commercially available food samples. The optimized values obtained from the Doehlert design using the focused microwave treatment were: T = 12 minutes; P = 240W and % H_2O_2 = 42%. The accuracy of the optimized method was evaluated by the analysis of certified reference materials and comparison with a closed vessel microwave oven dissolution method. Here, the application of a t-test showed no significant differences between the two methods and good recoveries were shown for all food samples using the focused microwave technique.

CONCLUSIONS

Experimental design and optimization methodology are important in modern research and development efforts. In combination, these two strategies can help in optimizing experimental procedures in a reduced number of studies as well as providing essential information for appropriate decisions of the future of said procedures. This approach is opposite to the classical univariate approach. Univariate methods are time consuming in that the response is investigated for each factor while all other factors are held at a constant level. This approach is relatively simple and suitable for factors that are independent. However, univariate methods do not take interactive effects between factors into account. If the effects are additive in nature, then experimental designs are the optimum choice and require fewer measurements. In order to address the above concerns, and meet the demands of modern research, proper experimental design techniques considering all factors and their possible interactions must be performed.

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