Handbook of Near-Infrared Analysis

Third Edition

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30 Pharmaceutical Applications of Near-Infrared Spectroscopy

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30.1 INTRODUCTION

Although the pharmaceutical industry was slow in adopting this technique, a number of pharmaceutical applications of near-infrared (NIR) have been reported. This chapter will discuss the development, application, and validation of NIR spectroscopy for the pharmaceutical industry; the software and multivariate methods used for this work are covered extensively in other chapters and will be mentioned in passing.

Though reported by Herschel in 1800, this spectral region was largely ignored until the late 1950s. The first publications describing pharmaceutical applications of NIR spectroscopy appeared approximately 10 years later. Several articles on this topic were published during the 1970s and early 1980s; the late 1980s brought a distinct increase in the frequency of published articles that, again, increased sharply in 2006 (see Figure 30.1). This reflects the growing popularity and increasing maturity of the technique for pharmaceutical analyses over the past few decades with the marked increase of the mid-2000s resulting from the process of analytical technology initiative from FDA [1]. General reviews of NIR spectroscopy have been published [2–8] and contain references to a number of earlier reviews of the technique and applications.

Several texts on NIR analysis are also available [9–16]. Three of the texts, those edited by Patonay, by Burns and Ciurczak, and by Ciurczak and Drennen contain chapters dedicated to pharmaceutical applications of NIR spectroscopy. Ciurczak also authored a comprehensive review of

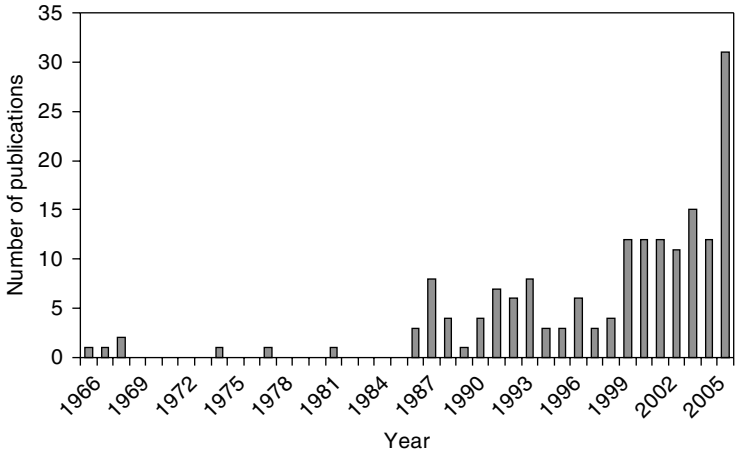

FIGURE 30.1 Number of pharmaceutical publications utilizing NIR spectroscopy.

the pharmaceutical applications of this method, and other papers discussing selected topics in NIR analysis of pharmaceuticals have been published [2,17–20].

The NIR region of the electromagnetic spectrum is generally defined as between 700 and 2500 nm. Absorption bands in this region are due to overtones and combinations of the fundamental mid-IR bands. Most compounds have low molar absorptivities in the NIR region, exhibiting broad, overlapping absorbance peaks. The low absorptivities, once considered a shortcoming of the NIR region, have become a primary reason the method is used extensively in the pharmaceutical industry. NIR absorbances arise primarily from anharmonic oscillations arising from C—H, O—H, and N—H bonds, common to most drugs.

Several qualities of the NIR method have made it appealing as an alternative to traditional analytical techniques for pharmaceutical products: samples can be scanned as is, requiring little or no preparation prior to analysis; analyses of complex matrices are performed rapidly, with results often obtained in a few seconds or less; and, unlike many other methods, NIR spectroscopy does not use expensive, dangerous solvents. These attributes make the technique well-suited for routine laboratory and process control applications.

30.2 APPLICATIONS IN THE ANALYSIS OF TABLETS AND SOLID PHARMACEUTICAL DOSAGE FORMS

The earliest publications of NIR assays of pharmaceuticals by NIR appeared in the late 1960s. In most cases, the drug was extracted from the dosage form and analyzed in solution. In several of these early studies, spectra of solid state samples of drug reported.

In 1966, Sinsheimer and Keuhnelian [21] investigated a number of pharmacologically active amine salts both in solution and in the solid state by NIR spectroscopy. Quantitative analysis of the samples in solution was performed using the 2150 to 2320 nm region. Solid-state samples consisted of compressed pellets composed of an amine salt/KCl mixture and pellets of amine salt only. Spectra of the solid samples collected in the 1050 to 2800 nm region were analyzed qualitatively using peak assignments. Several spectral features were noted as showing promise for the quantitation of drugs in the solid state, although no calibrations were developed. This was the first report of the analysis of pharmaceutical solids by NIR.

In 1967, Oi and Inaba [22] published an article on the quantitation of allylisopropylacetureide and phenacetin in pharmaceutical preparations. The samples were dissolved in chloroform and the concentrations determined using absorbance values at 1983 nm for allylisopropylacetureide and

2019 nm for phenacetin. Concentrations of these two drugs in standard mixed samples and unknown samples were determined without interference from three other drugs present in the formulation.

In another application, Sinsheimer and Poswalk [23] investigated the technique for the determination of water in several matrices. Solid-state samples were analyzed, in which hydrous and anhydrous forms of strychnine sulfate, sodium tartrate, and ammonium oxalate were mixed with KCl and compressed into disks containing 100 mg KCl and 25 mg of drug. In some samples, the water absorbance band at 1940 nm was clearly seen in the spectra of the hydrates. Quantitation of water in these samples was not successful. In other samples, differences in the spectra above 2100 nm were not explained.

30.3 DETERMINATION OF TABLET ACTIVES BY NEAR-INFRARED SPECTROSCOPY

In the earliest NIR assays of solid dosage forms, tablets and capsules served as the starting point, but were not analyzed intact. The drugs were extracted from the dosage form and the concentrations determined in solution.

The first use of NIR for tablet drug content was reported in 1968 by Sherken [24]. In this study, an assay for meprobamate in tablet mixtures and commercially available preparations was established. A range of standard solutions of meprobamate analyzed by the NIR method was used for calibration development. He used the two wavelengths corresponding to the symmetric and asymmetric stretching modes of the primary amine group of the drug molecule. The new method was as accurate, but less tedious than the official assay and recommended as the official method for determination of meprobamate.

In a study conducted by Allen [25], NIR was used for the determination of carisoprodol, phenacetin, and caffeine content in tablets. Twenty tablets were pulverized and an aliquot, dissolved in chloroform. A series of standard solutions of carisoprodol, phenacetin, and caffeine were scanned between 2750 and 3000 nm. All exhibited to Beer's law behavior (maximum 200 mg/ml). Carisoprodol and phenacetin were determined simultaneously at 2820 nm for carisoprodol and 2910 nm for phenacetin. Caffeine concentrations were determined at 3390 nm. Since chloroform is a strong absorber, carbon tetrachloride was substituted as the solvent. The NIR method provided a coefficient of variation (CV) for all three drugs of 1.4% or less.

In many of these early examples of pharmaceutical analysis by NIR, the investigators worked in the 2800 to 3100 nm region, considered to be part of the mid-IR region. However, these were pioneering efforts in NIR pharmaceutical analysis. The NIR analyses of pharmaceuticals reported since these early studies have generally been performed in the 1100 to 2500 nm region, primarily owing to the design of currently marketed instrumentation.

In 1977, Zappala and Post [26] investigated the use of NIR in the analysis of meprobamate in four pharmaceutical preparations: tablets, sustained-release capsules, suspensions, and injectables. By the publication of the paper, a colorimetric method for the assay of meprobamate in tablets had been adopted in USP XIX. This colorimetric method was more rapid than the previous assay, but still required close control of reagent pH. NIR remained an attractive alternative for determination of meprobamate concentrations in dosage forms.

This new NIR method was an improvement over the NIR method introduced by Sherken nearly 10 years earlier. It took advantage of a meprobamate primary amine combination band at 1958 nm, not subject to the interference that the peak at 2915 nm suffered. The previous NIR method required removal of an alcohol preservative from the chloroform prior to analysis that interfered with the 2915 nm drug absorbance peak. The 1958 nm absorbance band, though weaker, was sufficient for calibration development.

The new method required 20 tablets or capsules to be pulverized and an aliquot of meprobamate dissolved in chloroform. Testing the calibration, nine commercial products from four different

manufacturers were analyzed. The CV was 0.7% for tablets and 1.3% for capsules, compared to 1.5% for the reference method.

Used as described above, NIR has a detection limit of 0.1% for solids. High-potency drugs constitute a small percentage of the dosage form, making accurate quantitation of drug by NIR difficult. In a 1990 paper by Corti et al. [27], an extraction procedure was used to improve the detection limit of oral contraceptives, small tablets with a low-concentration of drug.

Ethinyloestradiol and norethisterone, two synthetic hormones commonly used in oral contraceptives were analyzed in this study, both qualitatively and quantitatively. Tablets with a total mass of 80 mg, 0.05 mg ethinyloestradiol content, and 0.25 mg norethisterone content were used. Drug was extracted from the powdered samples with chloroform. Aliquots of the extracts were placed in an aluminum sample cup and scanned in triplicate with a Technicon InfraAlyzer 450 spectrometer.

For qualitative analysis, six wavelengths were used in the Mahalanobis distance calculation. The program was able to discriminate between samples, and to distinguish the ethinyloestradiol extracts even at concentrations below 0.05%. For the quantitative analysis, a method based upon multiple linear regression was employed. Twenty extracts were used for calibration development, with ethinyloestradiol and norethisterone concentrations varying over a 10% range. The correlations obtained for the two calibrations were high (ethinyloestradiol calibration $r^2 = .85$ and norethisterone calibration $r^2 = .86$). Given the low-drug concentrations in the samples, however, the standard errors of calibration were also high.

The researchers attempted to use the calibrations for the prediction of samples not extracted from the tablets with a calibration based upon tablets prepared in the laboratory. When the tablet-based calibrations were used to determine drug concentration in the synthetic samples, poor results were obtained. This was an early illustration of the importance of the similarity between calibration and test samples when an accurate prediction is required.

30.4 FOUNDATIONS OF ANALYSIS OF SOLID DOSAGE FORMULATIONS

The next development in NIR spectroscopic analysis of pharmaceuticals was the analysis of solid dosage formulations. This represented a significant advance in pharmaceutical analysis, because it increased the potential for the application of the method to a large number of pharmaceutical processes. NIR was no longer restricted to the analytical laboratory; rather, it could now be used on the production floor. In addition, it obviated the need for extraction with solvents like chloroform and carbon tetrachloride prior to NIR analysis. This technique has been used in the NIR analysis of blends, granules, encapsulation matrices, and milled tablets.

An early paper using NIR in the analysis of pharmaceutical mixed powders was published in 1981 by Becconsall et al. [28]. NIR and UV photoacoustic spectroscopies were employed in the analysis of propranolol/magnesium carbonate mixtures. In this study, complete spectra were collected in the 1300 to 2600 nm region, using carbon black as the reference sample. An aromatic C—H combination band at 2200 nm and an overtone band at 1720 nm were used in the quantitation of propranolol in the drug-excipient mixtures.

The composition of the mixtures was varied over a wide range and a spectrum collected at each concentration. While the calibration developed with UV exhibited nonlinearity, the calibrations obtained using the two NIR wavelengths were linear. This linearity in the NIR region was attributed to the decreased light scattering efficiency at longer wavelengths and the more equal absorption coefficients of propranolol and magnesium carbonate in this region.

The authors concluded that pharmaceutical quality control measurements could potentially be applied in the NIR region, but cautioned that spectral interferences from other components in the sample matrix could complicate attempts at quantitation.

From 1982 through 1985, little was published on NIR analysis of solid dosage forms. Since 1986, the rapid growth in popularity of NIR for the analysis of solid dosage forms has been reflected by the numerous publications on this topic.

The first was a 1986 paper by Ciurczak and Maldacker [29]. These investigators used NIR in the analysis of tablet formulation blends and examined three methods of data treatment: spectral subtraction, spectral reconstruction, and discriminant analysis. Blends were prepared in which active ingredients (aspirin, butalbital, and caffeine) were either omitted from the formulation or varied over a concentration range of 90 to 110% of labeled strength. All samples were ground in a powder mill to ensure homogeneity, then scanned in a sample cup on a model 500C InfraAlyzer.

The first experiment examined the use of spectral subtraction. Spectra of true placebos were subtracted from spectra of the complete formulation, yielding spectra very close to that of the omitted drug. This technique could be used in the identification of constituents present in a tablet matrix.

The second experiment also involved the qualitative identification of formulation constituents. Spectral reconstruction was performed with commercially available software. Using a series of mixtures of known concentrations, the program used modified multiterm linear regression equations to correlate spectral changes with drug concentration changes. The spectrum of the drug could then be reconstructed, providing identification of drugs present in the sample blend.

The third experiment involved the classification of samples by discriminant analysis. In one series of blends, the caffeine, butalbital, and aspirin concentrations were varied independently between 90 and 110% of labeled strength. In another series, one of the three drugs was excluded from the mixture, while the other two were varied between 90 and 110%. The Mahalanobis distance calculation was employed successfully in the classification of formulations into similar groups. This technique was also used in the analysis of samples from complete formulations (in which all three drugs were present at 100% of labeled strength), borderline formulations, and samples which lacked one of the three active components. Although all the samples were classified correctly by the software, the authors noted that the spectra were nearly indistinguishable, forcing the analyst to depend on the software, rather than visual spectral differences, to make the identification.

Chemometric techniques are excellent for differentiation between samples with subtle spectral differences. However, relying on these routines without an understanding of the underlying physical and chemical phenomena responsible for the spectral differences could lead to unstable calibration models or classification based upon extraneous spectral characteristics.

These experiments highlight another advantage of NIR in dosage form analysis. For products containing multiple active ingredients, individual reference tests must generally be conducted to verify that each ingredient is present in its correct concentration. NIR can be used to characterize the entire formulation simultaneously, making multiple reference tests unnecessary.

The following year, Ciurczak and Torlini [30] published a paper on the analysis of solid and liquid dosage forms by NIR. The authors contrasted the use of NIR in the development of calibrations for natural products with that for pharmaceutical dosage forms. They noted that natural products require the development of a reference method for calibration development, while pharmaceutical dosage forms could be synthetically prepared to develop a calibration.

One disadvantage to this technique is the fact that samples prepared in the laboratory are often spectrally different from production samples due to differences in preparation methods, processing equipment, or batch size, making calibration by this method more difficult. In other words, this approach to calibration development often leads to unsatisfactory results, and the use of production samples for calibration development is preferred when production samples will be tested. In either case, a calibration range wider than that expected for production samples is necessary, to minimize the likelihood of accepting a false sample. This subject will be discussed in greater detail later.

In the study by Ciurczak and Torlini, the performance of NIR was compared with high performance liquid chromatography (HPLC) for speed and accuracy of results in dosage form analysis. The effect of milling the samples prior to analysis was also investigated. Two solid dosage form matrices,

a caffeine–acetaminophen tablet mixture and an acetaminophen tablet mixture, were prepared. Acetaminophen tablet mixtures were analyzed after milling, and caffeine–acetaminophen tablet mixtures were analyzed with and without milling. The samples were placed into a sample cup and scanned by a model 500C InfraAlyzer. Multiple Linear Regression was used for the calibration with 80% of the samples used for calibration development and the remainder for validation.

The investigation of the effects of milling showed that this sample preparation procedure did not necessarily improve results. Milling of the caffeine–acetaminophen mixture significantly improved the determination of acetaminophen, but the determination of caffeine was virtually unchanged. The largest differences between the NIR determined concentrations and theoretical concentrations were attributed to sample handling variability.

The average difference between the theoretical and predicted drug concentrations was approximately 0.25%, competitive with HPLC determinations. The NIR method also provided the advantages of rapid analysis times and no solvent purchase or disposal costs.

Granules can be used as a final dosage form, or as a processing intermediate requiring tabletting or encapsulation. In a 1987 paper, Chasseur used NIR for the assay of cimetidine granules [31]. Batches of granules were prepared in which the cimetidine concentration ranged from 70 to 130% of label strength; spectra were collected on a Pacific Scientific filter-based instrument. For calibration development, both first and second derivative spectra were analyzed, with one or two wavelengths included in the model. A two-wavelength model using first derivative spectra provided the best results, giving a standard error of 1.75%.

These researchers also compared the performance of NIR with the UV reference method in the determination of cimetidine content. Samples of 100% label strength were prepared and analyzed by both methods. The standard errors for the NIR (2.73%) and the UV (2.97%) assays were virtually identical.

In a 1987 paper, Osborne investigated the use of NIR spectroscopy in the determination of nicotinamide in vitamin pre-mixes [32]. Although this was not specifically a tablet matrix, the study showed potential advantages of the method for pharmaceutical analysis. HPLC was the existing reference method for nicotinamide, requiring 3 days to analyze 36 samples. The proposed NIR method required only 30 min to analyze the same samples.

Twenty-five mixtures were used for calibration development, with nicotinamide concentrations ranging from 0 to 6% of the pre-mix formulation. Spectra were collected in duplicate on a Pacific Scientific Mk I 6350 at 2 nm intervals between 1200 and 2400 nm. The spectra were reference corrected using a standard ceramic reference. Second derivative spectra were calculated and the calibration obtained by regressing nicotinamide concentration against the ratio of the second derivative values at 2138 nm (a nicotinamide absorbance peak) and 2070 nm (a spectral minimum in the pre-mixes). The validity of the calibration was determined in two ways: first, 25 more samples were prepared in the laboratory and used to validate the model; second, 25 commercially prepared pre-mix samples with different batches of raw materials were analyzed by the NIR method and by the HPLC reference method.

The standard error of prediction (SEP) for the validation set was 0.56% w/w, with sample positioning variability accounting for half of the total sample variance. To further validate the calibration, commercial samples were collected over a period of several months and analyzed by HPLC and NIR. The results obtained by the two methods were not significantly different, verifying the NIR calibration.

In NIR calibration, the introduction of unexpected components (e.g., incorrect chemicals or contaminated raw materials) into a sample may go undetected, causing erroneous results. This type of false sample is potentially more serious than samples in which the correct constituents are present in the wrong concentrations. In a 1988 paper, Lodder and Hieftje [33] discussed in detail the application of a new algorithm, the quantile-BEAST (Bootstrap Error-Adjusted Single-sample Technique), which was more sensitive to false sample detection. The quantile-BEAST was proposed as a new method to assess pharmaceutical powder blends qualitatively.

The quantile-BEAST algorithm is a nonparametric bootstrap method based upon the work of Efron. In the 1988 study, four individual benzoic acid derivatives were analyzed as were mixtures of the four derivatives. The active ingredient concentrations were varied between 0 and 25% of the sample, with aluminum oxide used as a diluent. The samples were ground and passed through a 100-mesh screen prior to analysis. Spectra were collected in triplicate at three wavelengths on an InfraAlyzer 400.

After analysis by the bootstrap algorithm, the individual benzoic acid derivatives were classified into clusters using the measurement of nonparametric standard deviations (SDs), analogous to SDs in parametric statistics. A value of three SDs or less was expected for identical samples, while dissimilar groups were expected to have SDs greater than three. The four derivatives displayed a minimum distance of 39 SDs from each other. A "worst-case" analysis was conducted, in which acetylsalicylic acid was mixed in with the formulations at concentrations between 1 and 20%. Although three of the ten contaminated samples failed the three SD test for being false, none of the uncontaminated samples were incorrectly identified. All the compounds, including the contaminant, were closely related derivatives of benzoic acid, making their differentiation and identification at relatively low concentrations even more notable.

The most important factor in the accuracy (bias) of this method was the size of the training set, while the most important factor in determining the CV was the number of bootstrap replications of the training set.

Polymorphism is a phenomenon of great concern to pharmaceutical formulators. A number of drugs have been shown to undergo polymorphic transformation under compression, creating potential stability and bioavailability problems. Gimet and Luong [34] published results of a study in which NIR spectroscopy was used for the quantitative determination of polymorphs in a formulation matrix. Most polymorphs exhibit spectral differences in the mid-IR, and, since NIR spectra arise from overtones and combinations of mid-IR absorbances, NIR was also reasoned to be suitable for the analysis of polymorphs. An investigational drug was shown to have two polymorphic forms, with the more stable form transformed to a less stable polymorph under pressure. The researchers employed NIR spectroscopy during formulation development to quantify the more stable polymorph.

Spectra of the polymorphs displayed minor variations, but no distinguishing characteristics were obvious. The authors did not attempt to identify spectral differences according to polymorphic changes in crystalline structure. Had difference spectra (in which the spectra of one polymorphic form are subtracted from the spectra of the other form) been calculated and studied, the spectral variations between polymorphic forms may have been enhanced, providing further insight into the phenomena which allow polymorphic forms to be discernable by NIR spectroscopy. Further research should be conducted into this application of NIR spectroscopy.

Quantitative determination of indomethacin was studied by Otsuka et al. in 2003. Tablets containing α [alpha] and γ [gamma] forms of indomethacin were manufactured at laboratory scale. The sum of the two polymorphs in the tablets constituted 50% of the tablet. A calibration was developed to predict the percentage of γ of indomethacin in the tablets from 0 to 100%. Powder x-ray diffraction was used as the reference method for the determination of polymorph content.

Changes in the NIR spectrum that are indicative of the presence of a polymorphic form are frequently subtle, but are detectable with the proper processing of the spectra. In 2005, Li et al. [35] demonstrated the detection of a second polymorph (form B) of an API (proprietary) in a formulation following wet granulation. Subsequent spiking studies with the discovered polymorph over a range of 1 to 8% enabled researchers to quantitatively predict the fraction of the API that had been converted to form B during wet granulation.

Qualitative determination of the polymorphic form of an API is achievable by NIR. In 2006, Blanco et al. [36] demonstrated that the polymorph of the API used to manufacture a tablet was detectable in the finished dosage form. Thus, demonstrating the ability of NIR spectra to verify the correct form of the API was utilized in the manufacturing process. NIR was used to determine which crystalline form (A or B) of formulations of desketoprofen trometamol (DKP, 10 to 25% w/w

of dosage form) was used to manufacture a tablet. Investigations indicated that different manufacturing processes had the potential to modify the crystalline form of DKP which were detected by NIR.

The ability of NIR to detect changes in the API can facilitate investigations into quality failures. Bauer et al. [37] utilized NIR to detect the desolvation of erythromycin in a finished tablet following dissolution failures. NIR was used to follow the loss of water from erythromycin and the uptake of those waters of hydration by $Mg(OH)_2$. Although the method describes on the ability to monitor the reaction in a qualitative sense, the potential for quantitative determination of the exchange of water, and the subsequent prediction of dissolution behavior is apparent. This work also demonstrates the ability to follow a solid-state reaction by NIR without specific knowledge of the specific reaction species.

The application of NIR analysis to the quantitation of ketoprofen in a gel matrix and a powder matrix intended for encapsulation was reported by Corti et al. in 1989 [38]. The goal of the research was to compare the utility of a calibration developed over a wide concentration range with that of a calibration developed over a narrow range in the prediction of unknown samples.

The NIR spectrometer was a filter-based Technicon InfraAlyzer 450 and a sample cup was used to hold the powdered samples. Reproducibility of the assay was checked by scanning a single sample in one sample cup ten times; the CV was 0.941%. When the same sample was scanned in ten different sample cups, a CV of 1.214% was obtained.

For the narrow calibration range, 13 samples were prepared that fell within ±5% of the theoretical content (33% active) of the matrix. The wider calibration range consisted of the original thirteen samples and an additional seven samples whose ketoprofen concentration ranged from 3 to 30%.

Several characteristics of the two calibration approaches were noted. First, the SEP for both calibrations was approximately 2%, with no sample having an error greater than 3.5%. Second, the wider calibration did not offer any significant advantages, as neither the standard error of estimate (SEE) nor the SEP improved. When the samples to be analyzed fell into a narrow range of concentrations (typical of production samples), relatively few samples were needed to develop a calibration. When the potential for considerable variability of the matrix exists (such as in the NIR analysis of natural products), many more samples are needed to generate a valid calibration.

In the analysis of powders in which the ingredient of interest varies over a wide concentration range, deviations from linearity are possible and can be a significant source of calibration error. Researchers should consider this phenomenon when developing such calibrations. For example, even though it might be valuable to use a calibration ranging from 50 to 150% of desired active ingredient concentration for quantitation of drug in both good and bad samples, it is unlikely that a linear response would be obtained over such a large concentration range. The range over which linearity is possible will depend on the concentration of drug in the matrix and the nature of the dosage form matrix itself. Additionally, the range of a calibration may significantly affect the commonly reported chemometric statistics such as root mean square error of prediction (RMSEP). For calibrations of a wide range, the RMSEP may be acceptably low, but the ability to distinguish between similar concentrations may be absent.

The following year, Corti et al. [39] applied NIR reflection spectroscopy to the analysis of ranitidine and water content in tablets. These were production samples with a narrow distribution of concentrations, so the authors established two criteria for the calibration development. First, the narrow range of sample concentrations allowed fewer samples to be included in the calibration set. Second, production samples had a normal concentration range of 4%, and the calibration was expanded to cover a 10% concentration range. These samples were prepared by adding either filler or drug to a commercial mixture (a spiking study).

Near-infrared spectra were collected on powdered samples, obtained by crushing five tablets and underdosing or overdosing the samples as described. A Technicon InfraAlyzer 450 filter-based instrument was used for the analysis. Tablet drug content was determined by HPLC and water content by Karl Fischer titration.

For quantitation of tablet drug content, three calibrations using multiple linear regression were developed. The first used only laboratory samples and provided a low SEE, but a SEP of 8.4% when the unknown commercial samples were tested. The second calibration used only production samples and provided a SEP of 1% for production samples; the SEP was 6.4% for laboratory samples. The third calibration, built with production and laboratory-modified samples, had SEPs of approximately 1% for both production and laboratory samples. The results showed that progressive widening of the calibration range provided an advantage only up to a point, beyond which no improvement in SEE or SEP was seen. This optimum value was determined to be a calibration range of approximately 5%.

The calibration for determination of water content also employed production samples and modified production samples. Both SEE and SEP were less than 0.1%. In a test of production samples over 1 year, the NIR had the greatest error in moisture prediction of tablets with less than 1% moisture. When used qualitatively, in no case did the NIR method erroneously reject samples with a moisture content greater than 2%, the moisture level at which tablets were rejected. As in the previous publication, the results supported the hypothesis that for products with little variability, a small number of samples (∼10 to 20) is sufficient for calibration development.

One study in which NIR was investigated and not found to be a suitable analytical method was reported by Ryan et al. in 1991 [40]. The purpose of the study was to find a rapid and routine method for the verification of the correctness of clinical packaging. Both mid-IR and NIR were investigated for the analysis of two structurally similar cholesterol-lowering drugs, lovastatin, and simvastatin. For this study, a Pacific Scientific Model 6250 and a Bio-Rad FTS 40N were employed, with KBr powder used as a reference. Sample preparation involved grinding the samples to a uniform particle size prior to analysis. Both mid-IR and NIR offered a detection limit of approximately 1% (w/w) in the presence of excipients. NIR was unable to differentiate between the two drugs at low concentrations. Since it was conceivable that these two compounds would be present in low concentrations in the same clinical packaging, the NIR technique was discontinued. However, as reported in another chapter, this type of detection has been accomplished [41–43].

In 1992, Corti et al. [44] analyzed a variety of antibiotic compounds by NIR, including primary materials, partially processed granules, and an antibiotic cream. Spectral collection was performed on an InfraAlyzer 450. All samples were read in triplicate, with the sample cup emptied and refilled between readings. As in the authors' earlier work, multiple linear regression was used for the calibration development and Mahalanobis distances for qualitative analysis.

Qualitative analysis involved the differentiation among ten antibiotic preparations, including three types of ampicillin (amorphous, crystalline, and trihydrate) and blends of erythromycin powder and granules. All samples tested were easily differentiated based upon Mahalanobis distances. Calibrations were developed for each material, including 15 samples spanning a 5% concentration range. The SEE and SEP for each calibration were less than 2% and most were less than 1%.

A 1993 paper by Blanco et al. [45] addressed some of the concerns regarding the laboratory manipulation of production samples prior to NIR analysis. In this study, two different commercial preparations of ascorbic acid (vitamin C) were analyzed, one a granular product, the other an effervescent tablet. No less than five batches were used in the calibration development for each product.

Samples for calibration and validation were ground to a specific mesh size (250 or 100 μm) before analysis. To expand the calibration range, samples were either diluted, with the primary inactive ingredient added to each, or overdosed with ascorbic acid. Using calibration matrices containing increasing numbers of overdosed samples and comparing the sum of squared differences between the laboratory-determined and NIR-determined values, the authors verified that the underdosing and overdosing process did not affect the physical properties of the samples. Avoiding calibration problems in dilution or concentration of samples is possible by milling samples to a uniform particle size.

Spectra of the homogenized samples were collected in triplicate on a NIRSystems 6500 in reflectance mode. The effects of sample particle size on NIR spectra are often significant, but can

usually be minimized by preprocessing the spectra. Prior to analysis, three preprocessing methods were evaluated: multiplicative scatter correction (MSC), signal scaling, and first derivative. In this study, the first derivative spectra provided the best calibration results and were used throughout the experiment.

Two chemometric techniques were used for calibration development. The first was stepwise multiple linear regression (SMLR), using four wavelengths or fewer, and provided correlation coefficients greater than or equal to .99. The SEE and SEP for all calibrations were less than 2.4%. Calibrations were also developed using partial least squares (PLS) regression, on both full (1100 to 2500 nm) and reduced wavelength (1300 to 1800 nm) spectra. Generally, two or three factors were adequate for the calibrations and most of the models had SEE and SEP values less than 2%. SMLR gave more accurate results for the simpler granule preparation, while PLS was more accurate in drug quantitation for the more complex effervescent tablet formulation.

These reports of NIR analysis of blends and granules lend substantial support to the usefulness of this technology in the monitoring of intermediate pharmaceutical processes, such as blending, granulation, and qualification of bulk powders prior to tabletting or encapsulation. NIR analysis of powdered tablets and capsules takes advantage of the analytical speed of this method, but does not capitalize on the nondestructive nature of the technique or the versatility of its sampling capabilities. Over the past 20 years, research has first intensified in the area of intact dosage form analysis and subsequently attention has been turned to use of NIR as a tool for monitoring pharmaceutical manufacturing.

30.5 ANALYSIS OF SOLID DOSAGE FORMULATIONS DURING MANUFACTURING

The monitoring of pharmaceutical manufacturing process, as they occur has become a primary goal of many analytical science practitioners since the introduction of the draft process analytical technology (PAT) guidance in early 2000s and its finalization in 2004 [1]. Substantial work in this area has been completed in foods and petroleum industries (and to a limited degree, the pharmaceutical industry) prior to this time; however, only a few forward looking researchers were working in the application of NIR to pharmaceutical processing prior to the FDA initiative. While the goal of the guidance is not to encumber pharmaceutical companies with additional testing burdens, achievement of the objectives of PAT often require the use of analytical solutions for on-line monitoring of pharmaceutical processes. Hammond and Warman [46] stated that greater than 70% of the on-line analytical needs of Pfizer involve NIR spectroscopy. It cannot be over-emphasized that the implementation of a NIR method (or any other on-line technique) does not constitute a PAT application; however, rapid and accurate methods for determination of critical quality attributes of a product during processing are an essential element of a successful PAT program.

The process of combining dry pharmaceutical ingredients is critical to the ultimate success of the manufacturing process. The homogeneity (or blend uniformity) achieved during the operation is of the utmost importance. Blend uniformity is typically described in terms of the mass of the final dosage unit. As the typical consumer will not consume less than an entire dosage unit, homogeneity at a scale of scrutiny less than that is frequently not considered. Recent work in NIR imaging has demonstrated some instances of this practice to be an inappropriate perspective for the overall quality of performance of a solid dosage form, and such work is covered in the analysis by NIR Imaging section of this chapter. Considerations for the mass of material sampled during blending and thus the scale of scrutiny is an important consideration when implementing NIR as a means of monitoring blending. The criteria for considering materials "homogenous" is of prime importance and must be carefully defined.

Early reports of NIR for blending was by Cuesta et al. and Wargo and Drennen [47,48] wherein, the use of NIR as a means of establishing homogeneity between samples from different locations

within a blender at a given time was demonstrated. Direct, on-line monitoring of pharmaceutical blending was demonstrated by Hailey et al. [49] and published in 1996. In this publication, the blender utilized was a V-blender with a NIR probe interfaced through windows in blender. This was the interface used again in 1998 by De Maesschalck [50]. Two differences existed between these early reports of the use of NIR for the determination of blend uniformity. The first is the basis for determining blend uniformity; the former works utilized multiple samples at a single time point; while the latter compared the same point in a blend across time. The second difference between these groups is the means by which the blend end point is calculated, although each utilized a version of either a conformity index approach (spectral matching) or a SD of some characteristic of the blend over the data collected from the blender. Numerous other reports of blend monitoring are available and employ a variety of sampling, and end-point calculation methods [51–56].

The work by Duong et al. [56] is notable as it examined the ability of NIR to monitor the uniformity of magnesium stearate in a blend. A landmark series of papers by El-Hagrasy and Drennen [57–59] demonstrated the critical nature of the location of the NIR probe in a blender. In these reports, the blending of materials in a V-blender is monitored at a number of locations around the blender. Differences in the results from the various locations are noted and an overall pattern of good inter-shell mixing and poor intra-shell mixing is observed. Lowery et al. and later Popo et al. [60,61] suggest characterization of the blend by monitoring the powder as it flows from the blender into the feed-frame of a tablet press.

An important early contribution is an estimation of the quantity of material sampled by a NIR probe during blend monitoring. This work was undertaken by Cho et al. [62] and demonstrated that the quantity of material sampled was approximately 0.15 to 0.86 g. With a typical tablet having a mass of 0.2 to 1.0 g, this quantity of material is on the correct order of magnitude to meet FDA guidelines on blend monitoring.

Wet massing of pharmaceutical ingredients, or granulation, is a commonly employed technique in pharmaceutical manufacturing. The first reports of NIR spectroscopy monitoring of this process are by Rantanen et al. [63–65] in 2000. In this work, the degree of hydration of the granules is monitored by NIR using a fiber optic probe interfaced through the side of a high-shear granulator. The authors report that a successful calibration based on four wavelengths was employed. Such a method is predicated upon the strong absorption of water in the NIR region that was used to demonstrate the ability to monitor granulation during the process.

A subsequent report by Jorgensen et al. [66] followed granulation in a similar manner, excepting that the full NIR spectrum was utilized to detect the change in moisture content of the granules and the formation of a crystalline hydrate. A fiber optic probe interfaced through the side of the granulator was used to obtain the data. This interface is common for all of the granulation monitoring applications.

In 2003, Otsuka et al. [67] reported direct determination of granule size during the granulation process by NIR spectroscopy. This was an example of directly determining the quality attribute of the product that is a function of the specific unit operation being measured. Alteration of crystalline habit of a proprietary molecule during wet granulation was reported by Li et al. [35] in 2005. Blanco et al. [36] later reported the modification of the crystalline habit of dexketoprofen trometamol during wet granulation. The work by Li and Blanco illustrates the versatility of NIR as these methods could be almost as valuable in an off-line application as they are when applied on-line. However, the ability to determine the crystalline state of the API on-line has good potential to assist formulation development by providing information on the state of the molecule as it progresses through the unit operation.

Roller compaction is another frequently employed means of mixing and consolidating pharmaceutical ingredients prior to tabletting. The ability to follow this process by NIR spectroscopy was demonstrated in an off-line application by Miller [68] in 2000. Gupta et al. [69] demonstrated the ability of NIR to monitor roller compaction on-line in 2004. In this study, a fiber optic probe from a diode-array NIR was suspended above a ribbon as it exited a roller compactor. Spectra were processed to predict ribbon strength and particle size of the granules following milling. In 2005, the

ability to predict moisture content, compact density, tensile strength, and Young's modulus of roller compacted blends was demonstrated by Gupta et al. [70]. In a subsequent study, NIR was used to establish the effect of ambient moisture on the compaction behavior (during roller compaction) of microcrystalline cellulose [71]. In this application, the properties of the roller compaction ribbon were monitored in real time. It is the type of data that can be critical to control the process and consequently the success of a PAT application. Recently, Miller et al. [72] reported the application of these techniques to monitor the scale-up of a roller compaction process. This work demonstrates another important potential function of NIR in the pharmaceutical manufacturing arena: the ability to monitor a process for a given parameter at multiple scales. This capacity is not limited to roller compaction and has been demonstrated in other unit operations.

Determination of properties of film coated tablets was demonstrated by Kirsch and Drennen [73] in 1995. Here, the thickness of an ethylcellulose coating, T_{50} (50% dissolution time), and the hardness of the tablet were determined from NIR spectra measured on grating based and acousto-optic tunable filter (AOTF) spectrometers. While the performance of the grating based spectrometer was superior, the AOTF was demonstrated to perform adequately for most pharmaceutical applications and had the advantage of substantially shorter measurement times. This demonstration of the capacity for rapid tablet analysis was a critical step towards the implementation of such systems in an in-line tablet analysis system.

Further demonstrations of this potential for NIR were presented by several other researchers [52,74–78]. In 2000, Andersson et al. [79] demonstrated an in-line system for following the process of coating of tablet cores by placing a NIR-fiber optic probe inside a pan coater. It was noted that the calibration for determination of coating thickness was not only a function of the increasing signal due to the coating, but also the disappearance of signal from the core. Perez-Ramose et al. [80] utilized a similar measurement configuration and NIR monitoring of the process by a single wavelength to develop a model for the film growth on tablets.

The current literature has shown the capability NIR to monitor blending, granulation, and coating during the manufacturing process. Advances in the on-/in-line use of NIR frequently rely on characterization of in-process materials and finished dosage forms through other analytical techniques. On-line applications demonstrate the analytical potential of NIR as a sensor in pharmaceutical manufacturing; they allow the rapid chemical and physical characterization of materials during unit operations. Appropriate understanding of the product during manufacturing allows control of the production process that ultimately improves the quality of the pharmaceutical product. Briefly, NIR is a critical enabling technology for PAT.

30.6 ANALYSIS OF INTACT DOSAGE FORMS

In most cases, an entire batch of powder blend or granulation is encapsulated or tableted, then a random sample of capsules or tablets drawn and analyzed prior to final release. Failure of a sample often means rework or disposal of the entire batch. Characterization of intact dosage forms by NIR is a significant advantage because it offers the potential for on-line or at-line qualification of dosage forms. Loss of batches could be avoided, because problems could be detected and addressed immediately.

Consider the potential advantage of real-time monitoring of the tabletting process. A production of a batch of 2.5 million tablets may be sold at retail for several dollars per tablet, not an unusual price today. Assume the granulation segregates in the hopper feeding a high-speed tablet press. Without NIR, it could be days before the LC data is returned, long after the production run is complete, and the lot of tablets would have to be discarded or reworked (if it is even detected). If the process had been monitored by NIR, the first unusual tablets produced as a result of segregation of the granulation would have been recognized and the process shut down, allowing the granulation to be remixed and the lot ($7.5 million in retail value) saved.

Furthermore, a single NIR scan of one tablet, collected in a matter of seconds or even fractions of a second, may be used to qualify or quantify numerous tablet properties. NIR has shown value for monitoring drug and excipient concentrations, hardness, moisture content, dissolution rate, degradants, and so forth. Pattern recognition algorithms or other methods for qualitative analysis allow one yes/no decision to determine if a tablet meets all specifications of interest: the tablet is acceptable or the tablet is unacceptable. Given the increasing demand for correlation to a rapidly expanding list of tablet parameters, NIR is frequently a method of choice for its speed of analysis and richness of the data [1]. Because of the rapid and nondestructive nature of the method, a larger sample of a production lot can be analyzed, giving more statistical confidence to our acceptance or rejection of a lot.

The first report of NIR applied to the analysis of intact dosage forms came as a direct result of the deaths caused by cyanide-laced capsules in the early and middle 1980s. Following these poisonings, the Food and Drug Administration analyzed two million capsules by a variety of methods. In 1987, Lodder et al. [81] published a landmark paper in which intact capsules were analyzed by NIR. In this study, the quantile-BEAST cluster-analysis algorithm was used in the analysis of adulterated and unadulterated capsules.

A reproducible positioning system is critical to sampling intact capsules. To achieve this, investigators used a plastic blister glued to the center of an elliptical polished aluminum reflector. Much of the signal returned to the detector by this elliptical reflector was due to specular reflectance, revealing little about the sample. A new reflector was developed using a 90° conical reflector machined from a block of aluminum. This conical reflector minimized specular reflectance and maximized diffuse reflectance from the sample. A comparative study of the two sample configurations revealed that when the ratio of distance between spectra of dissimilar capsules to distance between spectra of similar capsules was calculated, it was nearly three times greater for the conical reflector than the elliptical reflector.

Spectra of 10 to 13 unadulterated capsules were collected at 18 wavelengths with an InfraAlyzer 400 and used as a training set. The model was tested with an equal number of unadulterated capsules. Potassium and sodium cyanide, aluminum shavings, arsenic trioxide, and other contaminants were incorporated into acetaminophen capsules and used to test the model. All the adulterated capsules were easily differentiated from unadulterated capsules, even with as few as two wavelengths.

Both capsule color and positioning of the adulterant affected the NIR analysis. The relative position of the adulterant in the capsule was predictable by the NIR. Many of the capsules studied had a white end and a colored end; the white ends of the capsules caused more light-scattering and a lower signal when oriented toward the light source, giving a first indication of the significance of sample positioning in NIR analysis of intact dosage forms.

A calibration was established for a quantile-BEAST determination of capsule KCN content. A detection limit of 2.6 mg of KCN was established in acetaminophen capsules whose average mass was 670 mg (<0.4% of the capsule weight). Nine milligrams of KCN added to an intact capsule caused the capsule to be classified as an outlier, nearly six SDs from the center of the training group.

The next paper, by Lodder and Hieftje [82], discussed NIR analysis of intact tablets. The sample cell described earlier was modified, with a smaller right circular cone at the vertex of the main cone and oriented in the opposite direction. This insert illuminated the side of the tablet away from the light source.

Commercially available aspirin tablets from two manufacturers were analyzed at 18 wavelengths using the modified sample apparatus and two other configurations. Data treatment involved principal component analysis followed by discriminant analysis with the quantile-BEAST algorithm. Cluster separation was greatest for the spectra collected with the modified sample cell and least for tablets which had been powdered and placed in a traditional sample cup. The single-reflecting cone results were slightly better than those of the powdered samples.

To further examine the utility of the double-reflecting cone, a hole was drilled in the side of a tablet and packed with 10 mg KCN. When scanned using a single reflecting cone, the sample fell into

the cluster with the training set (unadulterated) tablets. When the double-reflecting cone was used, the tablet fell well outside the unadulterated tablet cluster, revealing the value of the double-reflecting cone over the single-cone configuration.

Continuing the analysis of intact dosage forms with NIR and the quantile-BEAST algorithm, Lodder and Hieftje published an article using the technique for the quantitative and qualitative characterization of capsules with low concentrations of contaminants. Using quantile–quantile (QQ) plots, detection of subpopulations in NIR spectral clusters was possible. These subpopulations were defined as samples whose distance from the center of the training group was less than three SDs.

Ten to thirteen APAP capsules, from which the contents were removed and repacked (to minimize variation between training and test sample handling), were used for the training set and an equal number used to validate the model. Contaminated samples were produced by adding aluminum shavings (average of 208 mg per capsule) or floor sweepings (average of 221 ppm per capsule) to the capsules. Incorporating these data into QQ plots, the investigators found that the correlation coefficients for the plots containing the contaminated samples fell below the confidence level established by the QQ plots of the unadulterated training and test sets. Detection of trace contaminants was possible by this method with as few as one or two wavelengths.

In a paper published the same year, Jensen et al. [83] used NIR in the analysis of amiodarone tablets. Before spectral collection, the film-coated surfaces of the tablets were scraped off and the tablets glued to an anodized aluminum plate. In this study, the authors suggested that the interference of the film coating necessitated its removal prior to analysis. The spectrum of the pure drug was obtained to determine wavelengths of drug absorbance and was compared to the tablet spectra. Six wavelengths were chosen for the calibration. To develop the calibration, tablets ranging from 47 to 63% active ingredient were prepared in increments of 2%. The calibration provided an $r^2 = .996$ and SEE = 0.45.

Reproducibility of the method was determined by analyzing a group of tablets with the same concentration of active ingredient. Samples were analyzed on a variety of sample backings and at three storage conditions (room temperature, 40°C, and room temperature with storage in a desiccator). Although the results were slightly less variable for samples stored at 40°C, NIR prediction for samples stored under all three conditions was virtually identical. Subsequent reports demonstrated the lack of necessity of removal of a tablet film coat prior to NIR analysis. An early demonstration of analysis through a tablet coating was reported by Kirsch and Drennen [73].

An investigation into the determination of degradation products by NIR was published in 1990 by Drennen and Lodder [84]. The major degradation process in aspirin tablets is the hydrolysis of aspirin to salicylic acid. One of two USP methods must be performed to verify tablet aspirin content and both are time-consuming. A second analysis by HPLC must be performed to verify that salicylic acid levels do not exceed 0.3% of the tablet mass.

In this study, tablets were stored in a hydrator for up to 168 h with tablets withdrawn at regular intervals. After removal from the hydrator, the tablets were weighed and NIR spectra collected prior to the HPLC analysis. Spectra of the intact tablets were collected on an InfraAlyzer 500 in the 1100 to 2500 nm region, using the double-reflecting sample apparatus described by Lodder and Hieftje [82]. The spectra were processed by principal component analysis, and the scores analyzed by the quantile-BEAST algorithm.

The study had three objectives: first, changes in NIR spectra were correlated to the time aspirin tablets spent in the hydrator (the calibration had a correlation coefficient of 0.95 and SEE of 18.8 h); second, a calibration was developed for the prediction of tablet salicylic acid content (the researchers ensured that prediction of salicylic acid was based on changes in salicylic acid concentration, and not some related process, such as absorption of moisture, by evaluation of loadings spectra from principal component analysis of the data. The HPLC-determined salicylic acid levels ranged from 0.36 to 1.66 mg, and the NIR method allowed prediction of the degradant with a standard error of 144 mg); and third, the mass of water absorbed by the tablets was determined by NIR spectroscopy.

Given the strong absorbance of water in the NIR region, a correlation between NIR spectra and water absorbed is not surprising. However, the amount of water absorbed by the tablets, determined by weighing the tablets on an analytical balance, was <2.5 mg for all samples. In this study, the amount of water absorbed was predicted with a standard error of 163 μg. Thus, even very small changes in dosage form moisture content can be detected by this method.

Two book chapters discussing NIR analysis of tablets were published in 1991. The first was from work presented at the 4th International Conference on Near Infrared Spectroscopy [85]. In this paper, Stark used a newly developed diode array spectrometer which scanned the 520 to 1800 nm region, useful in the analysis of macro- and micro-specimens. Samples were placed on a glass slide prior to analysis, maintained at an angle to the probe. With this configuration, light reflected by the sample was detected, but light reflected by the glass was not. Spectra were collected from a 1 mg sample of acetaminophen powder. The sample was subsequently divided into 500, 250, and 125 mg; reasonable spectra were collected from all these samples. Diffuse reflectance spectra of intact acetaminophen, ibuprofen, and antacid tablets were successfully collected. No comparative studies or quantitative or qualitative analyses of the spectra were performed.

Monfre and DeThomas [86] published a chapter describing a NIR calibration for QC monitoring of a marketed vasodilator. The NIR analysis was performed on a NIRSystems Model 6500, using an aperture plate to assist in tablet positioning. For the calibration, individual tablets were crushed to a fine powder and scanned. Second derivative spectra were used in the analysis to minimize the light scattering differences between the samples. A bias correction was introduced to factor in the scattering efficiency of the tablets vs. the powders. Because the excipient concentrations were not constant, a drug absorbance wavelength was ratioed by an absorbance wavelength primarily due to the formulation matrix, and these normalized values were then used for the calibration.

HPLC was used as the reference method and the tablets were found to vary between 96 and 102% of labeled strength. To determine the accuracy and precision of the method, one tablet was analyzed ten times with sample removal and replacement between scans. The NIR-determined value was within 0.5 mg of the HPLC-determined value, indicating the accuracy of the method. Tablet placement on the spectrometer played an important role in the precision of the method, even with the use of the aperture plate.

An increasing rate of publications on the determination of properties of finished dosage forms followed this initial work; between 1995 and 2005 more than 100 papers were published on this subject. Early work was concerned with the comparison of transmission and reflectance. An example of this is found in Gottfries et al. [87] from 1996, where transmission measurements were found to have a lower RMSEP (1.06 vs. 2.83) than reflection measurements for the determination of metoprolol succinate. Merckle and Kovar [88] compared transmission and reflection measurements of acetylsalicylic acid formulations and found both performed adequately without a significant difference between them. A comparison by Thosar et al. [89] indicated an advantage in using transmission, but noted that both methods demonstrated performance that was suitable to task. Cogdill et al. [90] indicated that while both reflection and transmission were suitable for determination of API in tablets in a system designed for in-line application, reflection was demonstrated to be much less sensitive to sample position.

The late 1990s represented a significant maturation of the use of NIR for tablet analysis, a summary of the analytes, ranges and NIR mode used is found in Table 30.1. Many researchers published accounts of successfully calibrating for tablet properties by NIR analysis [89,91–108]. Ebube et al. [109] reported an analysis of magnesium stearate at concentrations down to 0.25% in compacts of microcrystalline cellulose. Gustafsson et al. [110] reported the use of IR and NIR for the determination of compact density, particle shape, tablet axial tensile strength, and drug release characteristics. This work is an example of combining multiple spectral ranges (IR and NIR) and process data (compaction behavior) to create comprehensive models for tablet performance (ideas well aligned with PAT).

TABLE 30.1
Reported NIR Calibrations of Pharmaceutical Analytes and the Associated Sampling Method for Each Method

Analyte	Range (or lowest level studied, % w/w)	Sampling	Reference
Potassium cyanide (in acetaminophen capsules)	0.4	Reflection with a conical reflector accessory	[81]
Potassium cyanide (in aspirin tablets)	2	Reflection with a double reflecting conical accessory	[82]
Aluminum shavings	31	Reflection with a double reflecting conical accessory	[114]
Floor sweepings	33	Reflection with a double reflecting conical accessory	[114]
Amiodarone	47–63	Reflection (tablet coating removed)	[83]
Salicylic acid in Aspirin tablets	0.07–0.3	Reflection with a double reflecting conical accessory	[84]
Metoprolol	20–25	Transmission and reflection	[87]
Aspirin	7.0–21	Transmission and reflection	[88]
Paracetamol	76–93	Reflection	[91]
Magnesium stearate	0.25–2.0	Reflection	[109]
Paracetamol	76–93	Reflection	[111]
Gemfibrozil	67–89	Reflection	[93]
Steroid (Proprietary)	2.9–18	Transmission	[94]
Theophylline	1–40	Transmission and reflection	[89]
Ibuprofen	49–90	Transmission	[95]
Caffeine	13.7	Reflection	[98]
Water	1.74–5.32	Transmission	[99]
Sulfamethazine	60	Reflection	[100]
Bromazepam	0.60–3.9	Reflection	[101]
Clonazepam	1.4–2.6	Reflection	[101]
Paracetamol	45	Reflection	[102]
Amantadine hydrochloride	17	Reflection	[102]
Cimetidine	66–86	Reflection	[103]
Aminopyrine	28	Reflection	[104]
Phenacetin	28	Reflection	[104]
Roxithromycin	19.5	Reflection	[105]
Erythromycin	28.1	Reflection	[105]
Riboflavin	0.41–2.3	Transmission	[106]
Ibuprofen	0.7	Transmission	[107]
Mirtazapine	5.5–14.5	Reflection	[108]

Research through the 1990s dealt primarily with demonstration of the ability of NIR to determine different tablet properties in a regulatory environment (validation). An account of a NIR analytical methods validation by Moffat et al. [111] was published in 2000. The target of this investigation was to demonstrate that a NIR analytical method met the criteria established by ICH Q2 [112,113]. The accuracy, precision, specificity, detection limit, quantification limit, linearity, range, robustness, and system suitability testing were demonstrated to be appropriate for use in routine testing. While this type of validation is based upon the needs of other analytical techniques, it served as an example of the validation of a NIR-based method for tablet analysis. Other researchers followed in validating NIR analyses of tablets for use in release testing [95–98].

Prediction of the dissolution rate of tablets is another area of application of NIR spectroscopy. Carbamazepine is used for the treatment of epilepsy and consistent dissolution of the dosage form is critical in the maintenance of therapeutic blood levels of drug. In a 1991 study by Zannikos et al. [115], dissolution profiles of brand-name and generic carbamazepine tablets were compared after storage in conditions of high humidity. The calibration was based upon the percentage of drug in solution after 1 h in a USP Dissolution Apparatus II.

Spectra of intact tablets were collected on an InfraAlyzer 500, after which the dissolution rates of the tablets were determined. The NIR spectra and dissolution profiles of the generic tablets changed little during the 5 days of high-humidity storage, but the spectra and dissolution profiles of the brand-name tablets changed significantly. A calibration based upon principal component analysis followed by the bootstrap algorithm was developed for the brand-name tablets. The R was .99, and the SEP for extent of dissolution after 1 h was 6.8%.

Further storage of the tablets did not affect the dissolution profiles or the NIR spectra of the tablets appreciably, but after 5 days other absorbance peaks began to appear in the NIR spectra, which were attributed to degradation products in the tablet. The authors surmised that the peaks could have resulted from a chemical or physical change that altered the dissolution rate.

Drennen and Lodder [116] published a paper in 1991 comparing the performance of the improved quantile-BEAST algorithm with that of the Mahalanobis distance in the qualitative analysis of carbamazepine tablets. While the Mahalanobis distance calculation assumes that spectral variations associated with both the calibration and test set are random, in complex pharmaceutical mixtures this may not be the case. The bootstrap algorithm, on the other hand, is a nonparametric test which can be used with nearly any spectral data distribution.

In this study, the dissolution profiles of carbamazepine tablets exposed to conditions of high humidity were classified according to the Mahalanobis distance calculation and the bootstrap method using both full (701 wavelength) and principal component spectra collected on an InfraAlyzer 500. This was the first report of the use of full spectra and required substantial computing power relative to readily accessible systems of the time. In multiple tests, the bootstrap calculation was shown to provide more accurate qualitative results than the Mahalanobis calculation. In one experiment, nine tablets with a slow dissolution rate were used as a training set. Twenty-one tablets with a variety of dissolution rates were used to test the model. The modified bootstrap calculation correctly identified all tablets with a faster dissolution rate than the training set, while the Mahalanobis calculation incorrectly identified 58% of the tablets with a higher dissolution rate. The quantile-BEAST algorithm gave better precision, accuracy, and speed than the Mahalanobis calculation in nearly all cases.

Recent reports of prediction of dissolution behavior are focused on prediction of specific, release testing criteria. Tatavarti Aditya et al. [100] have reported prediction of the quantity of drug released at 120 min (Q120). Donoso and Ghaly [117] and Freitas et al. [118] developed models to predict the behavior of the tablet in specific pH buffer at a specific time. Each of these authors developed individual calibration for the quantity of drug released at several times for each of 3 pH conditions for dissolution testing. Donoso [119] also reported using NIR as a means of predicting the disintegration time for a tablet.

30.7 HARDNESS

In a 1993 review of pharmaceutical applications of NIR spectroscopy, Drennen and Lodder [120] presented new research on the prediction of tablet hardness based upon NIR spectral changes. Tablets ranging in hardness from 0.3 to 10.75 kilopons (kp) were analyzed nondestructively by NIR, then subjected to the destructive reference test. Increasing tablet hardness was found to cause an upward shift in the spectral baselines, probably due to a reduction of light scattering from the tablet. It was surmised that a harder and smoother tablet surface reduced the light scattering from the surface, allowing more light to penetrate the sample and causing increased absorbance. Tablets were easily classified according to hardness using the quantile-BEAST algorithm. Spectral changes were found

to correlate well with variations in hardness. Prediction of hardness provided SEE and SEP values of approximately 0.6 kp.

Following the initial example of the use of NIR to predict hardness, researchers have used NIR to predict hardness [121] and related properties such as tensile strength of tablets [100,110], or have demonstrated the effect of compression force on tablets [108,109].

The use of NIR in the qualification of clinical batches of tablets is an application for which this technique is well suited. When medications are dispensed for use in clinical trials, the blister packs in which they are distributed often contain tablets with a range of doses. The tablets are usually identical in appearance, making visual classification nearly impossible. Verification of the correct tablet configuration in the packaging by a noninvasive and nondestructive method would be a significant advantage in the quality control inspection of such packages.

In the first study published on this tablet analysis application, Dempster et al. [122] used three sampling configurations to investigate the classification of an experimental drug present in tablets in 2, 5, 10, and 20% concentrations, a matching placebo, and a marketed drug used as a clinical comparator. The first method of tablet analysis required the removal of the tablets from the blister packs and scanning them directly through the spectrometer window. In the second approach, the tablets were scanned through the plastic packaging using the spectrometer window. With the third arrangement, the tablets were analyzed through the plastic blister packaging with a fiber-optic probe.

A NIRSystems Model 6500 was employed in the analysis with a ceramic disk used as the reference. Second-derivative spectra were used in the data analysis. The identification and classification algorithms used were supplied by the instrument vendor. In the first configuration, all but the 2% tablets were easily classified. The 2% tablets were not be differentiated from the placebo. Using the second and third configurations, only the 10 and 20%, placebo and clinical comparator tablets could be properly classified.

This lack of ability to identify and classify the range of dosages in the clinical batch indicates the need for further research in this area. Substituting another plastic for the white opaque blister packaging used may have improved the results of the analysis, since this packaging would be expected to be an excellent light scatterer, decreasing the signal of the tablet within the blister packaging.

The second application of NIR spectroscopy in the analysis of intact tablets from clinical batches was published in a 1994 paper by Aldridge et al. [123]. A NIRSystems Model 6500 with a custom sampling configuration was used for spectral collection of the blister-packed samples, and the second derivative spectra were used in the analysis. Spectralon™ was used for reference. Although certain peaks in the NIR spectra were attributed to the hydrocarbon functionality of the packaging material, the spectral features of the tablets within were clearly visible.

The Mahalanobis distance calculation was used for discriminant analysis. These distances were plotted in a control chart, revealing that several samples were in danger of being misclassified based upon the Mahalanobis distance calculation alone. When the residual ratios of the spectra were calculated and plotted in a control chart, however, the probability of misclassifying a sample was greatly diminished.

In a paper by Kirsch and Drennen [73], intact theophylline tablets coated with an ethylcellulose polymer were analyzed by grating-based and AOTF spectrometers. The purpose of the work was threefold. First, tablets were coated with increasing levels of ethylcellulose to vary the dissolution profiles of the dosage forms. After NIR spectra were collected, the tablets were subjected to dissolution in a USPDA II. The time required for 50% of the drug to enter solution was used as the measure of dissolution rate. Principal component regression was used to develop the calibration. The calibration provided a SEE of 2.8 min, a coefficient of determination of .977, and an SEP of 6.6 min, with time to 50% dissolution values ranging from 48 to 93 min.

Second, the potential of this method in the monitoring of the film-coating process was investigated. Tablets coated with 2 to 7% ethylcellulose were used to determine the utility of this method in the prediction of film coat thickness. NIR spectral changes were found to correlate well with film thickness. Using the first principal component, a SEE of 0.0002 in. was obtained, for coating

thicknesses ranging from 0.001 to 0.003 in. The use of NIR spectroscopy as a means of on-line evaluation of film-coating is currently under investigation in this laboratory.

The third experiment was a NIR-based determination of the hardness of coated tablets. Thirty-eight tablets whose hardness ranged from 6 to 12 kp were first analyzed spectroscopically, and then by the destructive reference method. Prediction of hardness with a standard error of 0.6 kp was possible even after removal of the baseline shifting with MSC. This research confirmed the results of hardness studies reported earlier by Drennen and Lodder [120]. Further research is being conducted to characterize this phenomenon.

In another paper by Lodder et al. [124], the qualification of a number of tablet characteristics was performed in a comparative study of two classification algorithms: soft independent modeling of class analogies (SIMCA) and the quantile-BEAST. The study involved qualitative classification of tablet hardness, moisture content, dissolution rate, and degradant concentration.

An evaluation of the performance of these algorithms in predictions using inside model space and outside model space was conducted. In principal component regression, principal axes highly correlated with sample constituents of interest are considered to be inside model space, while axes typically attributed to spectral noise are termed outside model space.

SIMCA provided highly variable results, occasionally offering optimum performance with outside model space, while the quantile-BEAST gave better results overall and more consistent prediction. The best results were obtained when the quantile-BEAST algorithm was used with full spectra, with no principal axis transformation prior to analysis.

In a later work, Cogdill, Anderson, and Drennen [125] demonstrated the use of Hotelling's T^2 and spectral residuals (Q_{res}), and proposed wavelength uncertainty and spectral noise tests to demonstrate the suitability of the measurement for prediction of a concentration. Hotelling's T^2 and Q_{res} were used to verify the overall suitability of the prediction of the API concentration. Factors influencing these two metrics include the interaction between the model and sample changes, sample interface changes, and spectrometer performance. The remaining two metrics were utilized as indicators of spectrometer performance and were not related to the model used to predict API concentration or hardness.

30.8 CONSIDERATIONS FOR INTACT DOSAGE FORM ANALYSIS

Numerous styles and brands of instruments and sample cells have been used for the analysis of tablets. The authors currently use several brands of instrumentation for tablet analysis, including filter based, diffraction-grating based, and AOTF-based instrumentation. Detector configurations for tablet analysis are evolving slowly towards an optimum design; however, the standard designs offered by most instrument manufacturers are suitable. Tablets have been successfully analyzed with integrating spheres and with a standard dual-angled detector configuration. Intact tablets are analyzed in both diffuse reflectance and transmission modes.

The first analyses of individual intact tablets and capsules involved the use of reflective aluminum sample cells, designed specifically for tablets [82] or capsules [81], that allowed illumination of all sample surfaces. Illumination of all sample surfaces has proven to offer enhanced sensitivity. The authors now prefer the latest configuration of the original sample cell, the CAPCELL™ (Optical Prototypes, Inc., Natrona Heights, PA 15065), for analysis of individual intact tablets and capsules.

Sample positioning variability is the single largest source of error in NIR analysis of tablets, regardless of whether diffuse reflectance or transmission measurements are used. Hardware, methodology, and mathematics may be used to reduce this error. Tablet-specific sample cells that allow consistent positioning of tablets are valuable in reducing this error. A method involving the collection of three spectra per tablet, with 120° rotation of the sample or the sample cell/sample combination between spectra, is used by the authors. Using the mean (or median) spectrum for each tablet

significantly reduces the spectral variability by averaging out the positioning effects. The median calculation results in less weighting by particularly odd spectra than does the mean.

The individual tablet spectra must then always be corrected for baseline shifting prior to analysis. Many techniques have been attempted, but the second derivative and MSC calculations are most common. This baseline correction is critical even if an average tablet spectrum is used.

Curved surfaces, debossing, and scoring are factors that affect the spectrum of a tablet as positioning is varied, but the spectral effects of such factors can be reduced by the methods just discussed. Natural variations in tablet mass and hardness will affect a tablet's spectrum, primarily through spectral baseline shifting. Some work by Baxter [126] involved a unique method of normalizing tablet weight variations.

Baxter concluded that because NIR spectra are "in essence a picture of active per unit area" and do not allow detection of differences in tablet weight, reference assay values should be normalized for tablet weight, multiplying the HPLC reference value by the theoretical tablet weight and dividing by the actual tablet weight. Values predicted from this calibration must then be denormalized by multiplying the NIR predicted value by the actual tablet weight, divided by the theoretical weight. Baxter observed a reduction in residual values from 2.17 to 1.57% for 228 tablets for which active ingredient concentrations were predicted.

In 1997, Candolfi et al. [41] studied the sources of variance in NIR measurements of tablets and capsules. The sources of variation studied were measurement repeatability, sample positioning, day-to-day variability, object-to-object variability within a batch, and batch-to-batch variability. For tablets, positioning and time between measurements were the least significant factors; for capsules, sample positioning and time contributed the most to the variability of the spectra. Further remarks indicate that the researchers doubt that the contents of the gelatin capsules were probed. This work gave one of the early indications of the importance of sample positioning that remains a central issue to the present.

Researchers validating or performing tests claiming to validate analytical methods based upon the chemometric analysis of NIR data must take care to establish the complete independence of the validation samples. Take, for example, a data set of eight spectra collected from each of 20 tablets sampled from 10 batches. Setting aside one of the eight spectra collected from each tablet does not constitute an independent validation of the method; similarly, setting aside half of the tablets from each batch does not constitute an independent validation set. Even though the number of batches is limited, the only independent validation set available is the removal of a number of batches from the training and testing set to the validation set. From a compliance perspective, once a batch of samples have been used to validate the method, changes to the method (particularly those based upon the results of testing the validation set) render those samples not longer suitable for validation. In this instance, testing to demonstrate validation will require a new set of samples from which the data must be collected.

30.9 NEAR-INFRARED IMAGING

Hyperspectral imaging (or NIR imaging) increases the information density of NIR spectroscopy by combining spectra with spatial information of the locations from which the spectra originate. NIR as a single-point measurement has been demonstrated to be a robust technique for analysis of pharmaceutical ingredients; however, it lacks any spatial information beyond the volume of the sample represented by the spectrum. In hyperspectral imaging, the spatial domain is much like the field of view for a photograph; the difference is that instead of a color or intensity at each point on the image an entire NIR spectrum is captured for that point (or pixel). NIR imaging spectroscopy is not a new technique application and examples have been reported in the mid-1990s [127,128], however, it has found new interest in the wake of the FDA's PAT initiative [1].

The role of hyperspectral imaging in pharmaceutical manufacturing was discussed by Clarke et al. [129–131]. In these reports, it is suggested that the combination of chemical and spatial information provided by NIR imaging can greatly enhance the knowledge of the product and the process that has produced it. A subsequent paper by Clarke et al. [132] described the depth of penetration in terms of the thickness of penetration at which 50% of the substrate signal has been attenuated by the layers of cellulose placed on top of it. The values for this condition varied with wavelength and per sample. At 2380 nm, the 50% attenuation was approximately 27 μm; at 1675 nm, 39 to 61 μm; and at 1100 nm, ca. 180 μm.

Lyon et al. [133] reported on the value of NIR imaging for trouble-shooting formulations and pharmaceutical processes by careful analysis of the images. In this work, the importance of image analysis techniques is emphasized as a means of utilizing the data for purposes beyond intuitive visual inspection. The use of histograms to describe populations within an image is a key tool to gain maximum knowledge from images. Further discussions of the application of imaging to solid-dosage processes [8,134]; wherein LaPlant and Lodder focused on the data processing requirements of imaging spectroscopy.

Hyperspectral imaging of freeze-dried solid-dosage formulations have been recently published [135]. The ability to characterize both the morphological and chemical properties of a freeze-dried formulation is a critical element to the complete description the dosage forms studied.

30.10 CONCLUSIONS

Tremendous advances have been made recently in the use of NIR spectroscopy for the analysis of pharmaceutical dosage forms. Just 25 years ago, NIR spectroscopy was used in a way that offered relatively few advantages over other analytical methods for the analysis of dosage form drug content, requiring extractions with organic solvents prior to sample analysis. With advances in instrumentation, software, and sample handling, rapid characterization of intact dosage forms has become a reality. The pharmaceutical industry is beginning to implement NIR methods to monitor many phases of the manufacturing process, from the arrival of bulk raw material at the loading dock, to the inspection of tablets for final release.

Myths about the "black box" nature of this method have been debunked, and as those involved in analytical methods development, process control, and quality assurance acquire a more thorough understanding of NIR spectroscopy and its capabilities, pharmaceutical applications will become even more widespread. NIR instruments are becoming faster, smaller, and less expensive, increasing their potential for application as process monitors in many phases of the manufacturing process. Similarly, increased computer storage and power are becoming rapidly cheaper. Pharmaceutical manufacturers are under increasing pressure to validate their processes and to provide extensive documentation of ongoing validation activities. NIR has proven itself to be a rapid and rugged analytical method capable of continuous on-line process monitoring, making it a valuable method to couple with ongoing validation activities.

The FDA has recognized the value of NIR spectroscopy, and has already approved the method for the analysis of lincomycin in a veterinary product [136]. More recently, NIR spectroscopy has been employed in the qualification of incoming raw materials, and the FDA is working with pharmaceutical manufacturers to develop and implement other NIR-based methods, with several having been approved.

In many ways, NIR spectroscopy is an ideal method for pharmaceutical process control, particularly for the analysis of intact dosage forms. As production costs, including analytical expenses, continue to increase, the advantages of NIR spectroscopy will become more attractive. With NIR spectroscopy, the pharmaceutical industry will move one step closer to "zero-defect" quality control, making the costs associated with the method's development well spent.

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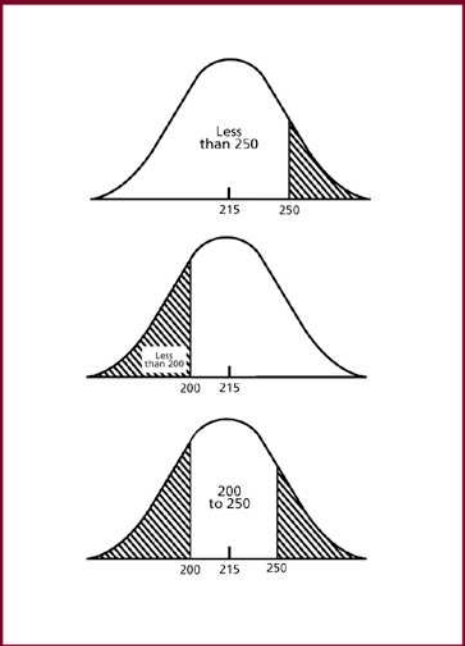
Pharmaceutical Statistics

Practical and Clinical Applications

Fourth Edition, Revised and Expanded



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(e.g., defects) within at most 1% of the true value. (The least precise estimate occurs when $p = 0.5$.)

3.4 CONTINUOUS DATA DISTRIBUTIONS

Another view of probability concerns continuous data such as tablet dissolution time. The probability that any single tablet will have a particular specified dissolution result is 0, because the number of possible outcomes for continuous data is infinite. Probability can be conceived as the ratio of the number of times that an event occurs to the total number of possible outcomes. If the total number of outcomes is infinite, the probability of any single event is zero. This concept can be confusing. If one observes a large number of dissolution results, such as time to 90% dissolution, any particular observation might appear to have a finite probability of occurring. Analogous to the discussion for discrete data, could we not make an equitable bet that a result for dissolution of exactly 5 min 13 sec, for example, would be observed? The apparent contradiction is due to the fact that data which are *continuous, in theory*, appear as *discrete* data *in practice* because of the limitations of measuring instruments, as discussed in Chapter 1. For example, a sensitive clock could measure time to virtually any given precision (i.e., to small fractions of a second). It would be difficult to conceive of winning a bet that a 90% dissolution time would occur at a very specific time, where time can be measured to any specified degree of precision (e.g., 30 min 8.21683475 ⋯ sec).

With *continuous* variables, we cannot express probabilities in as simple or intuitive a fashion as was done with discrete variables. Applications of calculus are necessary to describe concepts of probability with continuous distributions. Continuous cumulative probability distributions are represented by smooth curves (Fig. 3.7) rather than the steplike function shown in Fig. 3.5B. The area under the probability distribution curve (also known as the cumulative probability density) is equal to 1 for all probability functions. Thus the area under the normal distribution curve in Fig. 3.7A is equal to 1.

3.4.1 The Normal Distribution

The normal distribution is an example of a continuous probability density function. The normal distribution is most familiar as the symmetrical, bell-shaped curve shown in Fig. 3.8. A theoretical normal distribution is a continuous probability distribution and consists of an infinite number of values. In the theoretical normal distribution, the data points extend from positive infinity to negative infinity. It is clear that scientific data from pharmaceutical experiments cannot possibly fit this definition. Nevertheless, if real data conform reasonably well with the theoretical definition of the normal curve, adequate approximations, if not very accurate estimates of probability, can be computed based on normal curve theory.

The equation for the normal distribution (normal probability density) is

$$Y = \frac{1}{\sigma\sqrt{2\pi}} e^{-(1/2)(X-\mu)^2/\sigma^2} \tag{3.13}$$

where

σ = standard deviation
μ = mean
X = value of the observation
e = base of natural logarithms, 2.718⋯
Y = ordinate of normal curve, a function of X

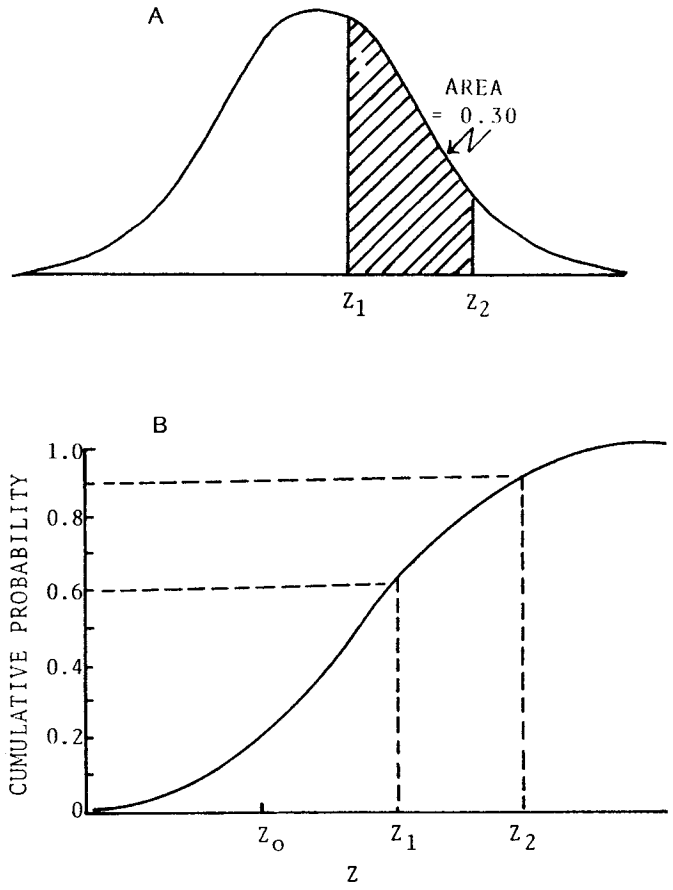

Figure 3.7 A normal distribution.

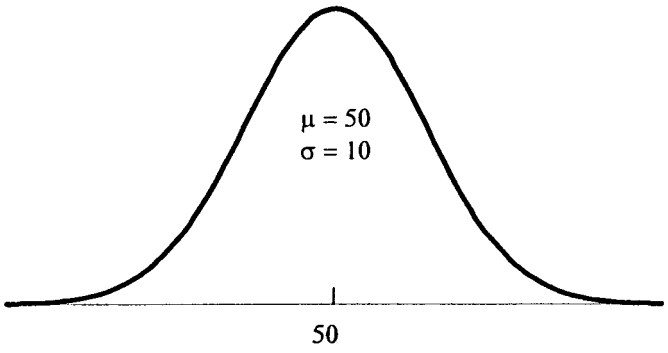

Figure 3.8 A typical normal curve.

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The normal distribution is defined by its mean, μ, and its standard deviation, σ [see Eq. (3.13)]. This means that if these two parameters of the normal distribution are known, all the properties of the distribution are known. There are any number of different normal distributions. They all have the typical symmetrical, bell-shaped appearance. They are differentiated only by their means, a measure of location, and their standard deviation, a measure of spread. The normal curve shown in Fig. 3.8 can be considered to define the distribution of the potencies of tablets in a batch of tablets. Most of the tablets have a potency close to the mean potency of 50 mg. The farther the assay values are from the mean, the fewer the number of tablets there will be with these more extreme values. As noted above, the spread or shape of the normal distribution is dependent on the standard deviation. A large standard deviation means that the spread is large. In this example, a larger s.d. means that there are more tablets far removed from the mean, perhaps far enough to be out of specifications (see Fig. 3.9).

In real-life situations, the distribution of a finite number of values often closely approximates a normal distribution. Weights of tablets taken from a single batch may be approximately normally distributed. For practical purposes, any continuous distribution can be visualized as being constructed by categorizing a large amount of data in small equilength intervals and constructing a histogram. Such a histogram can similarly be constructed for normally distributed variables.

Suppose that all the tablets from a large batch are weighed and categorized in small intervals or boxes (see Fig. 3.10). The number of tablets in each box is counted and a histogram plotted as in Fig. 3.11. As more boxes are added and the intervals made shorter, the intervals will eventually be so small that the distinction between the bars in the histogram is lost and a smooth curve results, as shown in Fig. 3.12. In this example, the histogram of tablet weights looks like a normal curve.

Areas under the normal curve represent probabilities and are obtained by appropriate integration of Eq. (3.13). In Fig. 3.7, the probability of observing a value between Z_1 and Z_2 is calculated by integrating the normal density function between Z_1 and Z_2.

This function is not easily integrated. However, tables are available that can be used to obtain the area between any two values of the variable, Z. Such an area is illustrated

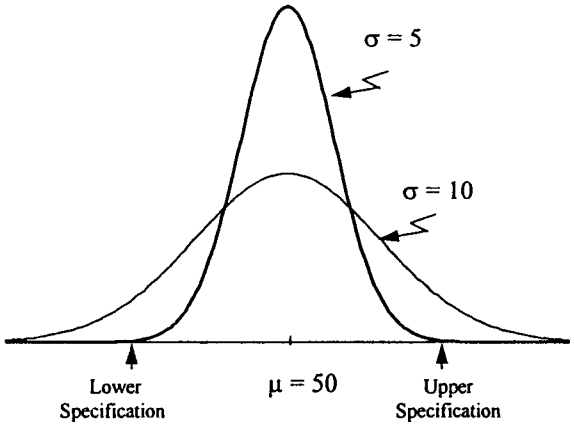

Figure 3.9 Two normal curves with different standard deviations.

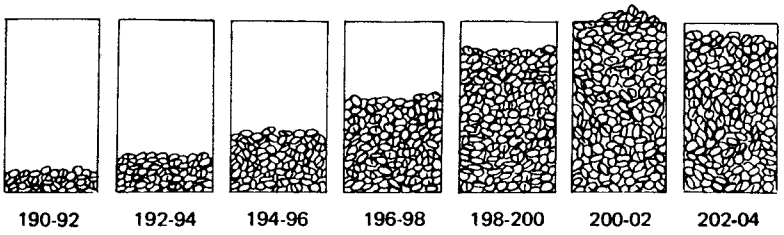

Figure 3.10 Categorization of tablets from a tablet batch by weight.

in Fig. 3.7A. If the area between Z_1 and Z_2 in Fig. 3.7 is 0.3, the probability of observing a value between Z_1 and Z_2 is 3 in 10 or 0.3. In the case of the tablet potencies, the area in a specified interval can be thought of as the proportion of tablets in the batch contained in the interval. This concept is illustrated in Fig. 3.13.

Probabilities can be determined directly from the cumulative distribution plot as shown in Fig. 3.7B (see Exercise Problem 9). The probability of observing a value below Z_1 is 0.6. Therefore, the probability of observing a value between Z_1 and Z_2 is $0.9 - 0.6 = 0.3$.

There are an infinite number of normal curves depending on μ and σ. However, the area in any interval can be calculated from tables of cumulative areas under the *standard normal curve*. The standard normal curve has a mean of 0 and a standard deviation of 1. Table IV.2 in App. IV is a table of cumulative areas under the standard normal curve, giving the area below Z (i.e., the area between $-\infty$ and Z). For example, for $Z = 1.96$, the area in Table IV.2 is 0.975. This means that 97.5% of the values comprising the

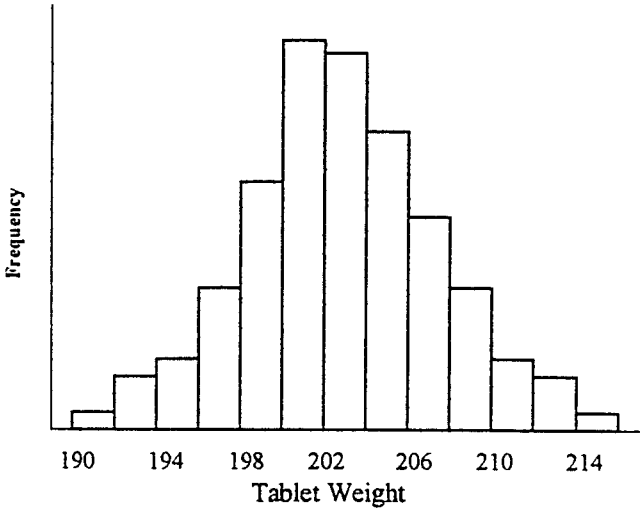

Figure 3.11 Histogram of tablet weights.

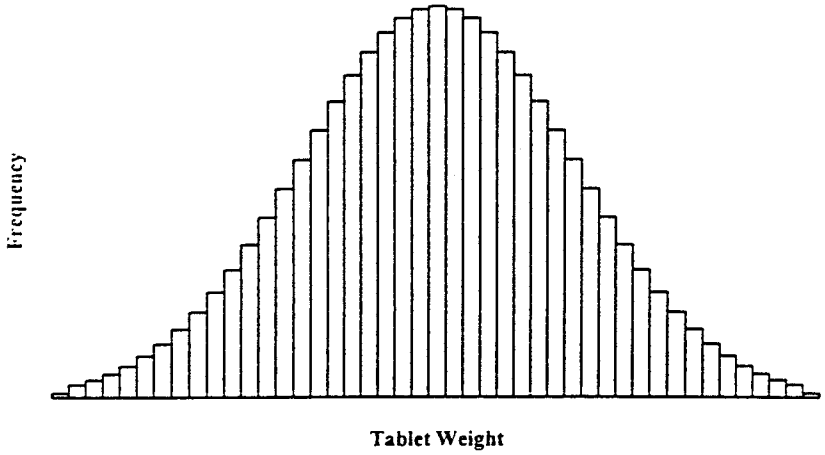

Figure 3.12 Histogram of tablet weights with small class intervals.

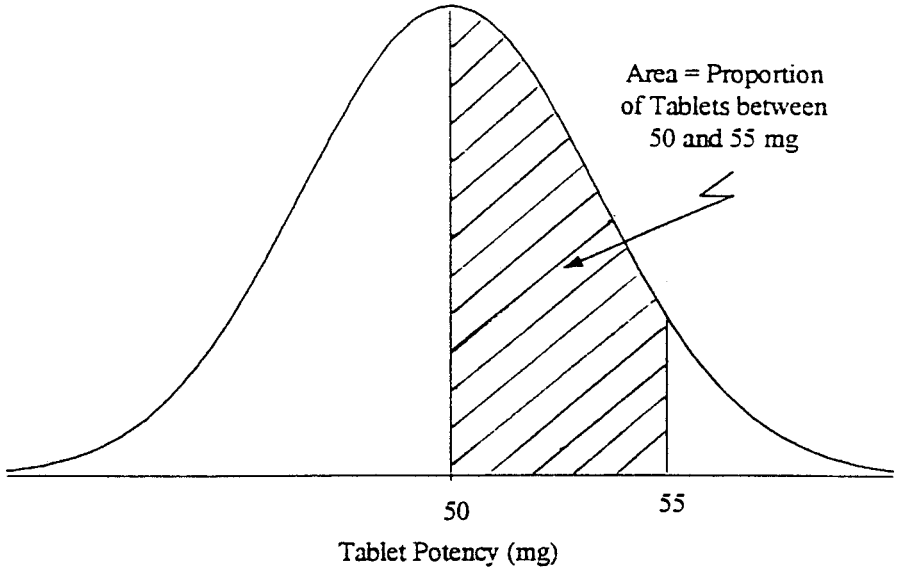

Figure 3.13 Area under normal curve as a representation of proportion of tablets in an interval.

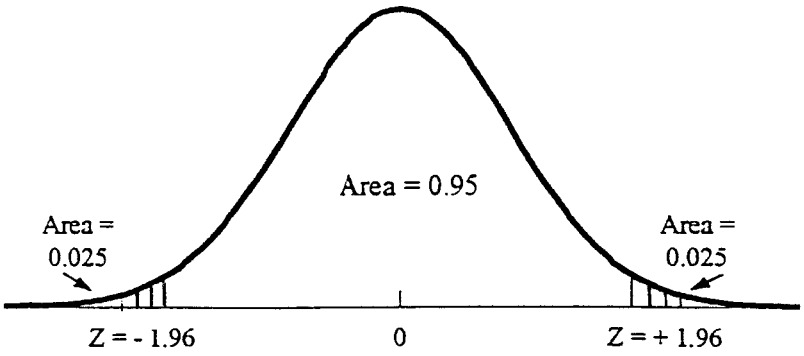

Figure 3.14 Symmetry of the normal curve.

standard normal curve are less than 1.96, lying between $-\infty$ and 1.96. The normal curve is symmetrical about its mean. Therefore, the area below -1.96 is 0.025 as depicted in Fig. 3.14. The area between Z equal to -1.96 and $+1.96$ is 0.95. Referring to Table IV.2, the area below Z equal to $+2.58$ is 0.995, and the area below $Z = -2.58$ is 0.005. Thus the area between Z equal to -2.58 and $+2.58$ is 0.99. It would be very useful for the reader to memorize the Z values and the corresponding area between $\pm Z$ as shown in Table 3.4. These values of Z are commonly used in statistical analyses and tests.

The area in any interval of a normal curve with a mean and standard deviation different from 0 and 1, respectively, can be computed from the standard normal curve table by using a transformation. The transformation changes a value from the normal curve with mean μ and standard deviation σ, to the corresponding value, Z, in the standard normal curve. The transformation is

$$Z = \frac{X - \mu}{\sigma} \tag{3.14}$$

The area (probability) between $-\infty$ and X (i.e., the area below X) corresponds to the value of the area below Z from the cumulative standard normal curve table. Note that

Table 3.4 Area Between $\pm Z$ for Some Commonly Used Values of Z

Z	Area between $\pm Z$
0.84	0.60
1.00	0.68
1.28	0.80
1.65	0.90
1.96	0.95
2.32	0.98
2.58	0.99

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if the normal curve which we are considering is the standard normal curve itself, the transformation results in the identity

$$Z = \frac{X - 0}{1} = X$$

Z is exactly equal to X, as expected. Effectively the transformation changes variables with a mean of μ and a standard deviation of σ to variables with a mean of 0 and a standard deviation of 1.

Suppose in the example of tablet potencies that the mean is 50 and the standard deviation is 5 mg. Given these two parameters, what proportion of tablets in the batch would be expected to have more than 58.25 mg of drug? First we calculate the transformed value, Z. Then the desired proportion (equivalent to probability) can be obtained from Table IV.2. In this example, $X = 58.25$, $\mu = 50$, and $\sigma = 5$. Referring to Eq. (3.14), we have

$$Z = \frac{X - \mu}{\sigma}$$
$$= \frac{58.25 - 50}{5} = 1.65$$

According to Table IV.2, the area between $-\infty$ and 1.65 is 0.95. This represents the probability of a tablet having 58.25 mg or less of drug. Since the question was, ''What proportion of tablets in the batch have a potency greater than 58.25 mg?'', the area above 58.25 mg is the correct answer. The area under the entire curve is 1; the area above 58.25 mg is $1 - 0.95$, equal to 0.05. This is equivalent to saying that 5% of the tablets have at least 58.25 mg (58.25 mg or more) of drug in this particular batch or distribution of tablets. This transformation is illustrated in Fig. 3.15.

One should appreciate that since the normal distribution is a perfectly symmetrical continuous distribution which extends from $-\infty$ to $+\infty$, real data never exactly fit this model. However, data from distributions reasonably similar to the normal can be treated as being normal, with the understanding that probabilities will be approximately correct. As the data are closer to normal, the probabilities will be more exact. Methods exist to

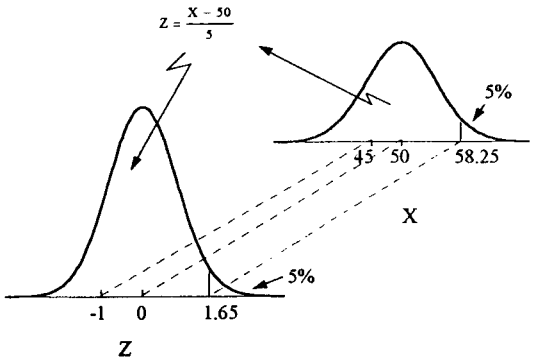

Figure 3.15 Z transformation for tablets with mean of 50 mg and s.d. of 5 mg.

test if data can reasonably be expected to be derived from a normally distributed population [1]. In this book, when applying the normal distribution to data we will either (a) assume that the data are close to normal according to previous experience or from an inspection of the data, or (b) that deviations from normality will not greatly distort the probabilities based on the normal distribution.

Several examples are presented below which further illustrate applications of the normal distribution.

Example 1: The U.S. Pharmacopia (USP) weight test for tablets states that for tablets weighing up to 100 mg, not more than 2 of 20 tablets may differ from the average weight by more than 10%, and no tablet may differ from the average weight by more than 20% [2]. To ensure that batches of a 100-mg tablet (labeled as 100 mg) will pass this test consistently, a statistician recommended that 98% of the tablets in the batch should weigh within 10% of the mean. One thousand tablets from a batch of 3,000,000 were weighed and the mean and standard deviation were calculated as 101.2 ± 3.92 mg. Before performing the official USP test, the quality control supervisor wishes to know if this batch meets the statistician's recommendation. The calculation to answer this problem can be made using areas under the standard normal curve if the tablet weights can be assumed to have a distribution that is approximately normal. For purposes of this example, the sample mean and standard deviation will be considered equal to the true batch mean and standard deviation. Although not exactly true, the sample estimates will be close to the true values when a sample as large as 1000 is used. For this large sample size, the sample estimates are very close to the true parameters. However, one should clearly understand that to compute probabilities based on areas under the normal curve, both the mean and standard deviation must be known. When these parameters are estimated from the sample statistics, other derived distributions can be used to calculate probabilities.

Figure 3.16 shows the region where tablet weights will be outside the limits, 10% from the mean ($\mu \pm 0.1\mu$), that is, 10.12 mg or more from the mean for an average tablet weight of 101.2 mg (101.2 ± 10.12 mg). The question to be answered is: What proportion

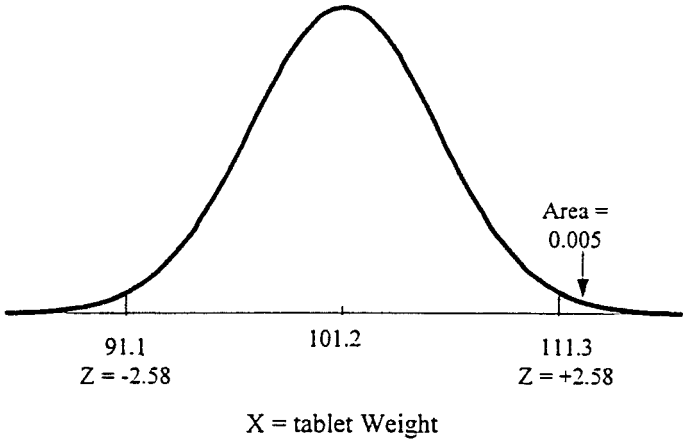

Figure 3.16 Distribution of tablets with mean weight 101.2 mg and standard deviation equal to 3.92.

of tablets is between 91.1 and 111.3 mg? If the answer is 98% or greater, the requirements are met. The proportion of tablets between 91.1 and 111.3 mg can be estimated by computing the area under the normal curve in the interval 91.1 to 111.3, the unshaded area in Fig. 3.16. This can be accomplished by use of the Z transformation and the table of areas under the standard normal curve (Table IV.2). First we calculate the areas below 111.3 using the Z transformation:

$$Z = \frac{X - \mu}{\sigma} = \frac{111.3 - 101.2}{3.92} = 2.58$$

This corresponds to an area of 0.995 (see Table IV.2). The area above 111.3 is (1 − 0.995) = 0.005 or 1/200. Referring to Fig. 3.16, this area represents the probability of finding a tablet that weighs 111.3 mg or more. The probability of a tablet weighing 91.1 mg or less is calculated in a similar manner

$$Z = \frac{91.1 - 101.2}{3.92} = -2.58$$

Table IV.2 shows that this area is 0.005; that is, the probability of a tablet weighing between $-\infty$ and 91.1 mg is 0.005. The probability that a tablet will weigh more than 111.3 mg or less than 91.1 mg is 0.005 + 0.005, equal to 0.01. Therefore, 99% (1.00 − 0.01) of the tablets weigh between 91.1 and 111.3 mg and the statistician's recommendation is more than satisfied. The batch should have no trouble passing the USP test.

The fact that the normal distribution is symmetric around the mean simplifies calculations of areas under the normal curve. In the example above, the probability of values exceeding Z equal to 2.58 is exactly the same as the probability of values being less than Z equal to -2.58. This is a consequence of the symmetry of the normal curve, 2.58 and -2.58 being equidistant from the mean. This is easily seen from an examination of Fig. 3.16.

Although this batch of tablets should pass the USP weight uniformity test, if *some* tablets in the batch are out of the 10 or 20% range, there is a chance that a random sample of 20 will fail the USP test. In our example, about 1% or 30,000 tablets will be more than 10% different from the mean (less than 91.1 or more than 111.3 mg). It would be of interest to know the chances, albeit small, that of 20 randomly chosen tablets, more than 2 would be ''aberrant.'' When 1% of the tablets in a batch deviate from the batch mean by 10% or more, the chances of finding more than 2 such tablets in a sample of 20 is approximately 0.001 (1/1000). This calculation makes use of the binomial probability distribution.

Example 2: During clinical trials, serum cholesterol, among other serum components, is frequently monitored to ensure that a patient's cholesterol is within the normal range, as well as to observe possible drug effects on serum cholesterol levels. A question of concern is: What is an abnormal serum cholesterol value? One way to define ''abnormal'' is to tabulate cholesterol values for apparently normal healthy persons, and to consider values very remote from the average as abnormal. The distribution of measurements such as serum cholesterol often have an approximately normal distribution.

The results of the analysis of a large number of ''normal'' cholesterol values showed a mean of 215 mg % and a standard deviation of 35 mg %. This data can be depicted as a normal distribution as shown in Fig. 3.17. ''Abnormal'' can be defined in terms of the proportion of ''normal'' values that fall in the extremes of the distribution. This may be thought of in terms of a gamble. By choosing to say that extreme values observed in a

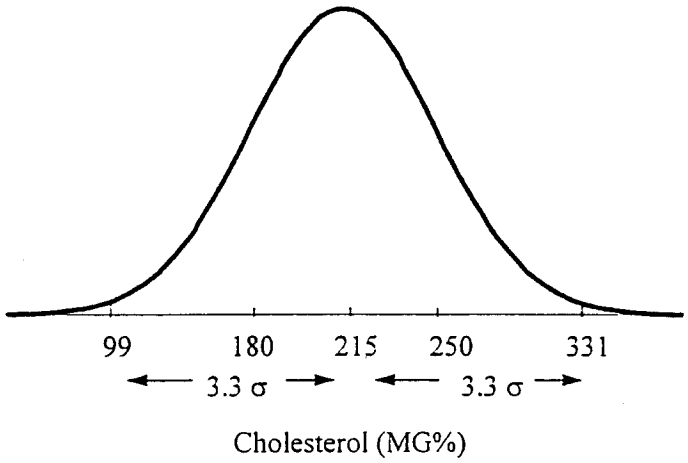

Figure 3.17 Distribution of ''normal'' cholesterol values.

new patient are abnormal, we are saying that persons observed to have very low or high cholesterol levels could be ''normal,'' but the likelihood or probability that they come from the population of normal healthy persons is small. By defining an abnormal cholesterol value as one that has a 1 in 1000 chance of coming from the distribution of values from normal healthy persons, cutoff points can be defined for abnormality based on the parameters of the normal distribution. According to the cumulative standard normal curve, Table IV.2, a value of Z equal to approximately 3.3 leaves 0.05% of the area in the upper tail. Because of the symmetry of the normal curve, 0.05% of the area is below $Z = -3.3$. Therefore, 0.1% (1/1000) of the values will lie outside the values of Z equal to ± 3.3 in the standard normal curve. The values of X (cholesterol levels) corresponding to $Z = \pm 3.3$ can be calculated from the Z transformation.

$$Z = \frac{X - \mu}{\sigma} = \frac{X - 215}{35} = \pm 3.3$$

$$X = 215 \pm (3.3)(35) = 99 \text{ and } 331$$

This is equivalent to saying that cholesterol levels which deviate from the average of ''normal'' persons by 3.3 standard deviation units or more are deemed to be abnormal. For example, the lower limit is the mean of the ''normals'' minus 3.3 times the standard deviation or $215 - (3.3)(35) = 99$. The cutoff points are illustrated in Fig. 3.17.

Example 3: The standard normal distribution may be used to calculate the proportion of values in any interval from any normal distribution. As an example of this calculation, consider the data of cholesterol values in Example 2. We may wish to calculate the proportion of cholesterol values between 200 and 250 mg %.

Examination of Fig. 3.18 shows that the area (probability) under the normal curve between 200 and 250 mg % is the probability of a value being less than 250 *minus* the probability of a value being less than 200. Referring to Table IV.2, we have:

Probability of a value less than 250:

$$\frac{250 - 215}{35} = 1 = Z \quad \text{probability} = 0.841$$

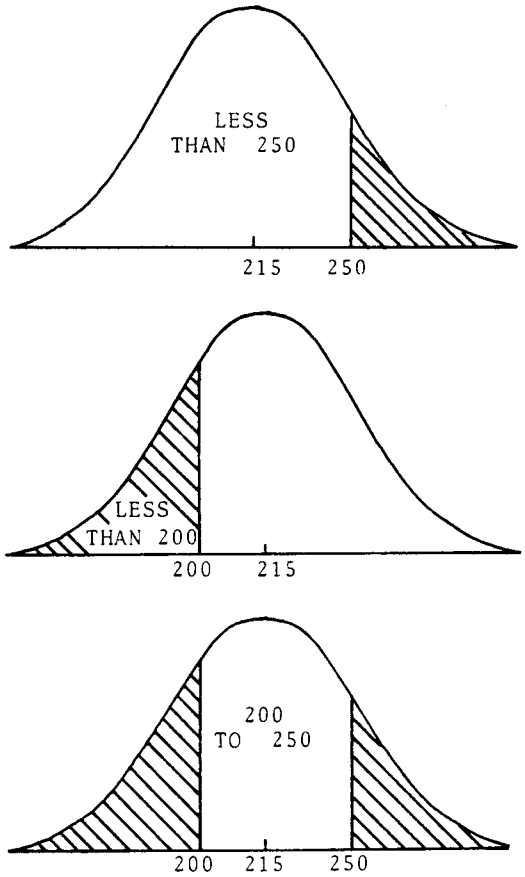

Figure 3.18 Illustration of the calculation of proportion of cholesterol values between 200 and 250 mg %.

Probability of a value less than 200:

$$\frac{200-215}{35} = -0.429 = Z \quad \text{probability} = 0.334$$

Therefore, the probability of a value falling between 250 and 200 is

$$0.841 - 0.334 = 0.507$$

3.4.2 Central Limit Theorem

''Without doubt, the most important theorem in statistics is the central limit theorem''[3]. This theorem states that the distribution of sample means of size N taken from *any* distribution with a finite variance σ^2 and mean μ tends to be *normal* with variance σ^2/N and mean μ. We have previously discussed the fact that a sample mean of size N has a variance equal to σ^2/N. The new and important feature here is that if we are dealing with means

of *sufficiently large sample size*, the means have a normal distribution, regardless of the form of the distribution from which the samples were selected.

How large is a ''large'' sample? The answer to this question depends on the form of the distribution from which the samples are taken. If the distribution is normal, any size sample will have a mean that is normally distributed. For distributions that deviate greatly from normality, larger samples will be needed to approximate normality than distributions which are more similar to the normal distributions (e.g., symmetrical distributions).

The power of this theorem is that the normal distribution can be used to describe most of the data with which we will be concerned, provided that the means come from samples of sufficient size. An example will be presented to illustrate how means of distributions far from normal tend to be normally distributed as the sample size increases. Later in this chapter we will see that even the discrete binomial distribution, where only a very limited number of outcomes are possible, closely approximates the normal distribution with sample sizes as small as 10 in symmetrical cases (e.g., $p = q = 0.5$).

Consider a distribution which consists of outcomes 1, 2, and 3 with probabilities depicted in Fig. 3.19. The probabilities of observing values of 1, 2, and 3 are 0.1, 0.3, and 0.6, respectively. This is an asymmetric distribution, with only three discrete outcomes. The mean is 2.5. Sampling from this population can be simulated by placing 600 tags marked with the number 3, 300 tags marked with the number 2, and 100 tags marked with the number 1 in a box. We will mix up the tags, select 10 (replacing each tag and mixing after each individual selection), and *compute the mean of the 10 samples*. A typical result might be five tags marked 3, four tags marked 2, and one tag marked 1, an average of 2.4. With a computer or programmable calculator, we can simulate this drawing of 10 tags. The distributions of 100 such means for samples of sizes 10 and 20 obtained from a computer simulation are shown in Fig. 3.20. The distribution is closer to normal as the sample size is increased from 10 to 20. This is an empirical demonstration of the central limit theorem. Of course, under ordinary circumstances, we would not draw 100 samples each of size 10 (or 20) to demonstrate a result that can be proved mathematically.

3.4.3 Normal Approximation to the Binomial

A very important result in statistical theory is that the binomial probability distribution can be approximated by the normal distribution if the sample size is sufficiently large (see

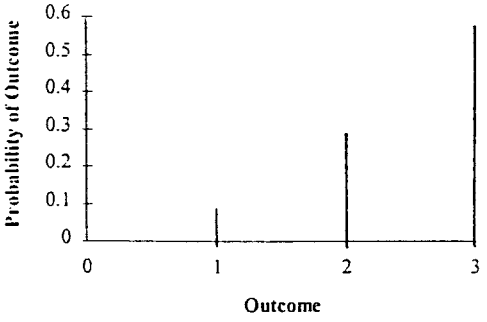

Figure 3.19 Probability distribution of outcomes 1, 2, and 3.

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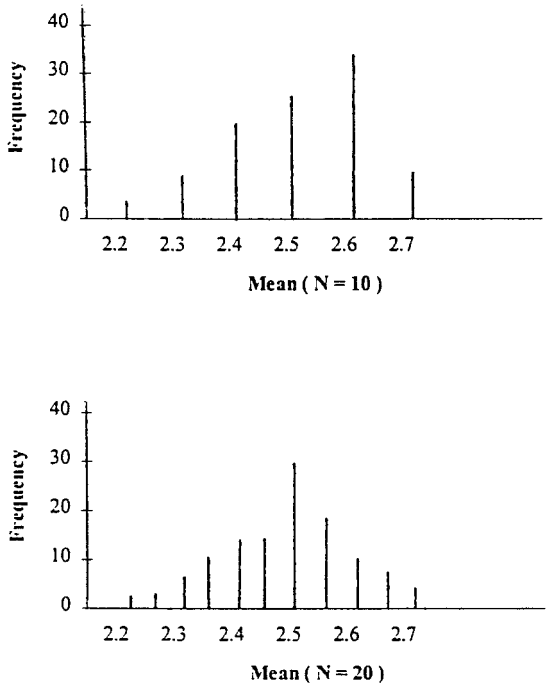

Figure 3.20 Distribution of means of sizes 10 and 20 from population shown in Fig. 3.19.

Sec. 3.4.2). A conservative rule of thumb is that if Np (the product of the number of observations and the probability of success) and Nq are both greater than or equal to 5, we can use the normal distribution to approximate binomial probabilities. With symmetric binomial distributions, when $p = q = 0.5$, the approximation works well for Np less than 5.

To demonstrate the application of the normal approximation to the binomial, we will examine the binomial distribution described above, where $N = 10$ and $p = 0.5$. We can superimpose a normal curve over the binomial with $\mu = 5$ (number of successes) and standard deviation $\sqrt{Npq} = \sqrt{10(0.5)(0.5)} = 1.58$, as shown in Fig. 3.21.

The probability of a discrete result can be calculated using the binomial probability [Eq. (3.9)] or Table IV.3. The probability of seven successes, for example, is equal to 0.117. In a normal distribution, the probability of a single value cannot be calculated. We can only calculate the probability of a range of values within a specified interval. The area that approximately corresponds to the probability of observing seven successes in 10 trials is the area between 6.5 and 7.5, as illustrated in Fig. 3.21. This area can be obtained using the Z transformation discussed earlier in this chapter [Eq. (3.14)]. The area between 6.5 and 7.5 is equal to the area below 7.5 minus the area below 6.5.

Area below 6.5:

$$Z = \frac{6.5 - 5}{1.58} = 0.948 \qquad \text{from Table IV.2, area} = 0.828$$

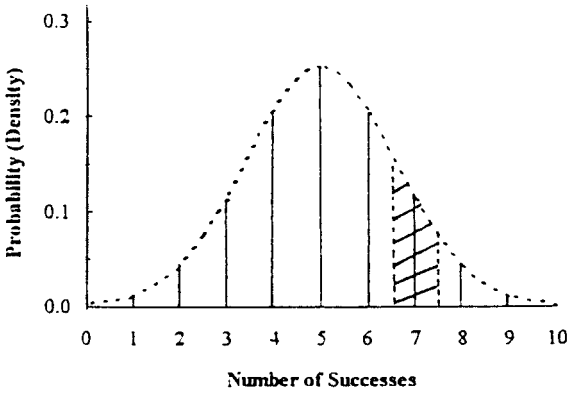

Figure 3.21 Normal approximation to binomial distribution: $Np = 5$ and s.d. $= 1.58$.

Area below 7.5:

$$Z = \frac{7.5 - 5}{1.58} = 1.58 \qquad \text{from Table IV.2, area} = 0.943$$

Therefore, the area (probability) between 6.5 and 7.5 is

$$0.943 - 0.828 = 0.115$$

This area is very close to the exact probability of 0.117.

The use of $X \pm 0.5$ to help estimate the probability of a discrete value, X, using a continuous distribution (e.g., the normal distribution) is known as a continuity correction. We will see that the continuity correction is commonly used to improve the estimation of binomial probabilities by the normal approximation (Chap. 5).

Most of our applications of the binomial distribution will involve data that allow for the use of the normal approximation to binomial probabilities. This is convenient because calculations using exact binomial probabilities are tedious and much more difficult than the calculations using the standard normal cumulative distribution (Table IV.2), particularly when the sample size is large.

3.5 OTHER COMMON PROBABILITY DISTRIBUTIONS

3.5.1 The Poisson Distribution

Although we will not discuss this distribution further in this book, the Poisson distribution deserves some mention. The Poisson distribution can be considered to be an approximation to the binomial distribution when the sample size is large and the probability of observing a specific event is small. In quality control, the probability of observing a defective item is often calculated using the Poisson. The probability of observing X events of a given kind in N observations, where the probability of observing the event in a single observation is P, is

$$p(X) = \frac{\lambda^X e^{-\lambda}}{X!} \tag{3.15}$$

where

$\lambda = NP$
e = base of natural logarithms (2.718 . . .)
N = number of observations

We may use the Poisson distribution to compute the probability of finding one defective tablet in a sample of 100 taken from a batch with 1% defective tablets. Applying Eq. (3.15), we have

$$N = 100 \quad P = 0.01 \quad NP = \lambda = (100)(0.01) = 1$$

$$P(1) = \frac{(1)^1(e^{-1})}{1!} = e^{-1} = 0.368$$

The exact probability calculated from the binomial distribution is 0.370. (See Exercise Problem 8.)

3.5.2 The *t* Distribution (''Student's *t*'')

The t distribution is an extremely important probability distribution. This distribution can be constructed by repeatedly taking samples of size N from a normal distribution and computing the statistic

$$t = \frac{\bar{X} - \mu}{S/\sqrt{N}} \tag{3.16}$$

where $\bar{X}$ is the sample mean, μ the true mean of the normal distribution, and S the sample standard deviation. The distribution of the t's thus obtained forms the t distribution. The exact shape of the t distribution depends on sample size (degrees of freedom), but the t distribution is symmetrically distributed about a mean of zero, as shown in Fig. 3.22A.

To elucidate further the concept of a sampling distribution obtained by repeated sampling, as discussed for the t distribution above, a simulated sampling of 100 samples each of size 4 ($N = 4$) was performed. These samples were selected from a normal distribution with mean 50 and standard deviation equal to 5, for this example. The mean and standard deviation of each sample of size 4 were calculated and a t ratio [Eq. (3.16)] constructed.

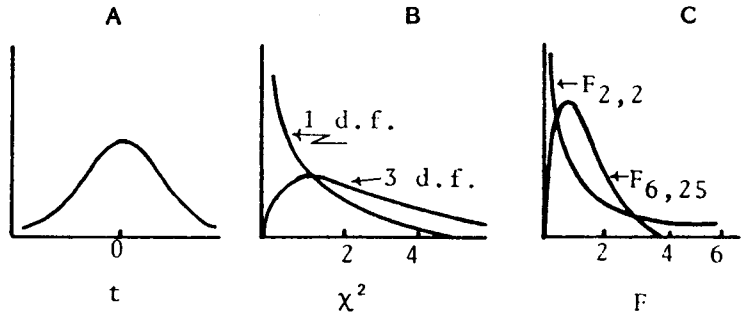

Figure 3.22 Examples of typical probability distributions.

Table 3.5 Frequency Distribution of 100 *t* Values Obtained by Simulated Repeat Sampling from a Normal Distribution with Mean 50 and Standard Deviation 5[a]

Class interval	Frequency
−5.5 to −4.5	1
−4.5 to −3.5	2
−3.5 to −2.5	2
−2.5 to −1.5	11
−1.5 to −0.5	18
−0.5 to +0.5	29
+0.5 to +1.5	21
+1.5 to +2.5	9
+2.5 to +3.5	4
+3.5 to +4.5	2
+4.5 to +5.5	1

[a] Sample size = 4.

The distribution of the 100 *t* values thus obtained is shown in Table 3.5. The data are plotted (histogram) together with the theoretically derived *t* distribution with 3 degrees of freedom ($N - 1 = 4 - 1 = 3$) in Fig. 3.23. Note that the distribution is symmetrically centered around a mean of 0, and that 5% of the *t* values are 3.18 or more units from the mean (theoretically).

3.5.3 The Chi-Square (X^2) Distribution

Another important probability distribution in statistics is the chi-square distribution. The chi-square distribution may be derived from normally distributed variables, defined as the sum of squares of independent normal variables, each of which has mean 0 and standard deviation 1. Thus, if Z is normal with $\mu = 0$ and $\sigma = 1$,

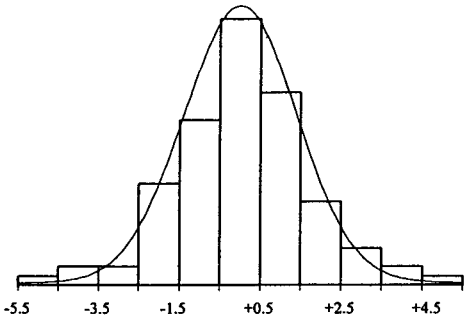

Figure 3.23 Simulated *t* distribution (d.f. = 3) compared to a theoretical *t* distribution.

$$\chi^2 = \sum Z_i^2 \tag{3.17}$$

Applications of the chi-square distribution are presented in Chapters 5 and 15. The chi-square distribution is often used to assess probabilities when comparing discrete values from comparative groups, where the normal distribution can be used to approximate discrete probabilities.

As with the *t* distribution, the distribution of chi-square depends on degrees of freedom, equal to the number of independent normal variables as defined in Eq. (3.17). Figure 3.22B shows chi-square distributions with 1 and 3 degrees of freedom.

3.5.4 The *F* Distribution

After the normal distribution, the *F* distribution is probably the most important probability distribution used in statistics. This distribution results from the sampling distribution of the ratio of two independent variance estimates obtained from the same normal distribution. Thus the first sample consists of N_1 observations and the second sample consists of N_2 observations:

$$F = \frac{S_1^2}{S_2^2} \tag{3.18}$$

The *F* distribution depends on two parameters, the degrees of freedom in the numerator ($N_1 - 1$) and the degrees of freedom in the denominator ($N_2 - 1$). This distribution is used to test for differences of means (analysis of variance) as well as to test for the equality of two variances. The *F* distribution is discussed in more detail in Chaps. 5 and 8 as applied to the comparison of two variances and testing of equality of means in the analysis of variance, respectively.

KEY TERMS

Binomial distribution
Binomial formula
Binomial trial
Central limit theorem
Chi-square distribution
Combinations
Conditional probability
Continuous distribution
Cumulative distribution
Density function
Discontinuous variable
Discrete distribution
Distribution
Equally likely
Event
Factorial
Failure
F distribution
Independent events
Multiplicative probability
Mutually exclusive
Normal distribution
Outcome
Poisson distribution
Population
Probability distribution
Proportion
Random
Randomly chosen
Standard normal distribution
Success
t distribution
Variability
Z transformation

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7

LINEAR REGRESSION AND CORRELATION

Simple linear regression analysis is a statistical technique that defines the functional relationship between two variables, *X* and *Y*, by the ''best-fitting'' straight line. A straight line is described by the equation, $Y = A + BX$, *where Y is the dependent variable* (ordinate), X *is the independent variable* (abscissa), and A *and* B *are the* Y *intercept* and *slope of the line*, respectively (see Fig. 7.1).* Applications of regression analysis in pharmaceutical experimentation are numerous. This procedure is commonly used:

1. To describe the relationship between variables where the functional relationship is known to be linear, such as in Beer's law plots, where optical density is plotted against drug concentration
2. When the functional form of a response is unknown, but where we wish to represent a trend or rate as characterized by the slope (e.g., as may occur when following a pharmacological response over time)
3. When we wish to describe a process by a relatively simple equation that will relate the response, *Y*, to a fixed value of *X*, such as in stability prediction (concentration of drug versus time).

In addition to the specific applications noted above, regression analysis is used to define and characterize dose-response relationships, for fitting linear portions of pharmacokinetic data, and in obtaining the best fit to linear physical-chemical relationships.

Correlation is a procedure commonly used to characterize quantitatively the relationship between variables. Correlation is related to linear regression, but its application and interpretation are different. This topic is introduced at the end of this chapter.

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* The notation $Y = A + BX$ is standard in statistics. We apologize for any confusion that may result from the reader's familiarity with the equivalent, $Y = mX + b$, used frequently in analytical geometry.

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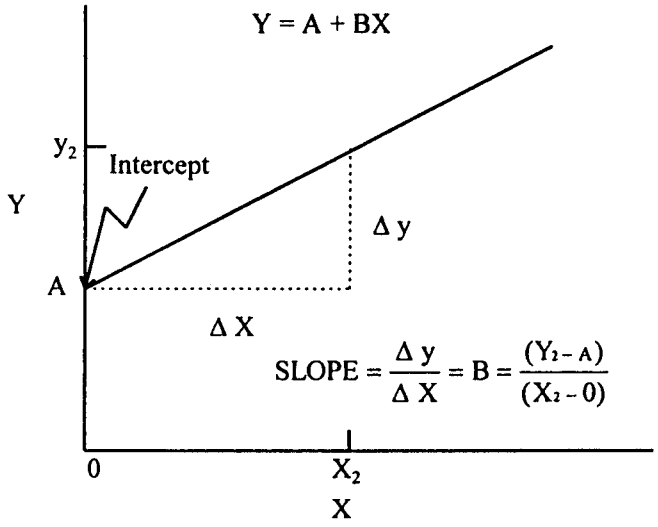

Figure 7.1 Straight-line plot.

7.1 INTRODUCTION

Straight lines are constructed from sets of data pairs, X and Y. Two such pairs (i.e., two points) uniquely define a stright line. As noted previously, a straight line is defined by the equation

$$Y = A + BX \tag{7.1}$$

where A is the Y intercept (the value of Y when $X = 0$) and B is the slope ($\Delta Y/\Delta X$). $\Delta Y/\Delta X$ is $(Y_2 - Y_1)/(X_2 - X_1)$ for any two points on the line (see Fig. 7.1). The slope and intercept define the line; once A and B are given, the line is specified. In the elementary example of only two points, a statistical approach to define the line is clearly unnecessary.

In general, with more than two X, y points,* a plot of y versus X will not *exactly* describe a straight line, even when the relationship is known to be linear. The failure of experimental data derived from truly linear relationships to lie exactly on a straight line is due to errors of observation (experimental variability). Figure 7.2 shows the results of four assays of drug samples of different, but known potency. The assay results are plotted against the known amount of drug. If the assays are performed without error, the plot results in a 45° line (slope $= 1$) which, if extended, passes through the origin; that is, the Y intercept, A, is 0 (Fig. 7.2A). In this example, the equation of the line $Y = A + BX$ is $Y = 0 + 1(X)$, or $Y = X$. Since there is no error in this experiment, the line passes exactly through the four X, Y points.

Real experiments are not error free, and a plot of X, y data rarely exactly fits a straight line, as shown in Fig. 7.2B. We will examine the problem of obtaining a line to fit data

* In the rest of this chapter, y denotes the experimentally observed point, and Y denotes the corresponding point on the least squares ''fitted'' line (or the true value of Y, according to context).

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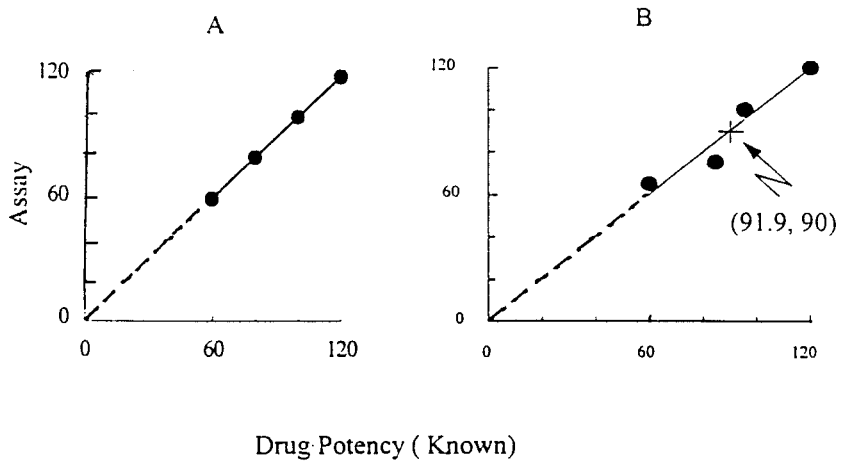

Figure 7.2 Plot of assay recovery versus known amount: theoretical and actual data.

that are not error free. In these cases, the line does not go exactly through all of the points. A ''good'' line, however, should come ''close'' to the experimental points. When the variability is small, a line drawn by eye will probably be very close to that constructed more exactly by a statistical approach (Fig. 7.3A). With large variability, the ''best'' line is not obvious. What single line would you draw to best fit the data plotted in Fig. 7.3B? Certainly, lines drawn through any two arbitrarily selected points will not give the best (or a unique) line to fit the totality of data.

Given N pairs of variables, X, y, we can define the best straight line describing the relationship of X and y as that line which minimizes the sum of squares of the vertical

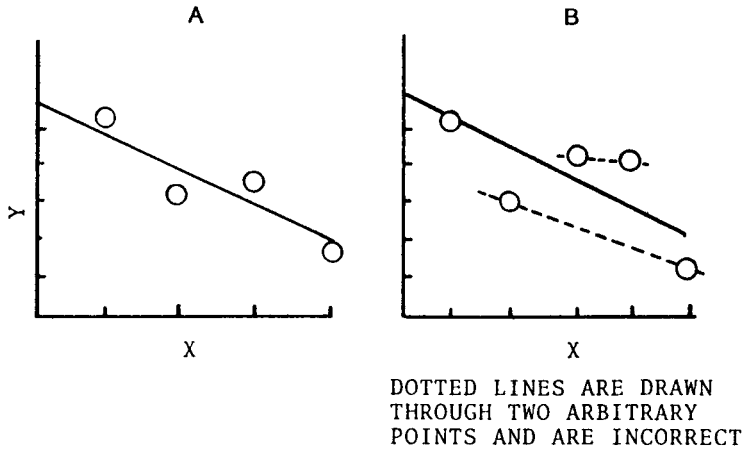

Figure 7.3 Fit of line with variable data.

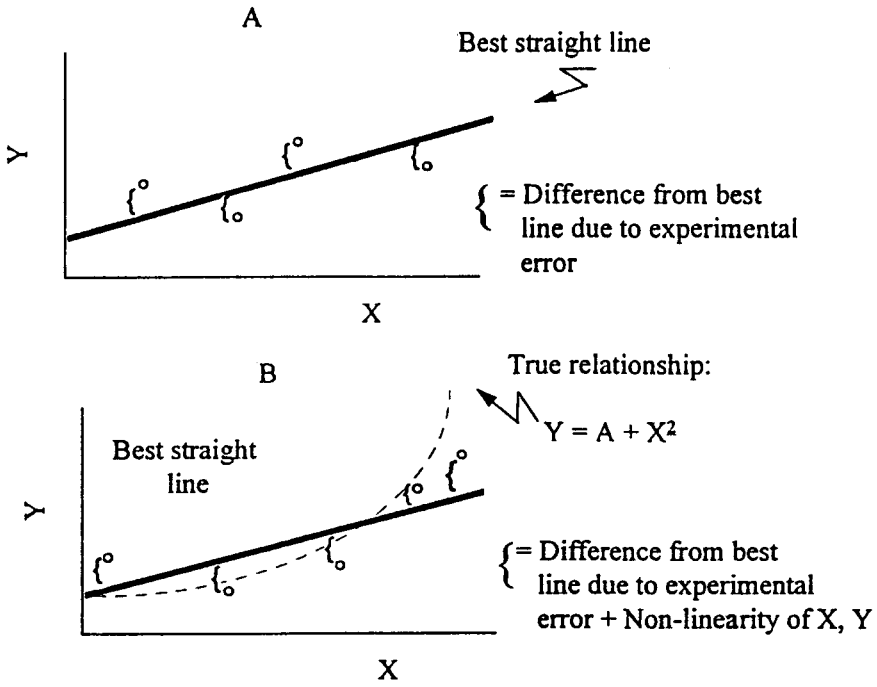

Figure 7.4 Lack of fit due to (A) experimental error and (B) nonlinearity.

distances of each point from the fitted line. The definition of ''sum of squares of the vertical distances of each point from the fitted line'' (see Fig. 7.4) is written mathematically as $\Sigma(y - Y)^2$, where y represents the experimental points and Y represents the corresponding points on the fitted line. The line constructed according to this definition is called the *least squares* line. Applying techniques of calculus, the slope and intercept of the least squares line can be calculated from the sample data as follows:

$$\text{Slope} = b = \frac{\sum (X - \bar{X})(y - \bar{y})}{\sum (X - \bar{X})^2} \tag{7.2}$$

$$\text{Intercept} = a = \bar{y} - b\bar{X} \tag{7.3}$$

Remember that the slope and intercept uniquely define the line.

There is a shortcut computing formula for the slope, similar to that described previously for the standard deviation:

$$b = \frac{N\sum Xy - \left(\sum X\right)\left(\sum y\right)}{N\sum X^2 - \left(\sum X\right)^2} \tag{7.4}$$

where N is the number of X, y pairs. The calculation of the slope and intercept is relatively simple, and can usually be quickly computed with a hand calculator. Some calculators have a built-in program for calculating the regression parameter estimates, a and b.*

* a and b are the sample estimates of the true parameters, A and B.

Table 7.1 Raw Data from Fig. 7.2A to Calculate the Least Squares Line

Drug potency, X	Assay, y	Xy
60	60	3,600
80	80	6,400
100	100	10,000
120	120	14,400
$\sum X = 360$	$\sum y = 360$	$\sum Xy = 34{,}400$
$\sum X^2 = 34{,}400$		

For the example shown in Fig. 7.2A, the line that exactly passes through the four data points has a slope of 1 and an intercept of 0. The line, $Y = X$, is clearly the best line for these data, an exact fit. The least squares line, in this case, is exactly the same line, $Y = X$. The calculation of the intercept and slope using the least squares formulas, Eqs. (7.3) and (7.4), is illustrated below. Table 7.1 shows the raw data used to construct the line in Fig. 7.2A.

According to Eq. (7.4) ($N = 4$, $\Sigma X^2 = 34{,}400$, $\Sigma Xy = 34{,}400$, $\Sigma X = \Sigma y = 360$),

$$b = \frac{(4)(3600 + 6400 + 10,000 + 14,000) - (360)(360)}{4(34,400) - (360)^2} = 1$$

a is computed from Eq. (7.3); $a = \bar{y} - b\bar{X}$ ($\bar{y} = \bar{X} = 90$, $b = 1$). $a = 90 - 1(90) = 0$. This represents a situation where the assay results exactly equal the known drug potency (i.e., there is no error).

The actual experimental data depicted in Fig. 7.2B are shown in Table 7.2. The slope b and the intercept a are calculated from Eqs. (7.4) and (7.3). According to Eq. (7.4),

$$b = \frac{(4)(33,600) - (360)(353)}{4(34,400) - (360)^2} = 0.915$$

According to Eq. (7.3),

Table 7.2 Raw Data from Fig. 7.2B Used to Calculate the Least Squares Line

Drug potency, X	Assay, y	Xy
60	63	3,780
80	75	6,000
100	99	9,900
120	116	13,920
$\sum X = 360$	$\sum y = 353$	$\sum Xy = 33{,}600$
$\sum X^2 = 34{,}400$	$\sum y^2 = 32{,}851$	

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$$a = \frac{353}{4} - 0.915(90) = 5.9$$

A perfect assay (no error) has a slope of 1 and an intercept of 0, as shown above. The actual data exhibit a slope close to 1, but the intercept appears to be too far from 0 to be attributed to random error. Exercise Problem 2 addresses the interpretation of these results as they relate to assay method characteristics.

This example suggests several questions and problems regarding linear regression analysis. The line that best fits the experimental data is an estimate of some true relationship between X and Y. In most circumstances, we will fit a straight line to such data only if we believe that the true relationship between X and Y is linear. The experimental observations will not fall exactly on a straight line because of variability (e.g., error associated with the assay). This situation (true linearity associated with experimental error) is different from the case where the underlying true relationship between X and Y is not linear. In the latter case, the lack of fit of the data to the least squares line is due to a combination of experimental error and the lack of linearity of the X, Y relationship (see Fig. 7.4). Elementary techniques of simple linear regression will not differentiate these two situations: (a) experimental error with true linearity and (b) experimental error and nonlinearity. (A design to estimate variability due to both nonlinearity and experimental error is given in App. II.)

We will discuss some examples relevant to pharmaceutical research which make use of least squares linear regression procedures. The discussion will demonstrate how variability is estimated and used to construct estimates and tests of the line parameters A and B.

7.2 ANALYSIS OF STANDARD CURVES IN DRUG ANALYSIS: APPLICATION OF LINEAR REGRESSION

The assay data discussed previously can be considered as an example of the construction of a *standard curve* in drug analysis. Known amounts of drug are subjected to an assay procedure, and a plot of percentage recovered (or amount recovered) versus amount added is constructed. Theoretically, the relationship is usually a straight line. A knowledge of the line parameters A and B can be used to predict the amount of drug in an unknown sample based on the assay results. In most practical situations, A and B are unknown. The least squares estimates a and b of these parameters are used to compute drug potency (X) based on the assay response (y). For example, the least squares line for the data in Fig. 7.2B and Table 7.2 is

$$Assay\ result = 5.9 + 0.915\ (potency) \tag{7.5}$$

Rearranging Eq. (7.5), an unknown sample which has an assay value of 90 can be predicted to have a true potency of

$$Potency = X = \frac{y - 5.9}{0.915}$$

$$Potency = \frac{90 - 5.9}{0.915} = 91.9$$

This point (91.9, 90) is indicated in Fig. 7.2 by a cross.

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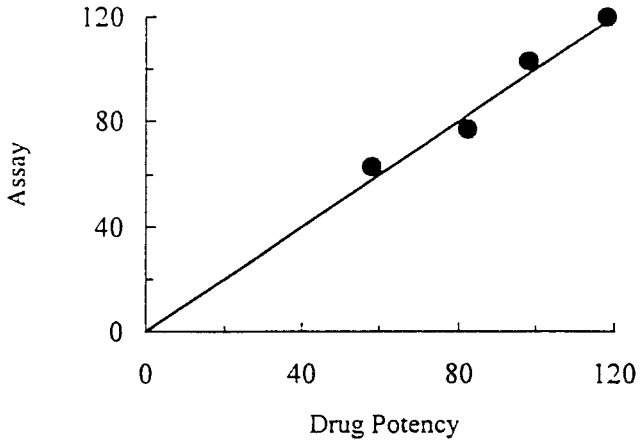

Figure 7.5 Plot of data in Table 7.2 with known (0, 0) intercept.

7.2.1 Line Through the Origin

Many calibration curves (lines) are known to pass through the origin; that is, the assay response must be zero if the concentration of drug is zero. The calculation of the slope is simplified if the line is forced to go through the point (0,0). In our example, if the intercept is *known* to be zero, the slope is (see also Table 7.2)

$$b = \frac{\sum Xy}{\sum X^2} = \frac{33,600}{60^2 + 80^2 + 100^2 + 120^2} = 0.977 \tag{7.6}$$

The least squares line fitted with the zero intercept is shown in Fig. 7.5. If this line were to be used to predict actual concentrations based on assay results, we would obtain answers which are different from those predicted from the line drawn in Fig. 7.2B. However, both lines have been constructed from the same raw data. ''Is one of the lines correct?'' or ''Is one line better than the other?'' Although one cannot say with certainty which is the better line, a thorough knowledge of the analytical method will be important in making a choice. For example, a nonzero intercept suggests either nonlinearity over the range of assays or the presence of an interfering substance in the sample being analyzed. The decision of which line to use can also be made on a statistical basis. A statistical test of the intercept can be performed under the null hypothesis that the intercept is 0 (H_0: $A = 0$, Sec. 7.4.1). Rejection of the hypothesis would be strong evidence that the line with the positive intercept best represents the data.

7.3 ASSUMPTIONS IN TESTS OF HYPOTHESES IN LINEAR REGRESSION

Although there are no prerequisites for fitting a least squares line, the testing of statistical hypotheses in linear regression depends on the validity of several assumptions.

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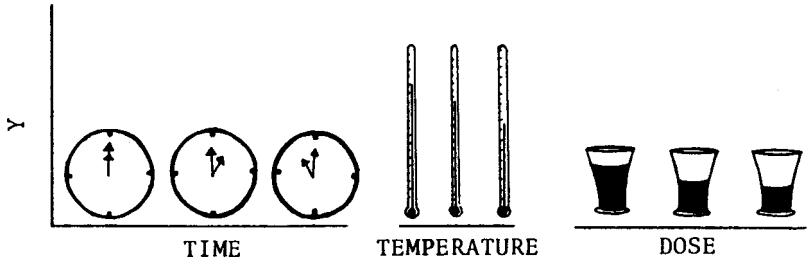
1. *The X variable is measured without error*. Although not always *exactly* true, *X* is often measured with relatively little error and, under these conditions this assumption can be considered to be satisfied. In the present example, *X* is the potency of drug in the ''known'' sample. If the drug is weighed on a sensitive balance, the error in drug potency will be very small. Another example of an *X* variable that is often used, which can be precisely and accurately measured, is ''time.''



2. *For each* X, *y is independent and normally distributed*. We will often use the notation *Y.x* to show that the value of *Y* is a function of *X*.
3. *The variance of* y *is assumed to be the same at each* X. If the variance of *y* is not constant, but is either known or related to *X* in some way, other methods [see Sec. 7.7] are available to estimate the intercept and slope of the line [1].
4. *A linear relationship exists between* X *and* Y. $Y = A + BX$, where *A* and *B* are the true parameters. Based on theory or experience, we have reason to believe that *X* and *Y* are linearly related.

These assumptions are depicted in Fig. 7.6. Except for location (mean), the distribution of *y* is the same at every value of *X*; that is, *y* has the same variance at every value of *X*. In the example in Fig. 7.6, the mean of the distribution of *y*'s decreases as *X* increases (the slope is negative).

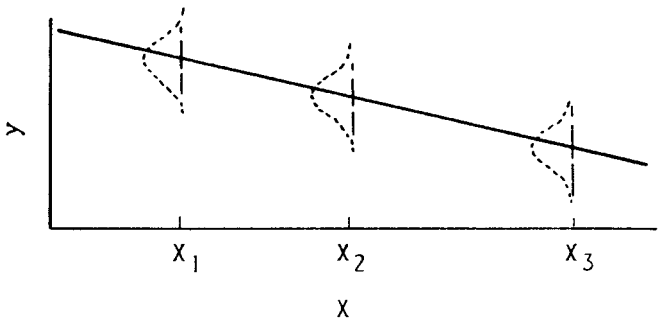


Figure 7.6 Normality and variance assumptions in linear regression.

8

ANALYSIS OF VARIANCE

Analysis of variance, also known as *ANOVA*, is perhaps the most powerful statistical tool. ANOVA is a general method of analyzing data from designed experiments, whose objective is to *compare two or more group means*. The *t* test is a special case of ANOVA in which only two means are compared. By *designed experiments*, we mean experiments with a particular structure. Well-designed experiments are usually optimal with respect to meeting study objectives. The statistical analysis depends on the design, and the discussion of ANOVA therefore includes common statistical designs used in pharmaceutical research. Analysis of variance designs can be more or less complex. The designs can be very simple, as in the case of the *t*-test procedures presented in Chapter 5. Other designs can be quite complex, sometimes depending on computers for their solution and analysis. As a rule of thumb, one should use the simplest design that will achieve the experimental objectives. This is particularly applicable to experiments otherwise difficult to implement, such as is the case in clinical trials.

8.1 ONE-WAY ANALYSIS OF VARIANCE

An elementary approach to ANOVA may be taken using the two-independent-groups *t* test as an example. This is an example of one-way analysis of variance, also known as a ''completely randomized'' design. (Certain simple ''parallel-groups'' designs in clinical trials correspond to the one-way analysis of variance design.) In the *t* test, the two treatments are assigned at random to different independent experimental units. In a clinical study, the *t* test is appropriate when two treatments are randomly assigned to different patients. This results in two groups, each group representing one of the two treatments. One-way ANOVA is used when we wish to test the equality of treatment means in experiments where two or more treatments are randomly assigned to different, independent experimental units. The typical null hypothesis is H_0: $\mu_1 = \mu_2 = \mu_3 = \cdots$ where μ_1 refers to treatment 1, and so on.

Suppose that 15 tablets are available for the comparison of three assay methods, five tablets for each assay. The one-way ANOVA design would result from a random assign-

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ment of the tablets to the three groups. In this example, five tablets are assigned to each group. Although this allocation (five tablets per group) is optimal with regard to the precision of the comparison of the three assay methods, it is not a necessary condition for this design. The number of tablets analyzed by each analytical procedure need not be equal for the purposes of comparing the mean results. However, one can say, in general, that symmetry is a desirable feature in the design of experiments. This will become more apparent as we discuss various designs. In the one-way ANOVA, symmetry can be defined as an equal number of experimental units in each treatment group.

We will pursue the example above to illustrate the ANOVA procedure. Five replicate tablets are analyzed in each of the three assay method groups, one assay per tablet. Thus we assay the 15 tablets, five tablets by each method, as shown in Table 8.1. If only two assay methods were to be compared, we could use a *t* test to compare the means statistically. If more than two assay methods are to be compared, the correct statistical procedure to compare the means is the one-way analysis of variance (ANOVA).

Analysis of variance is a technique of separating the total variability in a set of data into component parts, represented by a statistical model. In the simple case of the one-way ANOVA, the model is represented as

$$Y_{ij} = \mu + G_i + \varepsilon_{ij} \tag{8.1}$$

where

Y_{ij} = jth response in treatment group i (e.g., $i = 3$, $j = 2$, second tablet in third group)
G_i = deviation of the ith treatment (group) mean from the overall mean, μ
ε_{ij} = random error in the experiment (measurement error, biological variability, etc.) assumed to be normal with mean 0 and variance σ^2

The model says that the response is a function of the true treatment mean ($\mu + G_i$) and a random error that is normally distributed, with mean zero and variance σ^2. In the case of a clinical study, $G_i + \mu$ is the true average of treatment i. If a patient is treated with an antihypertensive drug whose true mean effect is a 10-mmHg reduction in blood pressure, then $Y_{ij} = 10 + \varepsilon_{ij}$, where Y_{ij} is the jth observation among patients taking the drug i. (Note that if treatments are identical, G_i is the same for all treatments.) The error, ε_{ij}, is a normally distributed variable, identically distributed for all observations. It is composed of many factors, including interindividual variation and measurement error. Thus the ob-

Table 8.1 Results of Assays Comparing Three Analytical Methods

Method A	Method B	Method C
102	99	103
101	100	100
101	99	99
100	101	104
102	98	102
$\bar{X}$ 101.2	99.4	101.6
s.d. 0.84	1.14	2.07

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served experimental values will be different for different people, a consequence of the nature of the assigned treatment and the random error, ε_{ij} (e.g., biological variation). Section 8.5 expands the concept of statistical models.

In addition to the assumption that the error is normal with mean 0 and variance σ^2, the errors must be independent. This is a very important assumption in the analysis of variance model. The fact that the error has mean 0 means that some people will show positive deviations from the treatment mean, and others will show negative deviations; but on the average, the deviation is zero.

As in the *t* test, statistical analysis and interpretation of the ANOVA is based on the following assumptions.

1. The errors are normal with constant variance.
2. The errors (or observations) are independent.

As will be discussed below, ANOVA separates the variability of the data into parts, comparing that due to treatments to that due to error.

8.1.1 Computations and Procedure for One-Way Analysis of Variance

Analysis of variance for a one-way design separates the variance into two parts, that due to *treatment differences* and that due to *error*. It can be proven that the *total sum of squares* (the squared deviations of each value from the overall mean)

$$\sum (Y_{ij} - \bar{Y})^2$$

is equal to

$$\sum (Y_{ij} - \bar{Y}_i)^2 + \sum N_i (\bar{Y}_i - \bar{Y})^2 \tag{8.2}$$

where $\bar{Y}$ is the overall mean and $\bar{Y}_i$ is the mean of the *i*th group. N_i is the number of observations in treatment group *i*. The first term in expression (8.2) is called the *within* sum of squares, and the second term is called the *between* sum of squares.

A simple example to demonstrate the equality in Eq. (8.2) is shown below, using the data of Table 8.2.

$$\sum (Y_{ij} - \bar{Y})^2 = \sum Y^2 - \frac{\left(\sum Y\right)^2}{N} = 160 - \frac{(24)^2}{6} = 64$$

$$\sum (Y_{ij} - \bar{Y}_i)^2 = (0-1)^2 + (2-1)^2 + (2-3)^2 + (4-3)^2 + (6-8)^2$$

$$= (10-8)^2 = 2 + 2 + 8 = 12$$

$$\sum N_i (\bar{Y}_i - \bar{Y})^2 = 2(1-4)^2 + 2(3-4)^2 + 2(8-4)^2 = 52$$

Thus, according to Eq. (8.2), 64 = 12 + 52.

Table 8.2 Sample Data to Illustrate Eq. (8.2)

Group I (Y_{1j})	Group II (Y_{2j})	Group III (Y_{3j})
0	2	6
2	4	10
$\bar{Y}_t$ 1	3	8

$\bar{Y} = (1 + 3 + 8)/3 = (0 + 2 + 2 + 4 + 6 + 10)/6 = 4$

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The calculations for the analysis make use of simple arithmetic with shortcut formulas for the computations similar to that used in the *t*-test procedures. Computer programs are available for the analysis of all kinds of analysis of variance designs from the most simple to the most complex. In the latter cases, the calculations can be very extensive and tedious, and use of computers may be almost mandatory. For the one-way design, the calculations pose no difficulty. In many cases, use of a pocket calculator will result in a quicker answer than can be obtained using a less accessible computer. A description of the calculations, with examples, are presented below.

The computational process consists first of obtaining the *sum of squares* (SS) for all of the data.

$$\text{Total sum of squares (SS)} = \sum (Y_{ij} - \bar{Y})^2 \tag{8.3}$$

The *total sum of squares* is divided into two parts: (a) the SS due to treatment differences (*between-treatment sum of squares*), and (b) the error term derived from the *within-treatment sum of squares*. The within-treatment sum of squares (within SS) divided by the appropriate degrees of freedom is the *pooled variance*, the same as that obtained in the *t* test for the comparison of two treatment groups. The ratio of the between-treatment mean square to the within-treatment mean square is a measure of treatment differences (see below).

To illustrate the computations, we will use the data from Table 8.1, a comparison of three analytical methods with five replicates per method. Remember that the objective of this experiment is to compare the average results of the three methods. We might think of method A as the standard, accepted method, and methods B and C as modifications of the method, meant to replace method A. As in the other tests of hypotheses described in Chap. 5, we first state the null and alternative hypotheses as well as the significance level, prior to the experiment. For example, in the present case*,

H_0: $\mu_A = \mu_B = \mu_c$ $\quad$ H_a: $\mu_i \neq \mu_j$ $\quad$ for any two means*

1. First, calculate the *total sum of squares* (total SS or TSS). Calculate $\Sigma\ (Y_{ij} - \bar{Y})^2$ [Eq. (8.3)] using all of the data, ignoring the treatment grouping. This is most easily calculated using the shortcut formula

$$\sum Y^2 - \frac{\left(\sum Y\right)^2}{N} \tag{8.4}$$

$(\Sigma Y)^2$ is the grand total of all of the observations squared, divided by the total number of observations N, and is known as the *correction term*, *C.T.* As mentioned in Chapter 1, the correction term is commonly used in statistical calculations, and is important in the calculation of the sum of squares in the ANOVA.

$$\begin{aligned}\text{Total sum of squares} &= \sum Y^2 - \frac{\left(\sum Y\right)^2}{N} \\ &= (102^2 + 101^2 + \ldots + 103^2 + \ldots \\ &\quad + 102^2) - \frac{(1511)^2}{15} \\ &= 152,247 - 152,208.07 = 38.93\end{aligned}$$

* Alternatives to H_0 may also include more complicated comparisons than $\mu_i \neq \mu_j$; see, for example, Sec. 8.2.1.

2. The *between-treatment sum of squares* (between SS or BSS) is calculated as follows:

$$\text{Between-treatment sum of squares} = \sum \frac{T_i^2}{N_i} - \text{C.T.} \tag{8.5}$$

T_i is the sum of observations in treatment group i and N_i is the number of observations in treatment group i. N_i need not be the same for each group. In our example, the BSS is equal to

$$\left(\frac{506^2}{5} + \frac{497^2}{5} + \frac{508^2}{5}\right) - 152,208.07 = 13.73$$

As previously noted, the *treatment* sum of squares is a measure of treatment differences. A large sum of squares means that the treatment differences are large. If the treatment means are identical, the treatment sum of squares will be exactly equal to zero (0).

3. The *within-treatment sum of squares* (WSS) is equal to the difference between the TSS and BSS; that is, TSS = BSS + WSS. The WSS can also be calculated, as in the t test, by calculating $\Sigma\ (Y_{ij} - \bar{Y}_i)^2$ within each group, and pooling the results.

$$\begin{aligned}\text{Within-treatment sum of squares} &= \text{Total SS} - \text{between SS} \\ &= 38.93 - 13.73 \\ &= 25.20\end{aligned} \tag{8.6}$$

Having performed the calculations above, the sum of squares for each ''source'' is set out in an ''analysis of variance table,'' as shown in Table 8.3. The ANOVA table includes the *source, degrees of freedom, sum of squares* (SS), *mean square* (MS) and the *probability* based on the statistical test (F ratio).

The degrees of freedom, noted in Table 8.3, are calculated as $N_{\text{i}} - 1$ *for the total* (N_{i} is the total number of observations); *number of treatments minus one for the treatments*; and for the *within error, subtract d.f. for treatments from the total degrees of freedom*. In our example,

Total degrees of freedom = 15 − 1 = 14
Between-treatment degrees of freedom = 3 − 1 = 2
Within-treatment degrees of freedom = 14 − 2 = 12

Note that for the within degrees of freedom, we have 4 d.f. from each of the three groups. Thus there are 12 d.f. for the within error term. The *mean squares* are equal to the sum of squares divided by the degrees of freedom.

Table 8.3 Analysis of Variance for the Data Shown in Table 8.1: Comparison of Three Analytical Methods

Source	d.f.	SS	MS	F
Between methods	2	13.73	6.87	$F = 3.27$*
Within methods	12	25.20	2.10	
Total	14	38.93		

* $0.05 < P < 0.10$.

Before discussing the statistical test, the reader is reminded of the assumptions underlying the analysis of variance model: *independence of errors, equality of variance*, and *normally distributed errors*.

Testing the Hypothesis of Equal Treatment Means

The *mean squares are variance estimates*. One can demonstrate that the variance estimated by the treatment mean square is a sum of the within variance plus a term that is dependent on treatment differences. If the treatments are identical, the term due to treatment differences is zero, and the between mean square (BMS) will be approximately equal to the within mean square (WMS) on the average. In any given experiment, the presence of random variation will result in nonequality of the BMS and WMS terms, even though the treatments may be identical. If the null hypothesis of equal treatment means is true, the distribution of the BMS/WMS ratio is described by the *F distribution*. Note that under the null hypothesis, both WMS and BMS are estimates of σ^2, the within-group variance.

The *F* distribution is defined by two parameters, degrees of freedom in the numerator and denominator of the *F* ratio:

$$F = \frac{\text{BMS(2 d.f.)}}{\text{WMS(12 d.f.)}} = \frac{6.87}{2.10} = 3.27$$

In our example, we have an *F* with 2 d.f. in the numerator and 12 d.f. in the denominator. A test of significance is made by comparing the observed *F* ratio to a table of the *F* distribution with appropriate d.f. at the specified level of significance. The *F* distribution is an asymmetric distribution with a long tail at large values of *F*, as shown in Fig. 8.1. (See also Secs. 3.5 and 5.3.)

To tabulate all the probability points of all *F* distributions would not be possible. Tables of *F*, similar to the *t* table, usually tabulate points at commonly used α levels. The cutoff points ($\alpha = 0.01, 0.05$) for *F* with n_1 and n_2 d.f. (numerator and denominator) are given in Table IV.6. the probabilities in this table (1% and 5%) are in the upper tail, usually reserved for one-sided tests. This table is used to determine statistical ''significance'' for the analysis of variance. Although the alternative hypothesis in ANOVA (H_a: at least two treatment means not equal) is two-sided, the ANOVA *F* test (BMS/WMS) uses the upper

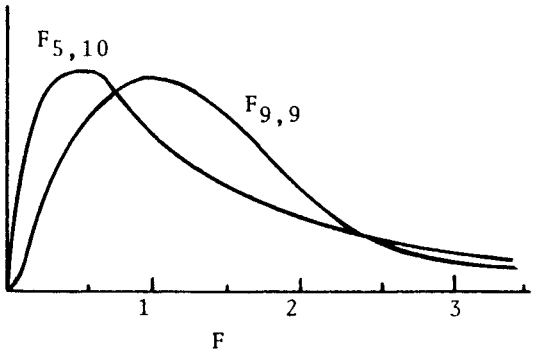

Figure 8.1 Some *F* distributions.

tail of the F distribution because, theoretically, the BMS cannot be smaller than the WMS.* (Thus the F ratio will be less than 1 only due to chance variability.) The BMS (between mean square) is composed of the WMS plus a possible ''treatment'' term. Only large values of the F ratio are considered to be significant. In our example, Table 8.3 shows the F ratio to be equal to 3.27. Referring to Table IV.6, the value of F needed for significance at the 5% level is 3.89 (2 d.f. in the numerator and 12 d.f. in the denominator). Therefore, we cannot reject the hypothesis that all means are equal: method A = method B = method C ($\mu_A = \mu_B = \mu_C$).

8.1.2 Summary of Procedure for One-Way ANOVA

1. Choose experimental design and state the null hypothesis.
2. Define the α level.
3. Choose samples, perform the experiment, and obtain data.
4. Calculate the total sum of squares and between sum of squares.
5. Calculate the within sum of squares as the difference between the total SS and the between SS.
6. Construct an analysis of variance table with mean squares.
7. Calculate the F statistic (BMS/WMS).
8. Refer the F ratio statistic to Table IV.6 (n_1 and n_2 d.f., where n_1 is the d.f. for the BMS and n_2 is the d.f. for the WMS).
9. If the calculated F is equal to or greater than the table value for F at the specified α level of significance, at least two of the treatments can be said to differ.

8.1.3 A Common but Incorrect Analysis of the Comparison of Means from More Than Two Groups

In the example in Sec. 8.1.1, if more than two assay methods are to be compared, the correct statistical procedure is a one-way ANOVA. A common error made by those persons not familiar with ANOVA is to perform three separate t tests on such data: comparing method A to method B, method A to method C, and method B to method C. This would require three analyses and ''decisions,'' which can result in apparent contradictions. For example, decision statements based on three separate analyses could read:

Method A gives higher results than method B ($P < 0.05$).
Method A is not significantly different from method C ($P > 0.05$).
Method B is not significantly different from method C ($P > 0.05$).

These are the conclusions one would arrive at if separate t tests were performed on the data in Table 8.1 (see Exercise Problem 1). One may correctly question: If A is larger than B, and C is slightly larger than A, how can C not be larger than B? The reasons for such apparent contradictions are (a) the use of different variances for the different comparisons, and (b) performing three tests of significance on the same set of data. Analysis of variance obviates such ambiguities by using a common variance for the single test

* This may be clearer if one thinks of the null and alternative hypotheses in ANOVA as H_0: $\sigma_B^2 = \sigma_w^2$; H_0 $\sigma_B^2 > \sigma_w^2$.

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9

FACTORIAL DESIGNS

Factorial designs are used in experiments where the effects of different factors, or conditions, on experimental results are to be elucidated. Some practical examples where factorial designs are optimal are experiments to determine the effect of pressure and lubricant on the hardness of a tablet formulation, to determine the effect of disintegrant and lubricant concentration on tablet dissolution, or to determine the efficacy of a combination of two active ingredients in an over-the-counter cough preparation. Factorial designs are the designs of choice for simultaneous determination of the effects of several factors and their interactions. This chapter introduces some elementary concepts of the design and analysis of factorial designs.

9.1 DEFINITIONS (VOCABULARY)

9.1.1 Factor

A *factor* is an *assigned variable* such as concentration, temperature, lubricating agent, drug treatment, or diet. The choice of factors to be included in an experiment depends on experimental objectives and is predetermined by the experimenter. A factor can be qualitative or quantitative. A *quantitative factor* has a numerical value assigned to it. For example, the factor ''concentration'' may be given the values 1%, 2%, and 3%. Some examples of *qualitative factors* are treatment, diets, batches of material, laboratories, analysts, and tablet diluent. Qualitative factors are assigned names rather than numbers. Although factorial designs may have one or many factors, only experiments with two factors will be considered in this chapter. Single-factor designs fit the category of one-way ANOVA designs. For example, an experiment designed to compare three drug substances using different patients in each drug group is a one-way design with the single factor ''drugs.''

9.1.2 Levels

The levels of a factor are the values or designations assigned to the factor. Examples of levels are 30° and 50° for the factor 'temperature,'' 0.1 molar and 0.3 molar for the factor ''concentration,'' and ''drug'' and ''placebo'' for the factor ''drug treatment.''

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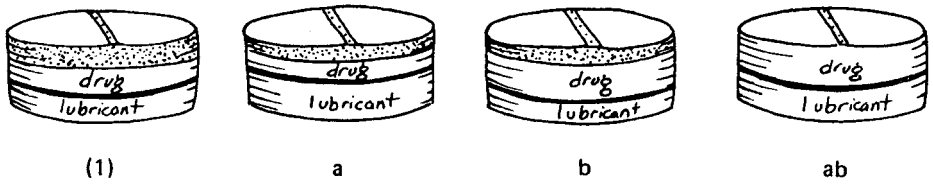
The *runs* or *trials* that comprise factorial experiments consist of all combinations of all levels of all factors. As an example, a two-factor experiment would be appropriate for the investigation of the effects of drug concentration and lubricant concentration on dissolution time of a tablet. If both factors were at two levels (two concentrations for each factor), four runs (dissolution determinations for four formulations) would be required, as follows:

Symbol	Formulation
(1)	Low drug and low lubricant concentration
a	Low drug and high lubricant concentration
b	High drug and low lubricant concentration
ab	High drug and high lubricant concentration

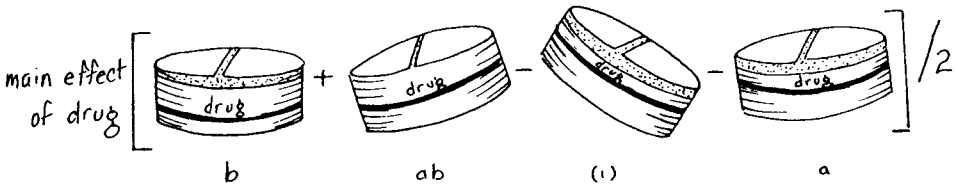
''Low'' and ''high'' refer to the low and high concentrations pre-selected for the drug and lubricant. (Of course, the actual values selected for the low and high concentrations of drug will probably be different from those chosen for the lubricant.) The notation (symbol) for the various combinations of the factors, (1), a, b, ab, is standard. When both factors are at their low levels, we denote the combination as (1). When factor A is at its high level and factor B is at its low level, the combination is called a. b means that only factor B is at the high level, and ab means that both factors A and B are at their high levels.



9.1.3 Effects

The *effect* of a factor is the change in response caused by varying the level(s) of the factor. The *main effect* is the *effect* of a factor *averaged over all levels of the other factors*. In the previous example, a two-factor experiment with two levels each of drug and lubricant, the main effect due to drug would be the difference between the average response when drug is at the high level (runs b and ab) and the average response when drug is at the low level [runs (1) and a]. For this example the main effect can

be characterized as a linear response, since the effect is the difference between the two points shown in Fig. 9.1.



More than two points would be needed to define more clearly the nature of the response as a function of the factor drug concentration. For example, if the response plotted against the levels of a quantitative factor is not linear, the definition of the main effect is less clear. Figure 9.2 shows an example of a curved (quadratic) response based on experimental results with a factor at three levels. In many cases, an important objective of a factorial experiment is to characterize the effect of changing levels of a factor or combinations of factors on the response variable.

9.1.4 Interaction

Interaction may be thought of as a lack of ''additivity of factor effects.'' For example, in a two-factor experiment, if factor A has an effect equal to 5 and factor B has an effect of 10, additivity would be evident if an effect of 15 (5 + 10) were observed when both

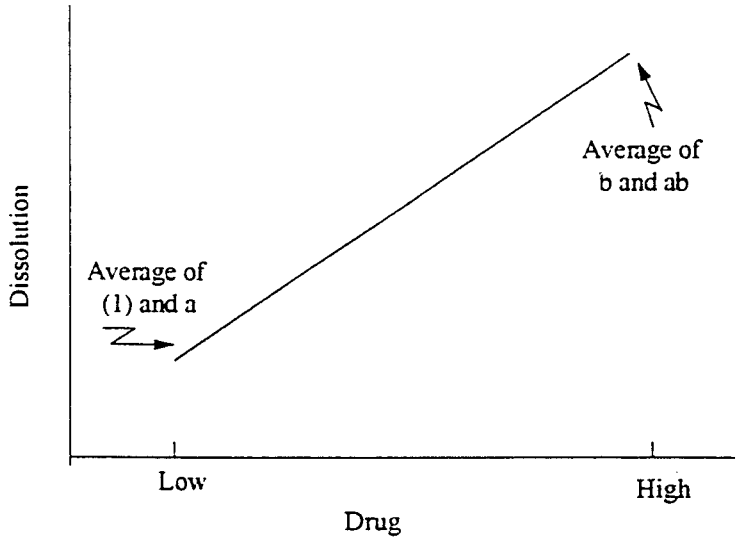


Figure 9.1 Linear effect of drug. a = lubricant; b = drug.

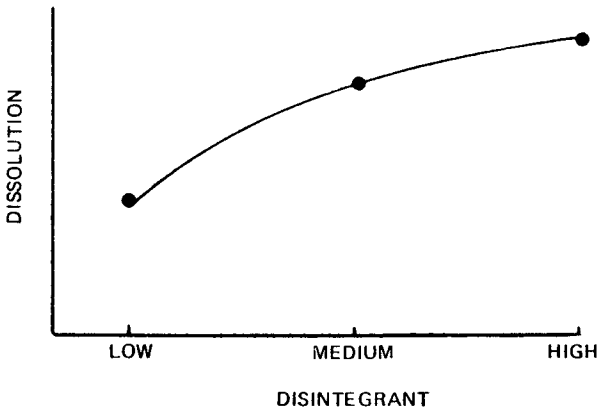


Figure 9.2 Nonlinear (quadratic) effect.

A and B are at their high levels (in a two-level experiment). (It is well worth the extra effort to examine and understand this concept as illustrated in Fig. 9.3.)

If the effect is greater than 15 when both factors are at their high levels, the result is *synergistic* (in biological notation) with respect to the two factors. If the effect is less than 15 when A and B are at their high levels, an *antagonistic* effect is said to exist. In statistical terminology, the lack of additivity is known as *interaction*. In the example above (two factors each at two levels), interaction can be described as the difference between the effects of drug concentration at the two lubricant levels. Equivalently, interaction is also the difference between the effects of lubricant at the two drug levels. More specifically,

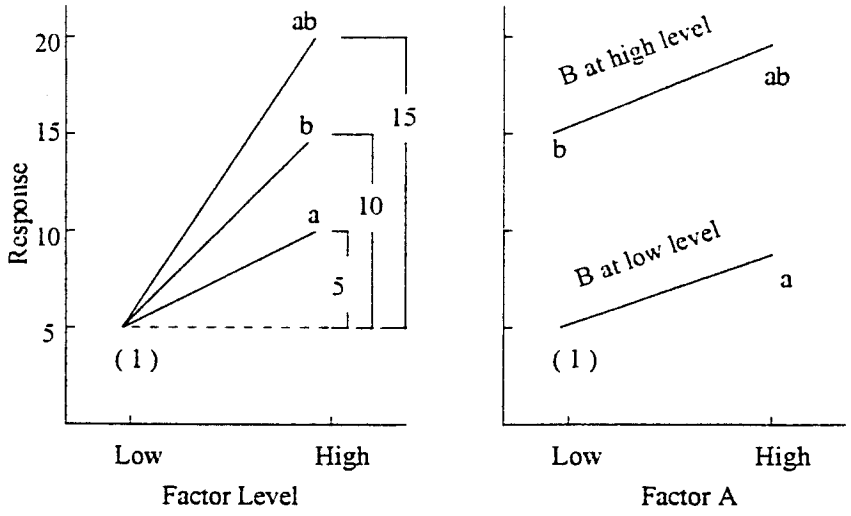


Figure 9.3 Additivity of effects: Lack of interaction.

this means that the drug effect measured when the lubricant is at the low level [a − (1)] is *different* from the drug effect measured when the lubricant is at the high level (ab − b). If the drug effects are the same in the presence of both high and low levels of lubricant, the system is additive, and no interaction exists. Interaction is conveniently shown graphically as depicted in Fig. 9.4. If the lines representing the effect of drug concentration at each level of lubricant are ''parallel,'' there is no interaction. Lack of parallelism, as shown in Fig. 9.4B, suggests interaction. Examination of the lines in Fig. 9.4B reveals that the effect of drug concentration on dissolution is dependent on the concentration of lubricant. The effects of drug and lubricant are not additive.

Factorial designs have many advantages [1]:

1. In the absence of interaction, factorial designs have maximum efficiency in estimating main effects.
2. If interactions exist, factorial designs are necessary to reveal and identify the interactions.
3. Since factor effects are measured over varying levels of other factors, conclusions apply to a wide range of conditions.
4. Maximum use is made of the data since all main effects and interactions are calculated from all of the data (as will be demonstrated below).
5. Factorial designs are orthogonal; all estimated effects and interactions are independent of effects of other factors. Independence, in this context, means that when we estimate a main effect, for example, the result we obtain is due only to the main effect of interest, and is not influenced by other factors in the experiment. In non-orthogonal designs (as is the case in many multiple-regression-type ''fits''—see App. III), effects are not independent. *Confounding* is a result of lack of independence. When an effect is confounded, one cannot assess how

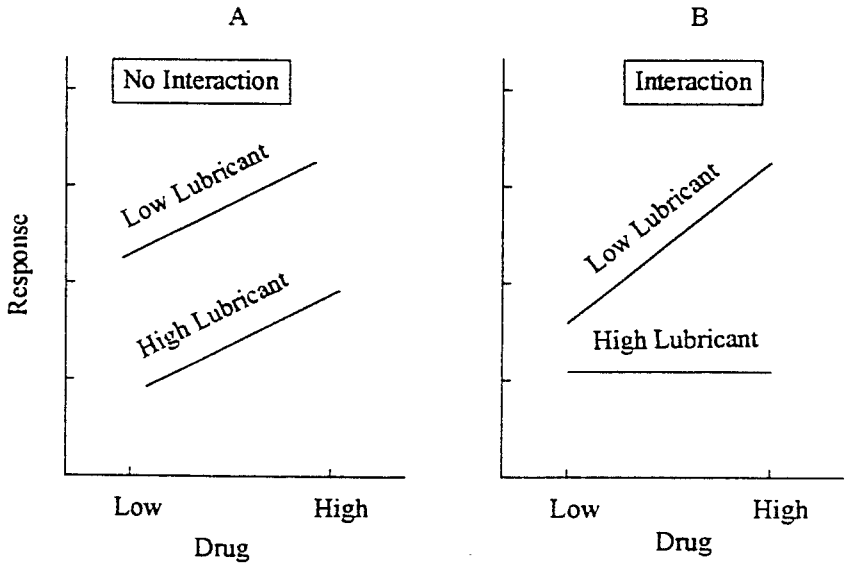


Figure 9.4 Illustration of interaction.

much of the observed effect is due to the factor under consideration. The effect is influenced by other factors in a manner that often cannot be easily unraveled, if at all. Suppose, for example, that two drugs are to be compared, with patients from a New York clinic taking drug A and patients from a Los Angeles clinic taking drug B. Clearly, the difference observed between the two drugs is confounded with the different locations. The two locations reflect differences in patients, methods of treatment, and disease state, which can affect the observed difference in therapeutic effects of the two drugs. A simple factorial design where both drugs are tested in both locations will result in an ''unconfounded,'' clear estimate of the drug effect if designed correctly, e.g., equal or proportional number of patients in each treatment group at each treatment site.

9.2 TWO SIMPLE HYPOTHETICAL EXPERIMENTS TO ILLUSTRATE THE ADVANTAGES OF FACTORIAL DESIGNS

The following hypothetical experiment illustrates the advantage of the factorial approach to experimentation when the effects of multiple factors are to be assessed. The problem is to determine the effects of a special diet and a drug on serum cholesterol levels. To this end, an experiment was conducted in which cholesterol changes were measured in three groups of patients. Group A received the drug, group B received the diet, and group C received both the diet and drug. The results are shown below. The experimenter concluded that there was no interaction between drug and diet (i.e., their effects are additive).

Drug alone: decrease of 10 mg %
Diet alone: decrease of 20 mg %
Diet + drug: decrease of 30 mg %

However, suppose that patients given *neither* drug nor diet would have shown a decrease of serum cholesterol of 10 mg % had they been included in the experiment. (Such a result could occur because of ''psychological effects'' or seasonal changes, for example.) Under these circumstances, we would conclude that drug alone has no effect, that diet results in a cholesterol lowering of 10 mg %, and that the combination of drug and diet is synergistic. The combination of drug and diet results in a decrease of cholesterol equal to 20 mg %. This concept is shown in Fig. 9.5.

Thus, without a fourth group, the control group (low level of diet and drug), we have no way of assessing the presence of interaction. This example illustrates how estimates of effects can be incorrect when pieces of the design are missing. Inclusion of a control group would have completed the factorial design, two factors at two levels. Drug and diet are the factors, each at two levels, either present or absent. The complete factorial design consists of the following four groups:

(1) Group on normal diet without drug (drug and special diet at low level)
a Group on drug only (high level of drug, low level of diet)
b Group on diet only (high level of diet, low level of drug)
ab Group on diet and drug (high level of drug and high level of diet)

The effects and interaction can be clearly calculated based on the results of these four groups (see Fig. 9.5).

Incomplete factorial designs such as those described above are known as the *one-at-a-time* approach to experimentation. Such an approach is usually very *inefficient*. By

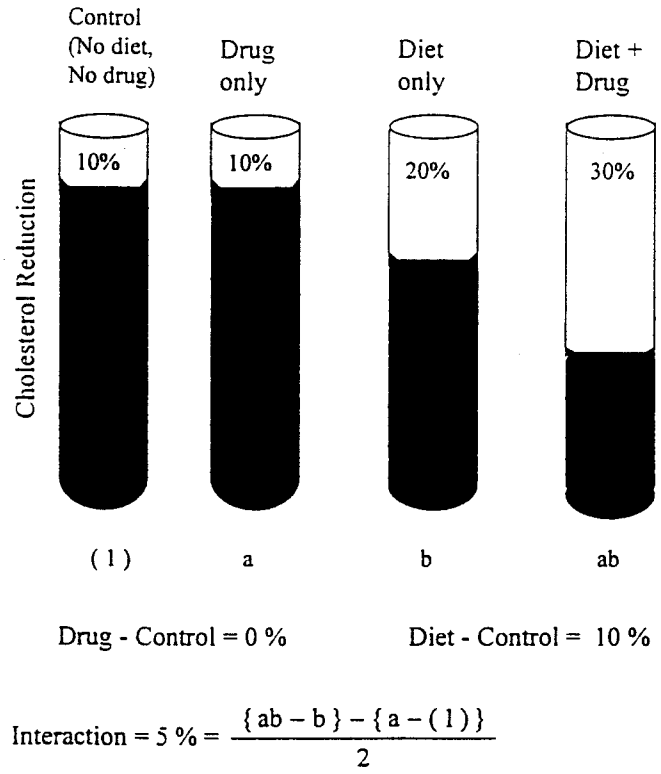


Figure 9.5 Synergism in cholesterol lowering as a result of drug and diet.

performing the entire factorial, we usually have to do *less work*, and we get *more* information. This is a consequence of an important attribute of factorial designs: effects are measured with maximum precision. To demonstrate this property of factorial designs, consider the following hypothetical example. The objective of this experiment is to weigh two objects on an insensitive balance. Because of the lack of reproducibility, we will weigh the items in duplicate. The balance is in such poor condition that the zero point (balance reading with no weights) is in doubt. A typical one-at-a-time experiment is to weigh each object separately (in duplicate) in addition to a duplicate reading with no weights on the balance. The weight of item A is taken as the average of the readings with A on the balance minus the average of the readings with the pans empty. Under the assumption that the variance is the same for all weighings, regardless of the amount of material being weighed, the variance of the weight of A is the sum of the variances of the average weight of A and the average weight with the pans empty (see App. I):

$$\frac{\sigma^2}{2}+\frac{\sigma^2}{2}=\sigma^2 \tag{9.1}$$

Note that the variance of the *difference* of the average of two weighings is the *sum of the variances* of each weighing. (The variance of the average of *two* weighings is $\sigma^2/2$.)

Similarly, the variance of the weight of B is $\sigma^2 = \sigma^2/2 + \sigma^2/2$. Thus, based on six readings (two weighings each with the balance empty, with A on the balance and with B on the balance), we have estimated the weights of A and B with variance equal to σ^2, where σ^2 is the variance of a single weighing.

In a factorial design, an extra reading(s) would be made, a reading with both A and B on the balance. In the following example, using a full factorial design, we can estimate the weight of A with the same precision as above using only 4 weighings (instead of 6). In this case the weighings are made without replication. That is, four weighings are made as follows:

(1)	Reading with balance empty	0.5 kg
a	Reading with item A on balance	38.6 kg
b	Reading with item B on balance	42.1 kg
ab	Reading with both items A and B on balance	80.5 kg

With a full factorial design, as illustrated above, the *weight of A* is estimated as (the main effect of A)

$$\frac{a-(1)+ab-b}{2} \tag{9.2}$$

Expression (9.2) says that the estimate of the weight of A is the average of the weight of A alone minus the reading of the empty balance [a − (1)] and the weight of both items A and B minus the weight of B. According to the weights recorded above, the weight of A would be estimated as

$$\frac{38.6-0.5+80.5-42.1}{2} = 38.25 \text{ kg}$$

Similarly, the weight of B is estimated as

$$\frac{42.1-0.5+80.5-38.6}{2} = 41.75 \text{ kg}$$

Note how we use *all the data* to estimate the weights of A and B; the weight of B alone is used to help estimate the weight of A, and vice versa!

Interaction is measured as the average difference of the weights of A in the presence and absence of B as follows:

$$\frac{(ab-b)-[a-(1)]}{2} \tag{9.3}$$

We can assume that there is no interaction, a very reasonable assumption in the present example. (The weights of the combined items should be the sum of the individual weights.) The estimate of interaction in this example is

$$\frac{(80.5-42.1)-(38.6-0.5)}{2} = 0.3$$

The estimate of interaction is not zero because of the presence of random errors made on this insensitive balance.

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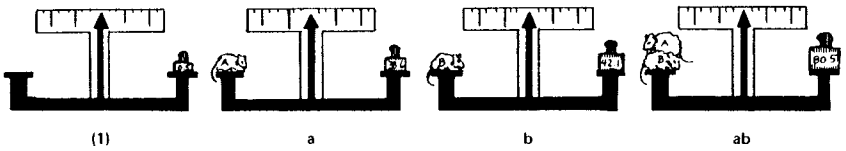
Table 9.1 Eight Experiments for a 2^3 Factorial Design[a]

Combination	A	B	C
(1)	−	−	−
a	+	−	−
b	−	+	−
ab	+	+	−
c	−	−	+
ac	+	−	+
bc	−	+	+
abc	+	+	+

[a] −, factor at low level; +, factor at high level.



In this example, we have made *four* weighings. The variance of the main effects (i.e., the average weights of A and B) is σ^2, *exactly the same variance as was obtained using six weightings in the one-at-a-time experiment!** We obtain the same precision with two-thirds of the work: four readings instead of six. In addition to the advantage of greater precision, if interaction were present, we would have had the opportunity to estimate the interaction effect in the full factorial design. *It is not possible to estimate interaction in the one-at-a-time experiment.*

9.3 PERFORMING FACTORIAL EXPERIMENTS: RECOMMENDATIONS AND NOTATION

The simplest factorial experiment, as illustrated above, consists of four trials, two factors each at two levels. If three factors, A, B, and C, each at two levels, are to be investigated, eight trials are necessary for a full factorial design, as shown in Table 9.1. This is also called a 2^3 experiment, three factors each at two levels.

As shown in Table 9.1, in experiments with factors at two levels, the low and high levels of factors in a particular run are denoted by the absence or presence of the letter, respectively. For example, if all factors are at their low levels, the run is denoted as (1). If factor A is at its high level, and B and C are at their low levels, we use the notation a. If factors A and B are at their high levels, and C is at its low level, we use the notation ab; and so on.

Before implementing a factorial experiment, the researcher should carefully consider the experimental objectives vis-à-vis the appropriateness of the design. The results of a

* The main effect of A, for example, is $[a - (1) + ab - b]/2$. The variance of the main effect is $(\sigma_a^2 + \sigma_{(1)}^2 + \sigma_{ab}^2 + \sigma_b^2)/4 = \sigma^2$. σ^2 is the same for all weighings (App. I).

factorial experiment may be used (a) to help interpret the mechanism of an experimental system; (b) to recommend or implement a practical procedure or set of conditions in an industrial manufacturing situation; or (c) as guidance for further experimentation. In most situations where one is interested in the effect of various factors or conditions on some experimental outcome, factorial designs will be optimal.

The choice of factors to be included in the experimental design should be considered carefully. Those factors not relevant to the experiment, but which could influence the results, should be carefully controlled or kept constant. For example, if the use of different technicians, different pieces of equipment, or different excipients can affect experimental outcomes, but are not variables of interest, they should not be allowed to vary randomly, if possible. Consider an example of the comparison of two analytical methods. We may wish to have a single analyst perform both methods on the same spectrophotometer to reduce the variability that would be present if different analysts used different instruments. However, there will be circumstances where the effects due to different analysts and different spectrophotometers are of interest. In these cases, different analysts and instruments may be designed into the experiment as additional factors.

On the other hand, we may be interested in the effect of a particular factor, but because of time limitations, cost, or other problems, the factor is held constant, retaining the option of further investigation of the factor at some future time. In the example above, one may wish to look into possible differences among analysts with regard to the comparison of the two methods (an analyst $\times$ method interaction). However, time and cost limitations may restrict the extent of the experiment. One analyst may be used for the experiment, but testing may continue at some other time using more analysts to confirm the results.

The more extraneous variables that can be controlled, the smaller will be the residual variation. The residual variation is the random error remaining after the ANOVA removes the variability due to factors and their interactions. If factors known to influence the experimental results, but of no interest in the experiment, are allowed to vary ''willy-nilly,'' the effects caused by the random variation of these factors will become part of the residual error. Suppose the temperature influences the analytical results in the example above. If the temperature is not controlled, the experimental error will be greater than if the experiment is carried out under constant-temperature conditions. The smaller the residual error, the more sensitive the experiment will be in detecting effects or changes in response due to the factors under investigation.

The choice of levels is usually well defined if factors are qualitative. For example, in an experiment where a product supplied by several manufacturers is under investigation, the levels of the factor ''product'' could be denoted by the name of the manufacturer: company X, company Y, and so on. If factors are quantitative, we can choose two or more levels, the choice being dependent on the size of the experiment (the number of trials and the amount of replication) and the nature of the anticipated response. If a response is known to be a linear function of a factor, two levels would be sufficient to define the response. If the response is ''curved'' (a quadratic response for example*), at least three levels of the quantitative factor would be needed to characterize the response. Two levels are often used for the sake of economy, but a third level or more can be used to meet experimental objectives as noted above. A rule of thumb used for the choice of levels in

* A quadratic response is of the form $Y = A + BX + CX^2$, where Y is the response and X is the factor level.

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two-level experiments is to divide extreme ranges of a factor into four equal parts and take the one-fourth (1/4) and three-fourths (3/4) values as the choice of levels [1]. For example, if the minimum and maximum concentrations for a factor are 1% and 5%, respectively, the choice of levels would be 2% and 4% according to this empirical rule.

The trials comprising the factorial experiment should be done in random order if at all possible. This helps ensure that the results will be unbiased (as is true for many statistical procedures). The fact that all effects are averaged over all runs in the analysis of factorial experiments is also a protection against bias.

9.4 A WORKED EXAMPLE OF A FACTORIAL EXPERIMENT

The data in Table 9.2 were obtained from an experiment with three factors each at two levels. There is no replication in this experiment. Replication would consist of repeating each of the eight runs one or more times. The results in Table 9.2 are presented in standard order. Recording the results in this order is useful when analyzing the data by hand (see below) or for input into computers where software packages require data to be entered in a specified or standard order. The standard order for a 2^2 experiment consists of the first four factor combinations in Table 9.2. For experiments with more than three factors, see Davies for tables and an explanation of the ordering [1].

The experiment that we will analyze is designed to investigate the effects of three components (factors)—stearate, drug, and starch—on the thickness of a tablet formulation. In this example, two levels were chosen for each factor. Because of budgetary constraints, use of more than two levels would result in too large an experiment. For example, if one of the three factors were to be studied at three levels, 12 formulations would have to be tested for a $2 \times 2 \times 3$ factorial design. Because only two levels are being investigated, nonlinear responses cannot be elucidated. However, the pharmaceutical scientist felt that the information from this two-level experiment would be sufficient to identify effects that would be helpful in designing and formulating the final product. The levels of the factors in this experiment were as follows:

Table 9.2 Results of 2^3 Factorial Experiment: Effect of Stearate, Drug, and Starch Concentration on Tablet Thickness[a]

Factor combination	Stearate	Drug	Starch	Response (thickness) (cm $\times 10^3$)
(1)	−	−	−	475
a	+	−	−	487
b	−	+	−	421
ab	+	+	−	426
c	−	−	+	525
ac	+	−	+	546
bc	−	+	+	472
abc	+	+	+	522

[a] −, factor at low level; +, factor at high level.

Factor	Low level (mg)	High level (mg)
A: Stearate	0.5	1.5
B: Drug	60.0	120.0
C: Starch	30.0	50.0

The computation of the main effects and interactions as well as the ANOVA may be done by hand in simple designs such as this one. Readily available computer programs are usually used for more complex analyses. (For n factors, an n-way analysis of variance is appropriate. In typical factorial designs, the factors are usually considered to be fixed.)

For two-level experiments, the effects can be calculated by applying the signs (+ or −) arithmetically for each of the eight responses as shown in Table 9.3. This table is constructed by placing a + or − in columns A, B, and C depending on whether or not the appropriate factor is at the high or low level in the particular run. If the letter appears in the factor combination, a + appears in the column corresponding to that letter. For example, for the product combination ab, a + appears in columns A and B, and a − appears in column C. Thus for column A, runs a, ab, ac, and abc have a + because in these runs, A is at the high level. Similarly, for runs (1), b, c, and bc, a − appears in column A since these runs have A at the low level.

Columns denoted by AB, AC, BC, and ABC in Table 9.3 represent the indicated interactions (i.e., AB is the interaction of factors A and B, etc.). The signs in these columns are obtained by multiplying the signs of the individual components. For example, to obtain the signs in column AB we refer to the signs in column A and column B. For run (1), the + sign in column AB is obtained by multiplying the − sign in column A times the − sign in column B. For run a, the − sign in column AB is obtained by multiplying the sign in column A (+) times the sign in column B (−). Similarly, for column ABC, we multiply the signs in columns A, B, and C to obtain the appropriate sign. Thus run ab has

Table 9.3 Signs to Calculate Effects in a 2^3 Factorial Experiment[a]

	Level of factor in experiment			Interaction[b]			
Factor combination	A	B	C	AB	AC	BC	ABC
(1)	−	−	−	+	+	+	−
a	+	−	−	−	−	+	+
b	−	+	−	−	+	−	+
ab	+	+	−	+	−	−	−
c	−	−	+	+	−	−	+
ac	+	−	+	−	+	−	−
bc	−	+	+	−	−	+	−
abc	+	+	+	+	+	+	+

[a] −, factor at low level; +, factor at high level.

[b] Multiply signs of factors to obtain signs for interaction terms in combination [e.g., AB at (1) = (−) × (−) = (+)].

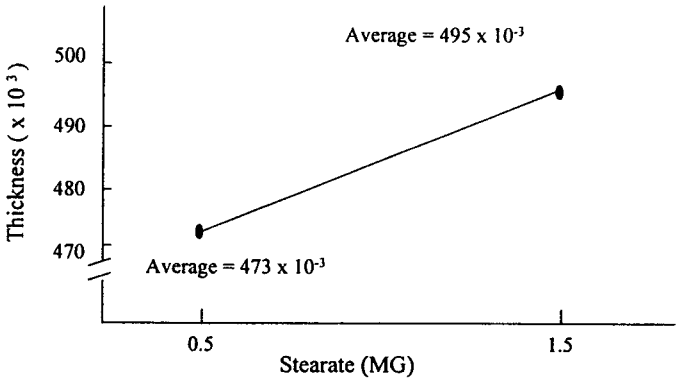

Figure 9.6 Main effect of the factor ''stearate.''

a − sign in column ABC as a result of multiplying the three signs in columns A, B, and C: (+) × (+) × (−).

The average effects can be calculated using these signs as follows. To obtain the average effect, multiply the response times the sign for each of the eight runs in a column, and divide the result by 2^{n-1}, where n is the number of factors (for three factors, 2^{n-1} is equal to 4). This will be illustrated for the calculation of the main effect of A (stearate). The main effect for factor A is

$$\frac{[-(1)+a-b+ab-c+ac-bc+abc]\times 10^{-3}}{4} \tag{9.4}$$

Note that the main effect of A is the average of all results at the high level of A minus the average of all results at the low level of A. This is more easily seen if formula (9.4) is rewritten as follows:

$$\text{Main effect of A} = \frac{a+ab+ac+abc}{4} - \frac{(1)+b+c+bc}{4} \tag{9.5}$$

''Plugging in'' the results of the experiment for each of the eight runs in Eq. (9.5), we obtain

$$\frac{[487+426+546+522-(475+421+525+472)]\times 10^{-3}}{4}$$

$$= 0.022 \text{ cm}$$

The *main effect of A is interpreted* to mean that the net effect of increasing the stearate concentration from the low to the high level (averaged over all other factor levels) is to increase the tablet thickness by 0.022 cm. This result is illustrated in Fig. 9.6.

The interaction effects are estimated in a manner similar to the estimation of the main effects. The signs in the column representing the interaction (e.g., AC) are applied to the eight responses, and as before the total divided by 2^{n-1}, where n is the number of factors. The interaction AC, for example, is defined as one-half the difference between the effect

of A when C is at the high level and the effect of A when C is at the low level (see Fig. 9.7). Applying the signs as noted above, the AC interaction is estimated as

$$\text{AC interaction} = \frac{1}{4}\{(abc + ac - bc - c) - [ab + a - b - (1)]\} \tag{9.6}$$

The interaction is shown in Fig. 9.7. With starch (factor C) at the high level, 50 mg, increasing the stearate concentration from the low to the high level (from 0.5 mg to 1.5 mg) results in an increased thickness of 0.0355 cm.* At the low level of starch, 30 mg, increasing stearate concentration from 0.5 mg to 1.5 mg results in an increased thickness of 0.0085 cm. Thus stearate has a greater effect at the higher starch concentration, a possible starch × stearate interaction.

Lack of interaction would be evidenced by the same effect of stearate at both low and high starch concentrations. In a real experiment, the effect of stearate would not be identical at both levels of starch concentration in the absence of interaction because of the presence of experimental error. The statistical tests described below show how to determine the significance of observed nonzero effects.

The description of interaction is ''symmetrical.'' The AC interaction can be described in two equivalent ways: (a) the effect of stearate is greater at high starch concentrations, or (b) the effect of starch concentration is greater at the high stearate concentration (1.5 mg) compared to its effect at low stearate concentration (0.5 mg). The effect of starch at low stearate concentration is 0.051. The effect of starch at high stearate concentration is 0.078. (Also see Fig. 9.7.)

* (1/2)(abc + ac − bc − c).

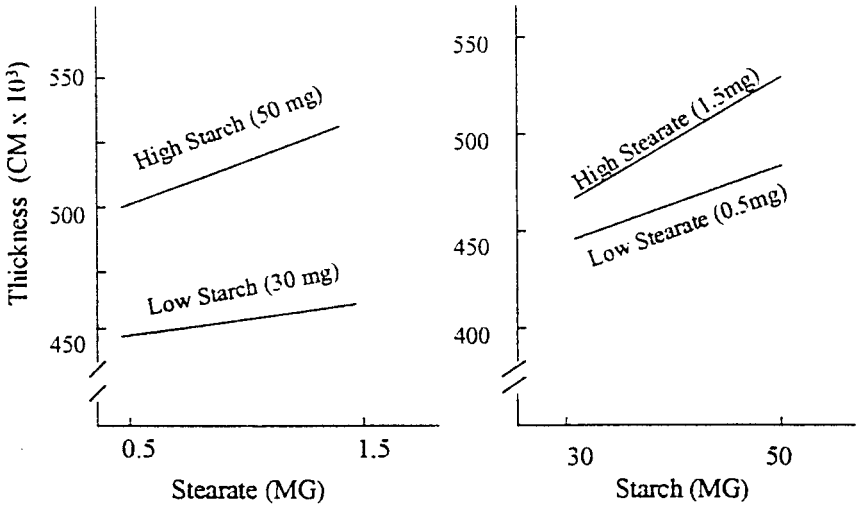

Figure 9.7 Starch × stearate interaction.

9.4.1 Data Analysis

Method of Yates

Computers are usually used to analyze factorial experiments. However, hand analysis of simple experiments can give insight into the properties of this important class of experimental designs. A method devised by Yates for systematically analyzing data from 2^n factorial experiments (n factors each at two levels) is demonstrated in Table 9.4. The data are first tabulated in standard order (see Ref. 1 for experiments with more than two levels). The data are first added in pairs, followed by taking differences in pairs as shown in column (1) in Table 9.4.

$$475 + 487 = 962$$
$$421 + 426 = 847$$
$$525 + 546 = 1071$$
$$472 + 522 = 994$$

$$487 - 475 = 12$$
$$426 - 421 = 5$$
$$546 - 525 = 21$$
$$522 - 472 = 50$$

This addition and subtraction process is repeated sequentially on the n columns. (Remember that n is the number of factors, three columns for three factors.) Thus the process is repeated in column (2), operating on the results in column (1) of Table 9.4. Note, for example, that 1809 in column (2) is 962 + 847 from column (1). Finally, the process is repeated, operating on column (2) to form column (3). Column (3) is divided by 2^{n-1} ($2^{n-1} = 4$ for 3 factors) to obtain the average effect. The mean squares for the ANOVA (described below) are obtained by dividing the square of column (n) by 2^n. For example, the mean square attributable to factor A is

$$\text{Mean square for A} = \frac{(88)^2}{8} = 968$$

Table 9.4 Yates Analysis of the Factorial Tableting Experiment for Analysis Variance

Combination	Thickness ($\times 10^3$)	(1)	(2)	(3)	Effect $(\times 10^3)(3)/4$	Mean square $(\times 10^6)(3)^2/8$
(1)	475	962	1809	3874	—	—
a	487	847	2065	88	22.0	968
b	421	1071	17	−192	−48.0	4608
ab	426	994	71	22	5.5	60.5
c	525	12	−115	256	64.0	8192
ac	546	5	−77	54	13.5	364.5
bc	472	21	−7	38	9.5	180.5
abc	522	50	29	36	9.0	162

The mean squares are presented in an ANOVA table, as discussed below.

Analysis of Variance

The results of a factorial experiment are typically presented in an ANOVA table, as shown in Table 9.5. In a 2^n factorial, each effect and interaction has 1 degree of freedom. The error mean square for statistical tests and estimation) can be estimated in several ways for a factorial experiment. Running the experiment with replicates is best. Duplicates are usually sufficient. However, replication may result in an inordinately large number of runs. Remember that replicates do not usually consist of replicate analyses or observations on the same run. A true replicate usually is obtained by repeating the run, from ''scratch.'' For example, in the 2^3 experiment described above, determining the thickness of several tablets from a single run [e.g., the run denoted by a (A at the high level)] would probably not be sufficient to estimate the experimental error in this system. The proper replicate would be obtained by preparing a new mix with the same ingredients, retableting, and measuring the thickness of tablets in this new batch.* In the absence of replication, experimental error may be estimated from prior experience in systems similar to that used in the factorial experiment. To obtain the error estimate from the experiment itself is always most desirable. Environmental conditions in prior experiments are apt to be different from those in the current experiment. In a large experiment, the experimental error can be estimated without replication by pooling the mean squares from higher-order interactions (e.g., three-way and higher-order interactions) as well as other interactions known to be absent, a priori. For example, in the tableting experiment, we might average the mean squares corresponding to the two-way interactions, AB and BC, and the three-way ABC interaction, if these interactions were known to be zero from prior considerations. The error estimated from the average of the AB, BC, and ABC interactions is

$$(60.5+180.5+162)\times\frac{10^{-6}}{3}=134.2\times10^{-6}$$

* If the tableting procedure in the different runs were identical in all respects (with the exception of tablet ingredients), replicates within each run would be a proper estimate of error.

Table 9.5 Analysis of Variance for the Factorial Tableting Experiment

Factor	Source	d.f.	Mean square ($\times10^6$)	F[a]
A	Stearate	1	968	7.2[b]
B	Drug	1	4608	34.3[c]
C	Starch	1	8192	61.0[c]
AB	Stearate × drug	1	60.5	
AC	Stearate × starch	1	364.5	2.7
BC	Drug × starch	1	180.5	
ABC	Stearate × drug × starch	1	162	

[a] Error mean square based on AB, BC, and ABC interactions, 3 d.f.
[b] $P < 0.1$.
[c] $P < 0.01$.

with 3 degrees of freedom (assuming that these interactions do not exist).

Interpretation

In the absence of interaction, the main effect of a factor describes the change in response when going from one level of a factor to another. If a large interaction exists, the main effects corresponding to the interaction do not have much meaning as such. Specifically, an AC interaction suggests that the effect of A depends on the level of C and a description of the results should specify the change due to A at each level of C. Based on the mean squares in Table 9.5, the effects which are of interest are A, B, C, and AC. Although not statistically significant, stearate and starch interact to a small extent, and examination of the data is necessary to describe this effect (see Fig. 9.7). Since B does not interact with A or C, it is sufficient to calculate the effect of drug (B), averaged over all levels of A and C, to explain its effect. The effect of drug is to *decrease* the thickness by 0.048 mm when the drug concentration is raised from 60 mg to 120 mg [Table 9.4, column (3)/4].

9.5 FRACTIONAL FACTORIAL DESIGNS

In an experiment with a large number of factors and/or a large number of levels for the factors, the number of experiments needed to complete a factorial design may be inordinately large. For example, a factorial design with 5 factors each at 2 levels requires 32 experiments; a 3-factor experiment each at 3 levels requires 27 experiments. If the cost and time considerations make the implementation of a full factorial design impractical, fractional factorial experiments can be used in which a fraction (e.g., $\frac{1}{2}$, $\frac{1}{4}$, etc.) of the original number of experiments can be run. Of course, something must be sacrificed for the reduced work. If the experiments are judiciously chosen, it may be possible to design an experiment so that effects which we believe are negligible are confounded with important effects. (The word ''confounded'' has been noted before in this chapter.) In fractional factorial designs, the negligible and important effects are indistinguishable, and thus confounded. This will become clearer in the first example.

To illustrate some of the principles of fractional factorial designs, we will discuss and present an example of a fractional design based on a factorial design where each of 3 factors is at 2 levels, a 2^3 design. Table 9.3 shows the 8 experiments required for the full design. With the full factorial design, we can estimate 7 effects from the 8 experiments, the 3 main effects (A, B, and C), and the 4 interactions (AB, AC, BC, and ABC). In a $\frac{1}{2}$ replicate fractional design, we perform 4 experiments, but we can only estimate 3 effects. With 3 factors, a $\frac{1}{2}$ replicate can be used to estimate the main effects, A, B, and C. The following procedure is used to choose the 4 experiments.

Table 9.6 shows the 4 experiments that define a 2^2 factorial design using the notation described in Sec. 9.3.

Table 9.6 2^2 Factorial Design

Experiment	A level	B level	AB
(1)	−	−	+
a	+	−	−
b	−	+	−
ab	+	+	+

To construct the ½ replicate with 3 factors, we equate one of the effects to the third factor. In the 2^2 factorial, the interaction, AB is equated to the third factor, C. Table 9.7 describes the ½ replicate design for 3 factors. The 4 experiments consist of (1) c at the high level (a, b at the low level); (2) a at the high level (b, c at the low level); (3) b at the high level (a, c at the low level); and (4) a, b, c all at the high level.

From Table 9.7, we can define the confounded effects, also known as aliases. An effect is defined by the signs in the columns of Table 9.7. For example, the effect of A is

$$(a + abc) - (c + b)$$

Note that the effect of A is exactly equal to BC. Therefore, BC and A are confounded (they are aliases). Also note that C = AB (by definition) and B = AC. Thus, in this design the main effects are confounded with the two factor interactions. This means that the main effects cannot be clearly interpreted if interactions are not absent or negligible. If interactions are negligible, this design will give fair estimates of the main effects. If interactions are significant, this design is not recommended.

Example 1: Davies [1] gives an excellent example of weighing 3 objects on a balance with a zero error in a ½ replicate of a 2^3 design. This illustration is used because interactions are zero when weighing two or more objects together (i.e., the weight of two or more objects is the sum of the individual weights). The three objects are denoted as A, B, and C; the high level is the presence of the object to be weighed, and the low level is the absence of the object. From Table 9.7, we would perform 4 weighinings: A alone, B alone, C alone, and A, B, and C together (call this ABC).

1. The weight of A is the [weight of A + the weight of ABC − the weight of B − weight of C]/2.
2. The weight of B is the [weight of B + the weight of ABC − the weight of A − weight of C]/2.
3. The weight of C is the [weight of C + the weight of ABC − the weight of A − weight of B]/2.

As noted by Davies, this illustration is not meant as a recommendation of how to weigh objects, but rather to show how the design works in the absence of interaction. (See Exercise Problem 5 as another way to weigh these objects using a ½ replicate fractional factorial design.)

Example 2. A ½ replicate of a 2^4 experiment: Chariot et al. [5] reported the results of a factorial experiment studying the effect of processing variables on extrusion-spheronization of wet powder masses. They identified 5 factors each at 2 levels, the full factorial

Table 9.7 One-Half Replicate of 2^3 Factorial Design

Experiment	A level	B level	C = AB	AC	BC
c	−	−	+	−	−
a	+	−	−	−	+
b	−	+	−	+	−
abc	+	+	+	+	+

requiring 32 experiments. Initially, they performed a ¼ replicate, requiring 8 experiments. One of the factors, extrusion speed, was not significant. To simplify this discussion, we will ignore this factor for our example. The design and results are shown in Table 9.8. A = spheronization time, B = spheronization speed, C = spheronization load, and D = extrusion screen.

Note the confounding pattern shown in Table 9.8. The reader can verify these confounded effects (see Exercise Problem 6 at the end of this chapter). Table 9.8 was constructed by first setting up the standard 2^3 factorial (Table 9.3) and substituting D for the ABC interaction. For the estimated effects to have meaning, the confounded effects should be small. For example, if BC and AD were both significant, the interpretation of BC and/or AD would be fuzzy.

To estimate the effects, we add the responses multiplied by the signs in the appropriate column and divide by 4. For example, the effect of AB is

$$[75.5 + 55.5 - 92.8 - 45.4 - 19.7 + 11.1 + 55.0]/4 = -1.825$$

Estimates of the other effects are (see Exercise Problem 7)

A = +23.98
B = −12.03
C = +2.33
D = −34.78
AB = −1.83
AC = +21.13
AD = +10.83

We cannot perform tests for the significance of these parameters without an estimate of the error (variance). The variance can be estimated from duplicate experiments, nonexistent interactions, or experiments from previous studies, for example. Based on the estimate above, factor A, D, and AC are the largest effects. To help clarify the possible confounding

Table 9.8 One-Half Replicate of 2^4 Factorial Design (Extrusion–Spheronization of Wet Powder Masses)

	Parameter							
Experiment	A (min)	B (rpm)	C (kg)	D (mm)	AB[a] = CD	AC = BD	AD = BC	Response
(1)	−	−	−	−	+	+	+	75.5
ab	+	+	−	−	+	−	−	55.5
ac	+	−	+	−	−	+	−	92.8
ad	+	−	−	+	−	−	+	45.4
bc	−	+	+	−	−	−	+	46.5
bd	−	+	−	+	−	+	−	19.7
cd	−	−	+	+	+	−	−	11.1
abcd	+	+	+	+	+	+	+	55.0

[a] Illustrates confounding.

effects, 8 more experiments can be performed. For example, the large effect observed for the interaction AC, spheronization time × spheronization load could be exaggerated due to the presence of a BD interaction. Without other insights, it is not possible to separate these 2 interactions (they are aliases in this design). Therefore, this design would not be desirable if the nature of these interactions are unknown. Data for the 8 further experiments that complete the factorial design are given in Exercise Problem 8.

The conclusions given by Chariot et al. are

1. Spheronization time (factor A) has a positive effect on the production of spheres.
2. There is a strong interaction between factors A and C (spheronization time × spheronization load). Note that the BD interaction is considered to be small.
3. Spheronization speed (factor B) has a negative effect on yield.
4. The interaction between spheronization speed and spheronization load (BC) appears significant. The AD interaction is considered to be small.
5. The interaction between spheronization speed and spheronization time (AB) appears to be insignificant. The CD interaction is considered to be small.
6. Extrusion screen (D) has a very strong negative effect.

Table 9.9 presents some fractional designs with up to 8 observations. To find the aliases (confounded effects), multiply the defining contrast in the table by the effect under consideration. Any letter that appears twice is considered to be equal to 1. The result is the confounded effect. For example, if the defining contrast is $-ABC$ and we are interested in the alias of A, we multiply $-ABC$ by $A = -A^2BC = -BC$. Therefore, A is confounded with $-BC$. Similarly, B is confounded with $-AC$ and C is confounded with $-AB$.

9.6 SOME GENERAL COMMENTS

As noted previously, experiments need not be limited to factors at two levels, although the use of two levels is often necessary to keep the experiment at a manageable size. Where factors are quantitative, experiments at more than two levels may be desirable when curvature of the response is anticipated. As the number of levels increase, the size of the experiment increases rapidly and fractional designs are recommended.

The theory of factorial designs is quite fascinating from a mathematical viewpoint. Particularly, the algebra and arithmetic lead to very elegant concepts. For those readers interested in pursuing this topic further, the book *The Design and Analysis of Industrial Experiments*, edited by O. L. Davies, is indispensable [1]. This topic is also discussed in some detail in Ref. 2. Applications of factorial designs in pharmaceutical systems have appeared in the recent pharmaceutical literature. Plaizier-Vercammen and De Neve investigated the interaction of povidone with low-molecular-weight organic molecules using a factorial design [3]. Bolton has shown the application of factorial designs to drug stability studies [4]. Ahmed and Bolton optimized a chromatographic assay procedure based on a factorial experiment (7).

KEY TERMS

Additivity
Aliases
Confounding
Main effect
One-at-a-time experiment
Replication

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Table 9.9 Some Fractional Designs for Up to 5 Factors

Observations	Factors	Fraction of full factorial	Defining contrast	Confounding	Design
4	3	1/2	−ABC	Main effects confused with 2-way interactions	(1), ab, ac, bc
8	4	1/2	ABCD	Main effects and three 2-way interactions are not confused	(1), ab, ac, bc, ad, bd, cd, abcd
8	5	1/4	−BCE −ADE	Main effects confused with 2-way interactions; see references note below	(1), ad, bc, abcd, abe, bde, ace, cde
16	5	1/2	ABCDE	Main effects and 2-factor interactions are not confused	(1), ab, ac, bc, ad, bd, cd, abcd, ae, be, ce, abce, de, abde, acde, bcde

See References 1 and 6 for more detailed discussion and other designs.

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Effects
Factor
Fractional factorial designs
Half replicate
Interaction
Level
Residual variation
Runs
Standard order
2^n factorials
Yates analysis

EXERCISES

1. A 2^2 factorial design was used to investigate the effects of stearate concentration and mixing time on the hardness of a tablet formulation. The results below are the averages of the hardness of 10 tablets. The variance of an average of 10 determinations was estimated from replicate determinations as 0.3 (d.f. = 36). This is the error term for performing statistical tests of significance.

Mixing time (min)	Stearate 0.5%	Stearate 1%
15	9.6 (1)	7.5 (a)
30	7.4 (b)	7.0 (ab)

   (a) Calculate the ANOVA and present the ANOVA table.
   (b) Test the main effects and interaction for significance.
   (c) Graph the data showing the possible AB interaction.
2. Show how to calculate the effect of increasing stearate concentration at low starch level for the data in Table 9.2. The answer is an increased thickness of 0.085 cm. Also, compute the drug × starch interaction.
3. The end point of a titration procedure is known to be affected by (1) temperature, (2) pH, and (3) concentration of indicator. A factorial experiment was conducted to estimate the effects of the factors. Before the experiment was conducted, all interactions were thought to be negligible except for a pH × indicator concentration interaction. The other interactions are to be pooled to form the error term for statistical tests. Use the Yates method to calculate the ANOVA based on the following assay results:

Factor combination	Recovery (%)	Factor combination	Recovery (%)
(1)	100.7	c	99.9
a	100.1	ac	99.6
b	102.0	bc	98.5
ab	101.0	abc	98.1

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(a) Which factors are significant?
(b) Plot the data to show main effects and interactions which are significant.
(c) Describe, in words, the BC interaction.

4. A clinical study was performed to assess the effects of a combination of ingredients to support the claim that the combination product showed a synergistic effect compared to the effects of the two individual components. The study was designed as a factorial with each component at two levels.

   Ingredient A: low level, 0; high level, 5 mg
   Ingredient B: low level, 0; high level, 50 mg

   Following is the analysis of variance table:

Source	d.f.	MS	F
Ingredient A	1	150	12.5
Ingredient B	1	486	40.5
A × B	1	6	0.5
Error	20	12	

   The experiment consisted of observing six patients in each cell of the 2^2 experiment. One group took placebo with an average result of 21. A second group took ingredient A at a 5-mg dose with an average result of 25. The third group had ingredient B at a 50-mg dose with an average result of 29, and the fourth group took a combination of 5 mg of A and 50 mg of B with a result of 35. In view of the results and the ANOVA, discuss arguments for or against the claim of synergism.

5. The 3 objects in the weighing experiment described in Sec. 9.5, Example 1, may also be weighed using the other 4 combinations from the 2^3 design not included in the example. Describe how you would weigh the 3 objects using these new 4 weighings. (Note that these combinations comprise a 1/2 replicate of a fractional factorial with a different confounding pattern from that described in Sec. 9.5. [Hint: See Table 9.9.]
6. Verify that the effects (AB = CD, AC = BD, and AD = BC) shown in Table 9.8 are confounded.
7. Compute the effects for the data in Sec. 9.5, example 2 (Table 9.8).

**8. In example 2 in Sec. 9.5 (Table 9.8), eight more experiments were performed with the following results:

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Experiment	Response
a	78.7
b	56.9
c	46.7
ab	21.2
abc	67.0
abd	29.0
acd	34.9
bcd	1.2

Using the entire 16 experiments (the 8 given here plus the 8 in Table 9.8), analyze the data as a full 2^4 factorial design. Pool the 3-factor and 4-factor interactions (5 d.f.) to obtain an estimate of error. Test the other effects for significance at the 5% level. Explain and describe any significant interactions.

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16

OPTIMIZATION TECHNIQUES AND SCREENING DESIGNS**

The optimization of pharmaceutical formulations with regard to one or more attributes has always been a subject of importance and attention for those engaged in formulation research. Product formulation is often considered an art, the formulator's experience and creativity providing the ''raw material'' for the creation of a new product. Given the same active ingredient and a description of the final marketed product, two different scientists will very likely concoct different formulations. Certainly, human input is an essential ingredient of the creative process. In addition to the *art* of formulation, techniques are available that can aid the scientist's choice of formulation components which will optimize one or more product attributes. These techniques have been traditionally applied in the chemical and food industries, for example, and in recent years have been applied successfully to pharmaceutical formulations. In this chapter we describe the application of factorial designs (and modified factorials) and simplex lattice designs to formulation optimization. When the effects of factors on a pharmaceutical process or response are unknown, the use of screening designs to estimate factor effects may be indicated.

16.1 INTRODUCTION

The pharmaceutical scientist has the responsibility to choose and combine ingredients that will result in a formulation whose attributes conform with certain prerequisite requirements. Often, the choice of the nature and quantities of additives (excipients) to be used in a new formulation is based on experience, for example, similar products previously prepared by the scientist or his or her colleagues. To break habits based on experience and tradition is difficult. Although there is much to be said for the practical experience of many years, we often become caught in the web of the past. The application of formulation optimization techniques is relatively new to the practice of pharmacy. When used intelli-

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gently, with common sense, these ''statistical'' methods will broaden the perspective of the formulation process.

Although several optimization procedures are available to the pharmaceutical scientist, a few frequently used methods will be presented in this chapter. The objective is to produce a mathematical model that describes the responses. In general, the procedure consists of preparing a series of formulations, varying the concentrations of the formulation ingredients in some systematic manner. These formulations are then evaluated according to one or more attributes, such as hardness, dissolution, appearance, stability, taste, and so on. Based on the results of these tests, a particular formulation (or series of formulations) may be predicted to be optimal. The ''proof of the pudding,'' however, is actually to prepare and evaluate the predicted *optimal* formulation.

If the formulation is optimized according to a single attribute, the optimization procedure is relatively uncomplicated. To optimize on the basis of two or more attributes, dissolution and hardness, for example, may not be possible. The formulation that is optimal for one attribute very well may be different from the formulation needed to optimize other attributes. In these cases, a compromise must be made, depending on the relative importance of each attribute. The final formulation, therefore, is suitably modified to attain an acceptable performance of all relevant attributes, if possible. We will discuss the optimization procedure based on a single attribute. More complex situations may require more complex designs, and the advice of an experienced statistician is recommended in these cases. Therefore, the use of the term, ''optimization'' may be a misnomer. An optimal response may not be a single response, but a region of responses that satisfy the requirements of the formulation. Once such a region is defined, the desired response may be defined using a range of factors.

In general, an advanced understanding of statistics is not necessary. One should be familiar with the following concepts as described elsewhere in this book.

16.1.1 Planning Experiments

Common sense should prevail. Design and choice of variables are discussed later in this chapter. In most cases, we have a reasonable idea of which variables are important, and their effective ranges. But, we may be surprised. If everything were known, we would not have to experiment. Also, we should be careful not to neglect potentially important variables. Screening designs may be useful if little is known of the system

16.1.2 Variables

Variables may be considered as Independent and Dependent (X,Y). Dependent variables (Y) are outcome variables (e.g., dissolution). Independent variables (X) are set in advance (e.g., lubricant level). Variables can be continuous or discrete. The number of experiments should be kept at a reasonable level. The more variables used, the more knowledge is gained, but expense and time should be taken into consideration.

16.1.3 Variability or Experimental Error

It is important to have an idea about variability of response (Y) and/or ''predicted response.'' Replication is typically needed to estimate variability, but this adds time and cost to the study. Estimates of variance can be obtained from replication, from ANOVA or from experience.

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16.1.4 Regression

For our purposes, regression is used to predict Responses, and/or to describe relationships. Either simple linear or multiple regression may be used to obtain optimized systems. We derive a response equation from the data (as described in this chapter), and predict a response within the bounds of the fixed independent variables, X. Prediction outside of the bounds of the independent variables are unreliable. Consider the following example.

Suppose that the theoretical response relationship (Y as a function of X_1 and X_2, where we have two independent variables) is $Y = 5 + 6X_1 + 7X_1^2 + 3X_2$. We obtain six values of Y as follows:

X_1	X_2	Y
1	1	21
2	1	48
1	2	24
2	2	57
3	1	89
1	3	45

Using multiple regression we obtain the following equation relating Y to the independent variables.

$$Y = -7 + 7.2X_1 + 7X_1^2 + 11.4X_2$$

This works well within the experimental space. But predictions outside are questionable. For example, if $X_1 = 4$ and $X_2 = 4$

Predicted = 179.4

Actual = 153

16.2 OPTIMIZATION USING FACTORIAL DESIGNS

The basic principles of factorial designs have been presented in Chapter 9. In factorial designs, levels of factors are independently varied, each factor at two or more levels. The effects that can be attributed to the factors and their interactions are assessed with maximum efficiency in factorial designs. Also, factorial designs allow for the estimation of the effects of each factor and interaction, unconfounded by the other experimental factors. Thus, if the effect of increasing stearic acid by 1 mg is to decrease the dissolution by 10%, in the absence of interactions, this effect is independent of the levels of the other factors. This is an important concept. If the levels of factors are allowed to vary haphazardly, as in an undesigned experiment, the observed effect due to any factor is dependent on the levels of the other varying factors. Generalities, or predictions, based on results of an undesigned experiment will be less reliable than those which would be obtained in a designed experiment, in particular, a factorial design. Screening designs use less runs, and estimate the main effects of factors. The latter part of this chapter will introduce screening designs. These designs are useful when a relatively large number of factors may affect the response or process. From a regulatory viewpoint, the data derived from factorial designs can be

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useful to predict responses when confronted with formulation or manufacturing modifications.

The optimization procedure is facilitated by construction of an equation that describes the experimental results as a function of the factor levels. A *polynomial* equation can be constructed, in the case of a factorial design, where the coefficients in the equation are related to the effects and interactions of the factors. For the present, we will restrict our discussion to factorial designs with factors at only two levels, called 2^n factorials, where n is the number of factors (see Chapter 9). These designs are simplest and often are adequate to achieve the experimental objectives. These designs estimate only linear effects. That is, if there is a curved response as a function of factor levels or combination, such effects will be missed. Sometimes, use of these smaller designs is imperative, for the sake of economy. Increasing the number of factor levels dramatically increases the number of formulations that are needed to complete the design. With a large number of factors, even designs where factors are restricted to two levels may result in a very large number of formulations to be prepared and tested. In such cases, *fractional* factorial designs may be used. Some information is lost when using fractional factorial designs, but one-half, one-fourth, or less of the formulations are needed compared to those needed to run a full factorial design. A brief description of fractional factorial designs is presented in Sec. 9.5. The theory and construction of these designs are presented in detail in *The Design and Analysis of Industrial Experiments*, edited by O. L. Davies [1]. Also see Ref. 12 for an example of optimization applied to an HPLC analytical method.

As noted above, the optimization procedure is facilitated by the fitting of an empirical polynomial equation to the experimental results. The equation constructed from a 2^n factorial experiment is of the following form:

$$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + \cdots + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{23}X_2X_3 + \cdots + B_{123}X_1X_2X_3 + \cdots \tag{16.1}$$

where Y is the measured response, X_i is the level (e.g., concentration) of the ith factor, B_i, B_{ij}, B_{ijk}, $\cdots$ represent coefficients computed from the responses of the formulations in the design, as will be described below. (B_0 represents the intercept.)

For example, in an experiment with three factors, each at two levels, we have eight formulations, a total of eight responses. The eight coefficients in Eq. (16.1) will be determined from the eight responses in such a way that each of the responses will be exactly predicted by the polynomial equation. For the present, to illustrate this concept we will look at the problem in reverse. Suppose that we already have an equation to predict the experimental results derived from a factorial design as follows:

$$Y = 5 + 2(X_1) + 3(X_2) + X_3 - 0.6(X_1X_2) - 0.4(X_1X_3) + 0.7(X_2X_3) + 0.12(X_1X_2X_3) \tag{16.2}$$

From Eq. (16.2), we can reconstruct the original data from the 2^3 experiment. Suppose that the levels (in mg) of the three factors in the design were as follows:

	Low level	High level
X_1 = stearate	0	2
X_2 = colloidal silica	0	1
X_3 = drug	0	5

Based on Eq. (16.2), the formulation with all factors at the low level will have a response of five. All factors are equal to 0, and all terms containing X_1, X_2, or X_3 are equal to 0. If X_1 is at the high level (2 mg), and X_2, and X_3 are at the low level (0), the predicted response is $Y = 5 + 2(X_1) = 5 + 2(2) = 9$. All other terms are equal to 0. If X_1 and X_2 are at the high level, and X_3 is at the low level, the response is

$$5 + 2(X_1) + 3(X_2) - 0.6(X_1X_2) = 5 + 2(2) + 3(1) - 0.6(2)(1) = 10.8$$

The results for all eight combinations (formulations) as predicted from Eq. (16.2) are shown in (Table 16.1).

Table 16.1 shows the results of the factorial experiment which were used to construct Eq. (16.2). The practical, more realistic problem is to construct the polynomial equation, given the experimental results. To solve this problem, we find the solution to eight equations with eight unknowns [the unknowns are the eight coefficients in Eq. (16.2)]. For example, in formulation 1 (Table 16.1),

$$X_1 = X_2 = X_3 = 0$$

Substituting $X_1 = X_2 = X_3 = 0$ into the general equation [Eq. (16.1)] results in

$$Y = B_0 \text{ (all other terms are 0)}$$

Since the response (Y) for formulation 1 (where $X_1 = X_2 = X_3 = 0$) is equal to 5,

$$Y = B_0 = 5$$

This is the simple solution for the first of the simultaneous equations.

In the second formulation, $X_1 = 2$, X_2 and X_3 are equal to 0 and Eq. (16.1) reduces to

$$Y = B_0 + B_1X_1 \text{ (all other terms are 0)} \tag{16.3}$$

The response, Y, for formulation 2 is 9 (Table 16.1). We can solve for B_1, using Eq. (16.3) ($B_0 = 5$ and $X_1 = 2$)

Table 16.1 Results of the 2^3 Factorial Experiment Which Led to the Construction of the Polynomial Equation (16.2)

Formulation	Factor level			Predicted response, Y
	X_1	X_2	X_3	
1	0	0	0	5
2	2	0	0	9
3	0	1	0	8
4	2	1	0	10.8
5	0	0	5	10
6	2	0	5	10
7	0	1	5	16.5
8	2	1	5	16.5

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$$9 = 5 + B_1(2) \qquad B_1 = 2$$

This procedure is continued, until we solve for all coefficients, B_i, B_{ij}, B_{ijk}, and so on.

In the example above, the solution for the coefficients for the polynomial equation is very simple, because the low level of all factors is zero. In general, the solution would be more difficult if the low level of all factors is not equal to zero. However, the general solution for the polynomial coefficients is not difficult for 2^n factorial designs, because of the independence (orthogonality) inherent in factorial designs. The first step in the solution is to code the levels of the factors so that the high level of each factor is $+1$, and the low level of each factor is -1. This procedure requires a transformation of each of the three variables, X_1, X_2, and X_3 to X'_1, X'_2, and X'_3, respectively, as follows:

For X_1, *let* $X'_1 = X_1 - 1$. Note that when $X_1 = 2$ (the high level), $X'_1 = +1$, and when $X_1 = 0$ (the low level), $X'_1 = -1$.
For X_2, *let* $X'_2 = 2X_2 - 1$.
For X_3, *let* $X'_3 = (2X_3 - 5)/5$.

In general, the formula for the transformation is

$$\frac{X - \text{the average of the two levels}}{\text{one-half the difference of the levels}} \tag{16.4}$$

After the transformation, the levels of the factors are as shown in Table 16.2 (see also Chapter 9).

Table 16.2 also contains ''transformed'' values for the interactions, represented by $+1$ or -1. These values are obtained by multiplying the values in the appropriate columns of X_1, X_2, and X_3. For example, in formulation 1, X_1X_2 is represented by $+1$, the product of -1 for X_1 and -1 for X_2 [$X_1X_2 = (-1)(-1) = +1$]. $X_1X_2X_3$ is represented by the product of $(-1)(-1)(-1) = -1$, derived from the values in the columns headed by X_1, X_2, and X_3. (See also Chapter 9 to clarify this procedure.) The ''total'' column contains only the value $+1$, and is used to calculate the intercept, B_0.

The coefficients for the polynomial equation (16.1) are calculated as $\Sigma\ XY/8$ ($\Sigma\ XY/2^n$, in general), where X is the value ($+1$ or -1) in the *column* appropriate for the coefficient being calculated, and Y is the response. An example should make the calculation

Table 16.2 Transformed Levels of Factors Showing Signs to Be Used to Determine Effects and Polynomial Coefficients

Formulation	X_1	X_2	X_3	X_1X_2	X_1X_3	X_2X_3	$X_1X_2X_3$	Total	Y
1[a]	−1	−1	−1	+1	+1	+1	−1	+1	5
2	+1	−1	−1	−1	−1	+1	+1	+1	9
3	−1	+1	−1	−1	+1	−1	+1	+1	8
4	+1	+1	−1	+1	−1	−1	−1	+1	10.8
5	−1	−1	+1	+1	−1	−1	+1	+1	10
6	+1	−1	+1	−1	+1	−1	−1	+1	10
7	−1	+1	+1	−1	−1	+1	−1	+1	16.5
8	+1	+1	+1	+1	+1	+1	+1	+1	16.5

[a] Note that X_1, X_2, and X_3 are at their low levels (0). Transformed values are -1, -1, and -1.

clear. For the coefficient corresponding to X_1 (B_1), the calculation is performed as follows. We multiply each value in the column headed X_1 ($+1$ or -1) by the corresponding response, Y. The sum of these products ($\Sigma\, XY$) divided by 8 (2^n) is the coefficient, B_1.

$$[(-1)(5)+(+1)(9)+(-1)(8)+(+1)(10.8)+(-1)(10)+(+1)(10)$$
$$+(-1)(16.5)+(+1)(16.5)] = \frac{6.8}{8} = 0.85$$

The coefficient, B_2, is calculated using the values ($+1$ or -1) in the second column, the X_2 column.

$$[(-1)(5)+(-1)(9)+(+1)(8)+(+1)(10.8)+(-1)(10)$$
$$+(-1)(10)+(+1)(16.5)+(+1)(16.5) = \frac{17.8}{8} = 2.225$$

The coefficient for $X_1X_2X_3$ is B_{123}, and is calculated using the values in the column headed by $X_1X_2X_3$ as follows.

$$[(-1)(5)+(+1)(9)+(+1)(8)+(-1)(10.8)+(+1)(10)$$
$$+(-1)(10)+(-1)(16.5)+(+1)(16.5)] = \frac{1.2}{8} = 0.15$$

All of the coefficients are calculated in this manner. B_0 is the sum of all of the observations, Y, divided by 8 (10.725).* (Note that all of the values in the ''total'' column are $+1$; this column is used to obtain B_0 in the same manner as the other coefficients.) The final polynomial equation for predicting the response, Y, is

$$Y = 10.725 + 0.85(X_1) + 2.225(X_2) + 2.525(X_3)$$
$$-0.15(X_1X_2) - 0.85(X_1X_3) + 1.025(X_2X_3) + 0.15(X_1X_2X_3) \tag{16.5}$$

This equation looks entirely different from Eq. (16.2), which also predicts the responses in this experiment. However, the two equations predict the same response. Equation (16.5) uses the transformed levels of X_1, X_2, and X_3 ($+1$ or -1), and Eq. (16.2) uses the actual, observed, untransformed values. For example, if X_1 and X_2 are at their high levels, and X_3 is at the low level, we can solve for the response, Y, using Eq. (16.5) and the transformed values, $+1$, $+1$, and -1 for X_1, X_2, and X_3, respectively.

$$Y = 10.725 + 0.85(+1) + 2.225(+1) + 2.525(-1) - 0.15(+1)(+1)$$
$$-0.85(+1)(-1) + 1.025(+1)(-1) + 0.15(+1)(+1)(-1) = 10.8$$

The response with X_1 and X_2 at the high level is 10.8, exactly equal to the value obtained from Eq. (16.2), where X_1, X_2, and X_3 are the actual levels, 2, 1, and 0 mg, respectively.

To reiterate, the reason for the transformation (also called coding) is to allow for calculation of the coefficients in the polynomial equation.** The transformation of the high and low factor levels to $+1$ and -1 also results in easy calculation of the variance of the coefficients. Using the transformed levels, the variance of a coefficient is

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* $B_0 = \bar{Y}$.

** The coded values also result in orthogonality (independence) of effects.

$\sigma^2/8$ [$\sigma^2/\Sigma\ (X - \bar{X})^2$]. With an estimate of the variance, S^2, each coefficient can be tested for significance, using a t test. These tests are exactly equivalent to the testing of the effects of the ANOVA of a factorial design as explained in Chapter 9. If, for example, the X_1X_2 interaction were found to be nonsignificant in an ANOVA, the coefficient of X_1X_2, -0.15 in this example, will also be non-significant. Usually, when constructing the polynomial equation, only those terms which are statistically ''significant'' are retained. In the experiment above, an estimate of the standard deviation was available from previous similar experiments; *s.d. = 0.32* with 16 d.f. Therefore, the coefficients B_{12} and B_{123} (0.15) are not significant.

$$t = \frac{|0.15|}{0.32/\sqrt{8}} = 1.3\ (P > 0.05)$$

Omitting the ''nonsignificant'' B_{12} and B_{123} terms, the final equation is

$$\begin{aligned} Y = {} & 10.725 + 0.85(X_1) + 2.225(X_2) + 2.525(X_3) \\ & - 0.85(X_1X_3) + 1.025(X_2X_3) \end{aligned} \tag{16.6}$$

An advantage of the transformation described above is that the omission of the two coefficients, B_{12} and B_{123}, does not affect the values of the remaining coefficients, that is, recalculation of the polynomial equation results in the same coefficients. This result would not occur if Eq. (16.2) were used to describe the data. Equation (16.2) used the untransformed factor levels and would necessitate extensive computations if some terms were omitted, probably requiring use of a computer as a computing aid. Using the transformed values ensures that the factors are orthoganol. This means that the estimates of the coeficients are independent.

Having derived an equation (16.6) that describes the experimental system based on the results of the experimental formulations, we consider this equation to approximately predict the response within the experimental space. Figure 16.1 shows the space described by this design. The *prediction* of the response, Y, at $X_1 = 1$ mg, $X_2 = 1$ mg, and $X_3 = 2.5$ mg is 12.95 [Eq. (16.6)] (see Exercise Problem 1). How do we know that Eq. (16.6) will be a good predictor for responses other than those included in the factorial design? Without actually testing some ''extra-design'' formulations, we have no way of knowing that the derived empirical equation will be adequate to predict the results of yet-to-be-tested formulations. If the response is ''well behaved,'' the in-between points should be able to be accurately predicted from the response equation.

Usually, it is a good idea to test at least one formulation, not included in the design, as a *check point*. The observed results of the checkpoint formulation can then be compared to the predicted value to test the equation. In our example, a formulation was prepared with $X_1 = 1$ mg, $X_2 = 0.5$ mg, and $X_3 = 2.5$ mg. The transformed values are equal to zero for the three variables (see the transformation equation (16.4). Using Eq. (16.6), the predicted response is 10.725 (only the intercept term is not equal to 0). The factor values for the check point are the average of the low and high levels of the factors (X variables), and lie in the center of the cube in Fig. 16.1. This is called a ''Center Point.'' The actual observation made on this formulation was 10.5, very close to the predicted value. Extrapolation of predicted results outside the factor space, as shown in Fig. 16.1, is not recommended. A two-level design can make predictions only in a linear fashion, usually a gross approximation. If curvature is present, the response may be misrepresented both inside and outside the confines of the design.

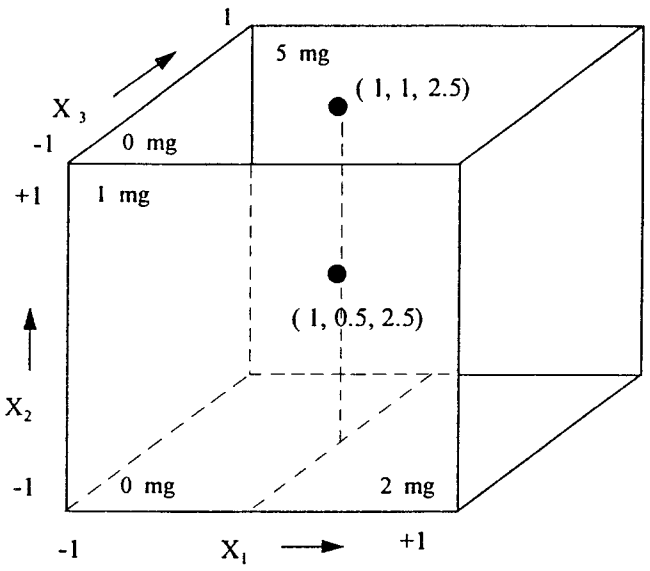

Figure 16.1 Factor space for experiment with factor levels shown in Table 16.1.

Once the polynomial-response equation has been established, an optimum formulation (or a region of optimum formulations) can be found by various techniques. Sometimes, inspection of the experimental results may be sufficient to choose the desired product. In the example above, if large values of the response are desirable, Formulations 7 and 8 may be chosen as ''best'' (Table 16.1). With the use of computers (programmable calculators will often do), a ''grid'' method may be used to identify optimum regions, and response surfaces may be depicted (see Fig. 16.2). The response surface is a geometrical representation of the response and the factor levels, similar to a contour map. For more than two factors, response surfaces cannot be easily represented in two-dimensional space. However, one can take slices of the surface, with all but two factors at fixed levels, as shown in Fig. 16.2. A computer can calculate the response, based on Eq. (16.1), at many combinations of the factor levels. The formulation(s) whose response has optimal characteristics based on the experimenter's specifications can then be chosen. To illustrate the grid method, a very rough grid with predicted responses based on Eq. (16.6) is shown in Table 16.3.

The experimental system analyzed above is a very simple example, but is a typical approach to the optimization process. More sophisticated designs may be used, such as the composite designs to be described below (Sec. 16.3), or fractional factorial designs. The principles are the same. All of these designs have orthogonal properties to allow for clear and simple estimation of the polynomial coefficients. For these designs, the magnitude of the coefficients is directly related to the magnitude of the response.

The polynomial coefficients may be calculated by techniques such as described here, or by using a multiple regression computer program (see App. III). For two-level experiments (2^n factorials), the factor levels should be transformed so that the low level is equal to -1 and the high level equal to $+1$, according to Eq. (16.4). (Experiments with factors at more than two levels should be analyzed with the help of a statistician.) The transformation

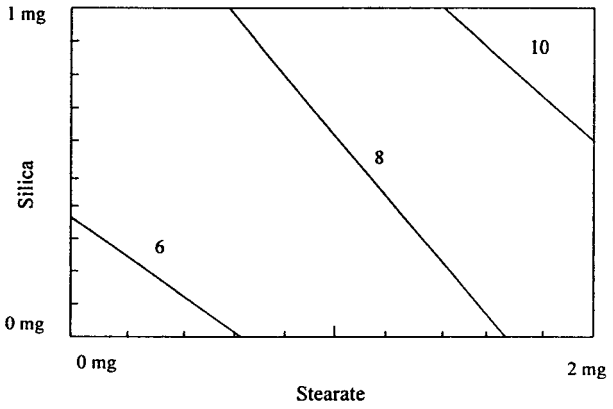

Figure 16.2 Response surface with drug (X_3) constant (low level) [Eq. (16.6)].

considerably reduces the complexity of the computations, and aids in the interpretation of the results. Each coefficient may be tested for significance discarding those coefficients that are not significant, although there are no firm rules regarding this procedure. In addition to the statistical criteria, scientific judgment may be used in making decisions about the ''significance'' of the coefficients. In order to statistically test the coefficients for significance, an estimate of the experimental error is required. This error estimate may be obtained from previous experience, but is best estimated by replicating runs. Replication, however, may result in a large number of experiments, which could be very costly. Replication, accomplished by performing duplicate assays on the same sample, for example, is usually *not* sufficient. The best procedure for replication consists of preparing each formulation or experiment in duplicate (or more), and randomizing the order of the experiments, if all formulations cannot be prepared and tested simultaneously. Methods are available to obtain an estimate of error from an unreplicated factorial experiment (e.g.,

Table 16.3 Grid Solutions for Responses (Y) Based on Eq. (16.6)

X_1[a]	X_2	X_3	Y	X_1	X_2	X_3	Y	X_1	X_2	X_3	Y
−1	−1	−1	5.3	0	−1	−1	7	+1	−1	−1	8.7
−1	−1	0	7.65	0	−1	0	8.5	+1	−1	0	9.35
−1	−1	+1	10	0	−1	+1	10	+1	−1	+1	10
−1	0	−1	6.5	0	0	−1	8.2	+1	0	−1	9.9
−1	0	0	9.875	0	0	0	10.725	+1	0	0	11.575
−1	0	+1	13.25	0	0	+1	13.25	+1	0	+1	13.25
−1	+1	−1	7.7	0	+1	−1	9.4	+1	+1	−1	11.1
−1	+1	0	12.1	0	+1	0	12.95	+1	+1	0	13.8
−1	+1	+1	16.5	0	+1	+1	16.5	+1	+1	+1	16.5

[a] Transformed values.

halfnormal plots [2,3], or from higher-order interactions as discussed in Chapter 9, but these procedures will not be discussed here.

Replication (*Sample Size*)

We may only want to find optimum conditions, or we may want to know that effects are real, and not just due to random error. In the latter case, we may want to perform statistical tests (or confidence intervals). To determine the sample size for hypothesis tests, we may use the approximate formula, $N = 4(S^2/\text{delta}^2)(10)$, where N is the sample size for the comparative groups ($N = 4$ for the 2^3 design), where alpha = 0.05 and beta = 0.8. Usually a sample size between 10 and 20 should be sufficient.

Note that for two-level designs, the variance of an effect is $4S^2/N$, where N is the number of runs.

Example:

A difference in response of 2.5 units is meaningful in a 2^3 experiment. The standard deviation is expected to be 1.5. What size sample should we use?

$N = 4(2.25/6.25)(10) =$ approximately 16

Extra (*Center*) *Points*

Often, it is useful to include an extra run as a ''prediction'' point, or to estimate curvature. A center point should be equal to the average of the ''run'' points if there is no curvature. If curvature is present, more runs will be needed to model the data.

The ANOVA for the following data set is shown below to illustrate the analysis of replicated data.

		LEVEL		
Experiment	A,B	P	D	Resonse
1 (1)	A	1	0.1	5,6
2 P	B	1	0.1	7,11
3 D	A	2	0.1	4,6
4 PD	B	2	0.1	8,11
5 A	A	1	0.2	12,12
6 PA	B	1	0.2	16,21
7 DA	A	2	0.2	11,12
8 PDA	B	2	0.2	24,29
9 Checkpoint	B	1.5	0.15	22

Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level
P	1	162	162	40.50	0.000380*
D	1	5.555555	5.555555	1.39	0.277097
PD	1	10.88889	10.88889	2.72	0.142947
A:	1	304.2222	304.2222	76.06	0.000052*
AP	1	26.88889	26.88889	6.72	0.035802*
AD	1	5.555555	5.555555	1.39	0.277097
APD	1	5.555555	5.555555	1.39	0.277097
S	7	28	4		
Total	14	456.9333			

* $p < 0.05$

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In the absence of replication, there is no proper error term to test significance of the effects. Sometimes we can use an estimate of error from previous experiments or pool the higher-order interaction terms. If the runs are replicated, we would have a new term in the ANOVA, residual or error. Then, we can perform F (or t) tests to test for significance.

We could also construct an equation to predict the response (assuming a linear response with factors at two levels). This will be discussed later.

Fractional factorial designs use a fraction of the full factorials (e.g., ½, ¼). The gain is that we use less runs in the experiment. The loss is that we confound some effects. We try to confound effects that we feel are not significant (or very small) with effects that we wish to measure. In this example, the smallest fractional design is a ½ replicate, using four of the eight runs. In four runs, we can only measure three effects. The logical choice of effects to measure are A, P, and D. We assume that all interactions are negligible. If our assumption is wrong, the measure of the main effects will be biased.

16.2.1 Optimization of a Combination Drug Product

The following example of a 2^2 factorial experiment is another illustration of the technique of ''optimization'' using factorial designs. In this experiment, a *combination* drug product was tested to obtain the dose of each drug which would result in an optimal response. The product contained two drugs, $A(X_1)$ and $B(X_2)$. The experiment consists of formulating combinations containing each drug at two dose levels. The doses for A were 5 mg and 10 mg; B was chosen at doses of 50 mg and 100 mg. These levels were carefully selected to cover a range of doses which would include an appropriate dose to be chosen as the prime candidate for the final marketed product. The full factorial consists of the four experiments shown in Table 16.4

The product is a local anesthetic, and the response (Y) is the average time to anesthesia for 12 patients per group. The high and low levels of drug A and drug B are transformed to $+1$ and -1 [Eq. (16.4)]. For drug A, the transformation is

$$\frac{\text{Potency} - 7.5}{2.5} \text{(high level is 10; low level is 5)}$$

For drug B, the transformation is

$$\frac{\text{Potency} - 75}{25} \text{(high level is 100; low level is 50)}$$

The response equation has the form

Table 16.4 Factorial Design for the Drug Combination Study

	Potency (mg)		Potency (transformed)			
Formulation	A (X_1)	B (X_2)	A (X_1)	B (X_2)	AB (X_1X_2)	Response, Y (min)
1	5	50	−1	−1	+1	9.7
2	10	50	+1	−1	−1	7.2
3	5	100	−1	+1	−1	8.4
4	10	100	+1	+1	+1	4.1

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Table 16.5 Predicted Values of Response to Anesthetic Combinations of Drugs A and B Based on Eq. (16.8)

		Dose of drug A[a]				
		−1	−0.5	0	+0.5	+1
Dose of drug B[a]	−1	9.7	9.075	8.45	7.825	6.2
	0	9.05	8.2	7.35	6.5	6.65
	+1	8.4	7.325	6.25	5.17	4.1

[a] Coded values of drug potency.

$$Y = B_0 + B_1(X_1) + B_2(X_2) + B_{12}(X_1)(X_2) \tag{16.7}$$

The coefficients are computed as described earlier in this section. For example, referring to Table 16.4, B_1 is:

Column A (X_1)	Y	X_1Y
−1	9.7	−9.7
+1	7.2	+7.2
−1	8.4	−8.4
+1	4.1	+4.1
		−6.8/4 = −1.7

(B_1 is the sum of X_1Y /4 = −1.7.) The polynomial equation is calculated as

$$Y = 7.35 - 1.7(X_1) - 1.1(X_2) - 0.45(X_1X_2) \tag{16.8}$$

The response, Y, is the time to anesthesia. Formulation 4, which has the high levels of both drugs, has the shortest time to anesthesia, and formulation 1 or 4 would be chosen as optimal if either a long time or a short time to anesthesia is desired. However, an intermediate time might be more desirable. For example, suppose that a time of 5 min is the most desirable time based on considerations such as the administration of the product and the type of conditions that are meant to be treated with the aid of the product. Table 16.5 is a rough grid of the predicted responses based on Eq. (16.8). Based on a time to anesthesia of approximately 5 min, a formulation containing 0.5 of *A* and 1 of *B* would be a candidate. Decoding the values result in a formulation containing 8.75 mg of *A* and 100 mg of *B*.

16.3 COMPOSITE DESIGNS TO ESTIMATE CURVATURE

In general, when looking for optimality, the response equation will be more reliable if it contains terms that reflect curvature. Physical systems are less satisfactorily described by

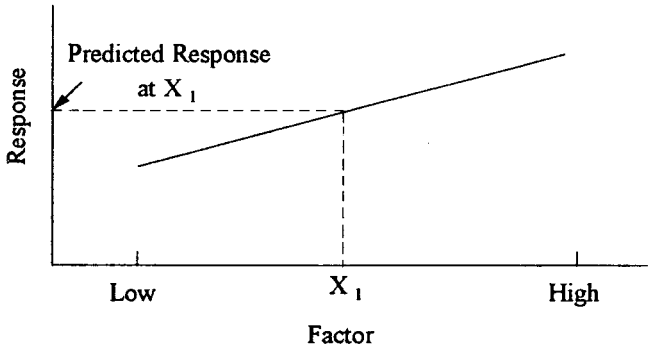

Figure 16.3 Figure showing linear response as a function of a single variable (factor).

empirical equations containing only linear terms. Figure 16.3 shows an example of a single factor, X, at two levels. Clearly, to interpolate the response, Y, at values of X between the low and high levels requires an assumption of linearity. These predictions would be very much in error if the response is curved, as shown in Fig. 16.4.

In order to estimate curvature, more than two levels of the factor must be included in the experiment. The presence of curvature would be reflected in the presence of terms with a power greater than 1 (e.g., X_1^2) in the response equation. Such equations are known as polynomials of order 2, and have the following form for a two-factor design:

$$Y = B_0 + B_1X_1 + B_{11}X_1^2 + B_2X_2 + B_{22}X_2^2 + B_{12}X_1X_2 + \cdots \tag{16.9}$$

Composite designs are effective designs to estimate second-order terms. These designs have a number of desirable features. In addition to allowing an estimate of curvature, composite designs give orthogonal estimates of the polynomial coefficients, and allow for the possibility of proceding with the experiment in a stepwise fashion rather than performing the entire experiment at once. The theory underlying composite designs is beyond the

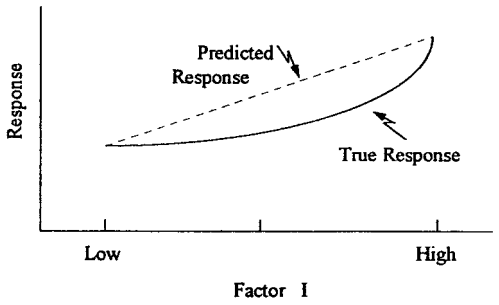

Figure 16.4 Figure showing curved response as a function of a single variable (factor).

Table 16.6 Orthogonal Composite Design with Two Factors (3^2 Design)

	Coded level						
Formulation	X_1	X_2	X_1X_2	$X^2{}_1 - 2/3$	$X^2{}_2 - 2/3$	Response, Y	Predicted response
1	−1	−1	+1	+1/3	+1/3	9.7	9.3
2	−1	0	0	+1/3	−2/3	9.0	9.4
3	−1	+1	−1	+1/3	+1/3	8.4	8.4
4	0	−1	0	−2/3	+1/3	5.3	5.6
5	0	0	0	−2/3	−2/3	4.8	5.0
6	0	+1	0	−2/3	+1/3	3.8	3.3
7	+1	−1	−1	+1/3	+1/3	8.2	8.3
8	+1	0	0	+1/3	−2/3	7.5	6.9
9	+1	+1	+1	+1/3	+1/3	4.1	4.6

scope of this book. An excellent description of this design and optimization procedure can be found in Chapter 11 of Ref. 1.

Although the following discussion is somewhat more advanced than the bulk of material presented in this book, for those who are interested in this subject, an example of a two-factor composite design will be presented to illustrate the technique. A two-factor composite design is identical to a 3^2 factorial design, that is, two factors each at three levels, a total of nine combinations (see Table 16.6).

In general, composite designs are not full factorials of the class 3^n, where n is the number of factors. These full factorial designs require a larger number of experiments. For example, a 3^n design with three factors requires 27 runs (27 formulations, for example), 3^3. With more than two factors, composite designs consist of the 2^n design, plus *extra-design* points. The extra points include a *center point* and 2^n extra points, appropriately chosen to maintain orthogonality of the design [1]. The two-factor composite design is shown in Fig. 16.5.

The coded values −1, 0, and +1 in Table 16.6 for the factor levels represent three *equally spaced* levels of each factor. The coded values in the column headed X_1X_2 are

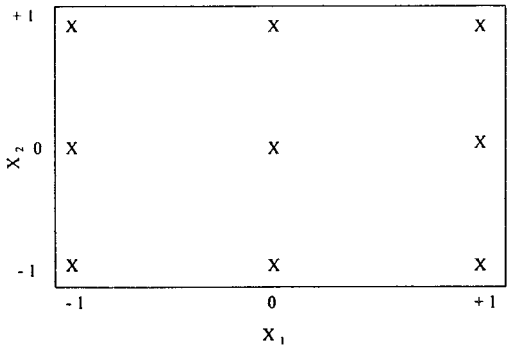

Figure 16.5 Two-factor composite design (3^2 factorial).

obtained by multiplying the corresponding values in the first two columns (X_1, X_2) as previously described. The values in the columns $X_1^2 - 2/3$ and $X_2^2 - 2/3$ are derived so that the product of corresponding values in any two columns of Table 16.6 sum to zero, resulting in orthogonality (independence) of effects. The special orthogonality obtained by transforming X_i^2 to $X_i^2 - 2/3$ allows for easy calculation of the coefficients and their variances. With this transformation, Eq. (16.9) is modified to

$$Y = B_0 + B_1X_1 + B_{11}(X_1^2 - 2/3) + B_2X_2 + B_{22}(X_2^2 - 2/3) + B_{12}X_1X_2 + \cdots \tag{16.10}$$

The data in Table 16.6 consist of the four formulations from Table 16.4 plus five new runs to complete the composite design. The doses of each drug (X_1 and X_2) were chosen such that the three doses are at equally spaced intervals. Thus the third dose, in addition to the two doses chosen for the 2^2 factorial, is 7.5 mg for X_1 (A) and 75 mg for X_2 (B). The experiment consists of evaluating the nine combinations of doses, 5, 7.5, and 10 mg for X_1 (A) and 50, 75, and 100 mg for X_2 (B). Note that the *center point* for the composite design is the combination 7.5 mg and 75 mg of X_1 and X_2, respectively.

The results of the nine runs are shown in Table 16.6. The results are shown schematically in Fig. 16.6A. The plane at the bottom of the figure shows the combinations of X_1 and X_2. The vertical ''sticks'' are the responses at each combination of X_1 and X_2. We will compute an equation of the form of Eq. (16.10) which represents a smooth curved surface based on the experimental data. In general, the equation can be obtained through the use of a multiple regression computer program.

The coefficients can also be calculated by ''hand'' (calculator) using the coded values in Table 16.6. The sum of the products of the coded values times the responses divided by the sum of the squared coded values in the column of interest gives the coefficient. For example, the coefficient B_{11} in Eq. (16.10) is calculated as follows:

$X_1'^2 = X_1^2 - 2/3$	Y	$(X_1'^2)(Y)$
+1/3	9.7	3.23
+1/3	9.0	3.00
+1/3	8.4	2.80
−2/3	5.3	−3.53
−2/3	4.8	−3.20
−2/3	3.8	−2.53
+1/3	8.2	2.73
+1/3	7.5	2.50
+1/3	4.1	1.37
$\Sigma X_1'^2 = 2$		sum = 6.37

The sum of squared values in the $(X_1^2 - 2/3)$ column is 2. Therefore, the coefficient, B_{11}, is 6.37/2 = 3.18. The intercept, B_0, is the average of the nine responses, $\bar{Y}$, equal to 6.756. The response equation is

$$Y = 6.756 - 1.22(X_1) + 3.18(X_1^2 - 2/3) - 1.15(X_2) - 0.52(X_2^2 - 2/3) - 0.7(X_1X_2) \tag{16.11}$$

Note that Eq. (16.11) is not an exact fit to the experimental data, as was the case with the polynomial fit described for factorial designs in Sec. 16.2. Had we included three more terms representing various interactions, the equation would exactly fit the data. Equation (16.11) is computed with the assumption that interactions are negligible. Because of the larger number of experiments and the estimation of only six coefficients, we have 2 d.f. for error. Although such an error estimate is not very reliable, it does gives us some information, albeit small. The response surface described by Eq. (16.11) is shown in Fig. 16.6B. If this equation does not adequately represent the experimental observations, more terms may be needed in the polynomial equation [Eq. (16.9)] to improve the fit.

The contour plot (similar to contour maps) shown in Fig. 16.6B allows the selection of combinations of X_1 and X_2 to satisfy given levels of the response. If a maximum response

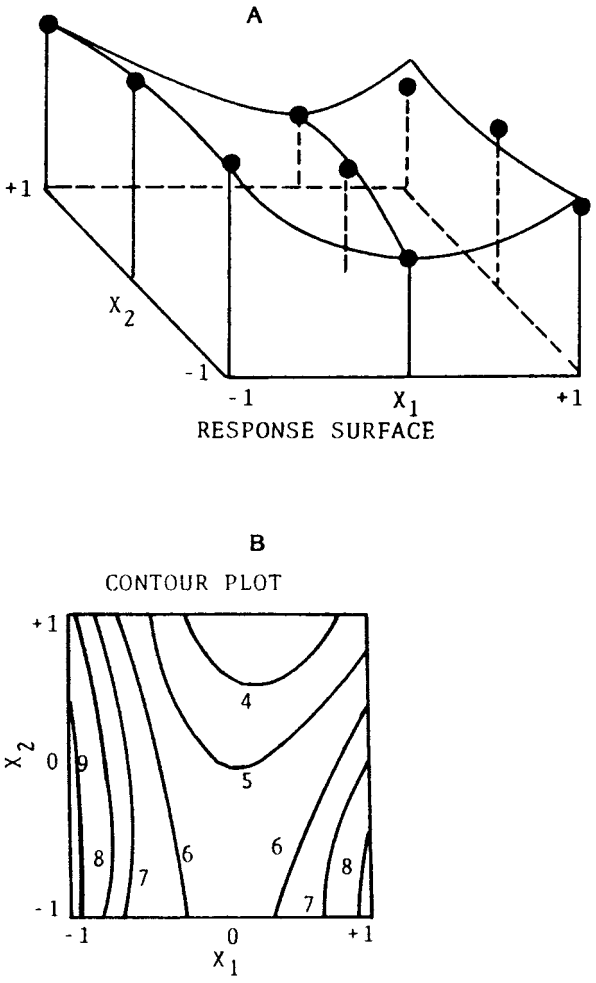

Figure 16.6 Results of composite design experiment from Table 16.6 and response surface computed from Eq. (16.11).

is desired, the X_1, X_2 combinations are limited to a small area of the $X_1 - X_2$ space. If a response of approximately 5 min is desired, various combinations of X_1 and X_2 will satisfy the requirements. The ultimate choice will probably depend on other factors, as well, such as cost, toxicity, and so on.

Use of factorial designs in tablet formulation optimization has been presented by Schwartz et al. [4], Fonner et al. [5], and Lindberg et al. [6]. These papers discuss designs somewhat more complex than that presented here. However, for those interested in pursuing this topic further, these papers and the books *The Design and Analysis of Industrial Experiments* [1] and *Statistics for Experimenters* [3] are recommended.

16.4 THE SIMPLEX LATTICE

Response surfaces and optimal regions for *formulation* characteristics are frequently obtained from the application of simplex lattice designs. This class of designs is particularly appropriate in formulation optimization procedures where the *total* quantity of the different ingredients under consideration must be *constant*. Therefore, these are also called ''Mixture Designs.'' For example, suppose that in a liquid formulation, the active ingredient and solvent compose 90% of the product. The remaining 10% of the formulation consists of preservatives, coloring agents, and a surfactant. We wish to prepare a formulation with a certain optimal attribute(s) which is dependent on the relative concentrations of preservative, color, and surfactant. In order to determine optimal regions, we vary the concentrations of these three ingredients in a systematic manner, with the restriction that the total concentration of these ingredients is 10%. This approach differs from the previous procedures (Secs. 16.2 and 16.3) in that a constraint is imposed on the total amount of the varying ingredients. In this example, the total amount of the varying components is maintained at 10%. Given the concentration of two of the ingredients, the third ingredient is fixed where in this example $C = 10\% - A - B$.

Implementation of the simplex design consists of preparing various formulations containing different combinations of the variable ingredients. The combinations are prepared in a manner such that the experimental data can be used to predict the responses over the simplex space* in a simple and efficient manner. The combinations (formulations) in a simplex design are chosen to cover the space of interest in a symmetrical manner. The experimental results are used to compute a polynomial (simplex) equation which can be used to estimate the response surface. As is true with all optimization and so-called response surface procedures, extrapolation to combinations outside the range included in the experimental design is not recommended. The equation resulting from the experiment, the simplex equation, is an empirical equation which approximately describes the response pattern in the simplex space. There is no reason to believe that the equation has any physical meaning, other than the fact that the complex response patterns resulting from the varying formulations can often be approximated by simple polynomial equations.

Figure 16.7 representing a two-component system (A and B) is useful to help clarify some concepts of simplex designs. One can consider components A and B to be two solvents, which together comprise the entire solvent system of a drug product. We wish to mix A and B in the correct proportion to optimize the solubility of the drug.

* The simplex space is the region enclosed by the various combinations of ingredients chosen for the experiment. See Fig. 16.8, for example.

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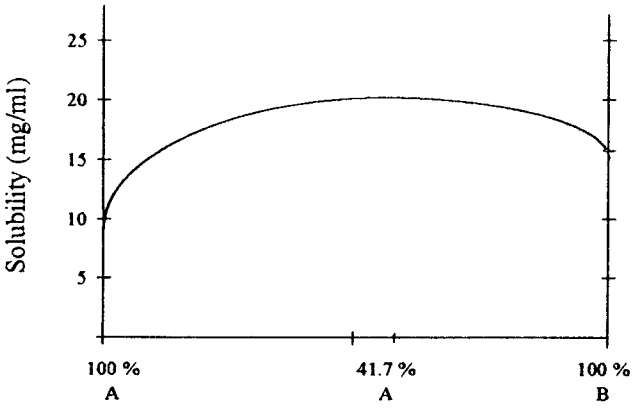

Figure 16.7 Two-component solvent system used to illustrate the simplex approach to optimization.

Figure 16.7 is familiar as a solubility phase diagram. This system can also be visualized as an elementary simplex system. The constraint is that the concentrations of *A* and *B* must add to 100%. This experiment consists of observing responses (solubility) at three points, *100%A*, *100%B*, and a *50 − 50 mixture of A and B*, an elementary simplex design. According to Fig. 16.7, the solubilities of the drug at the three simplex points, 100% *A*, 100% *B*, and 50% *A* − 50% *B*, are 10 mg/ml, 15 mg/ml, and 20 mg/ml, respectively. In the simplex approach, we construct an equation of the form

$$Y = B_1(A) + B_2(B) + B_{12}(A)(B) \tag{16.12}$$

where *Y* is the response (solubility in this example), and (*A*) and (*B*) are the concentrations (proportions) of *A* and *B*, respectively. The coefficients, B_1, B_2, and B_{12}, are calculated from the experimental observations. The response, *Y*, can then be predicted for all combinations of *A* and *B*, where (*A*) + (*B*) = 1.0 (100%). (The proportion of each component is usually indicated as a decimal rather than as a percentage.) The form of the simplex design allows for easy calculation of the coefficients. In this example, the coefficients are simply calculated as follows:

B_1 = response at (*A*) equal to 1.0 (100%) = 10
B_2 = response at (*B*) equal to 1.0 (100%) = 15
B_{12} = 4 (response at 0.5 − 0.5 mixture of *A* − *B*) − 2 (sum of responses at *A* = 1.0 and *B* = 1.0)
$B_{12} = 4(20) - 2(10 + 15) = 30$

The response equation is

$$Y = 10(A) + 15(B) + 30(A)(B) \tag{16.13}$$

The solution above for the three coefficients is a result of the solution of three simultaneous equations:

With A = 1.0 and $B = 0$, from Eq. (16.12), $B^*_1 = 10$
With $A = 0$ and $B = 1.0$, from Eq. (16.12), $B_2 = 15$
With $A = 0.5$ and $B = 0.5$, from Eq. (16.12),

$$20 = 0.5B_1 + 0.5B_2 + 0.25B_{12} \text{ or } B_{12} = 4(20) - 2(B_1 + B_2) = 30$$

We will see that in more complex simplex designs, the polynomial coefficients are, similarly, easily calculated as linear combinations of experimental results.

Equation (16.13) exactly predicts the observed points: a fit of a polynomial with three terms to three experimental points. We can always construct an equation with N coefficients which will exactly pass through N points. For example, for the 50 − 50 mixture,

$$Y = 10(0.5) + 15(0.5) + 30(0.5)(0.5) = 20$$

The *response equation* predicts responses at extra-design points, those formulations not included in the experiment but which lie within the simplex space, 100% *A* to 100% *B* in this example. For example, what solubility would be predicted in a solvent system containing 75% *A* and 25% *B*? (Note that $A + B$ must equal 100%.) Applying Eq. (16.13), we have

$$Y = 10(0.75) + 15(0.25) + 30(0.75)(0.25) = 16.875$$

See also Fig. 16.7. The entire response may be sketched in by predicting solubilities along the curve, as shown in the figure.

The primary experimental objective in experiments such as that described above may be the determination of the solvent combination that results in maximum drug solubility. The optimum solubility can be computed by calculating the predicted solubility at many solvent combinations so as to clearly define the response over the solvent mixture continuum. This may seem an indirect and tedious approach, but with the ready availability of computers, this is often the most expeditious route. The maximum solubility is predicted to occur at 41.67% *A*. In this simple example, the maximum can easily be calculated by setting the first derivative of Eq. (16.13) equal to 0 (see Exercise Problem 6).

In general, the simplex design is usually applied to formulation problems in which a mixture of three or more components is to be investigated. The design is conveniently represented by regular-sided figures, which can be visualized for three- or four-component systems. For more than four components, a single figure cannot be conveniently constructed, but can be theoretically conceived as an N-sided figure in $(N - 1)$-dimensional space. For example, Fig. 16.8 shows the three-component system which is represented as an equilateral triangle in two-dimensional space. A regular simplex design for a three-component mixture system consists of six or seven formulations:

- *Three* formulations, one each at each vertex, *A*, *B*, and *C*. These formulations represent formulations with the pure components, *A*, *B*, and *C*, respectively.
- *Three* formulations are prepared with 50 − 50 mixtures of each pair of components, *AB*, *AC*, and *BC*.
- A *seventh* formulation may be prepared with one-third of each component. This lies in the *center* of the design.

An example of a simplex design for four components consisting of 15 formulations is shown in Fig. 16.8. The 15 formulations consist of:

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* The response, *Y*, with *A* equal to 1.0 (100%) is 10.

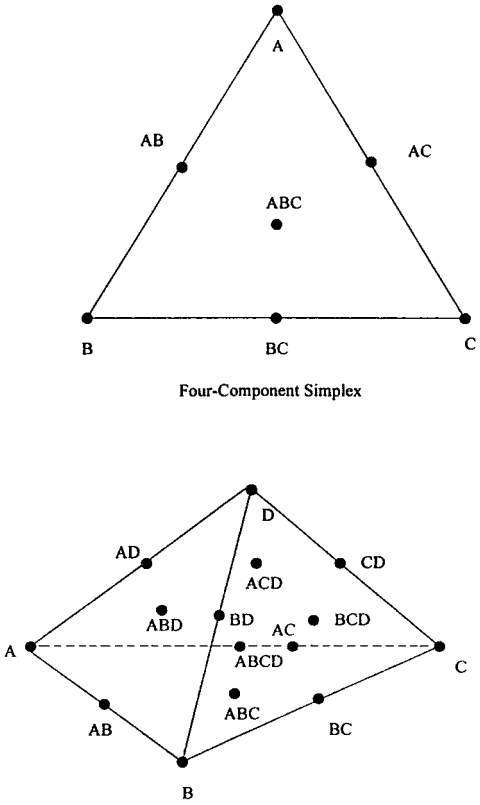


Figure 16.8 Three-component simplex lattice design and four-component simplex lattice design.

Four formulations each with 100% of each of the four pure components *Six* formulations of 50 − 50 mixtures of component pairs (*AB*, *AC*, *AD*, *BC*, *BD*, and *CD*)
Four formulations consisting of one-third mixtures of combinations of three components (*ABC*, *ABD*, *ACD*, *BCD*)
A mixture containing 25% of each of the four components (*ABCD*)

The simplex design is arranged so that the experimental space is well covered in a symmetrical fashion. In addition, the symmetrical spacing of the points allows for an easy computation of the response equation coefficients. The general equation for the response based on a simplex design contains terms for pure components and all mixtures of components as follows:

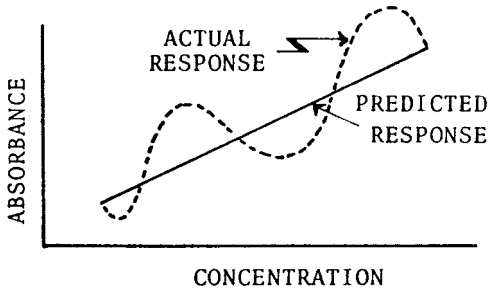
$$Y = B_a(A) + B_b(B) + B_c(C) + \cdots + B_{ab}(A)(B) + B_{ac}(A)(C) + B_{bc}(B)(C) + \cdots + B_{abc}(A)(B)(C) + \cdots \tag{16.14}$$

where (A), (B), and (C) are the proportions of components A, B, and C, and $(A) + (B) + (C) + \cdots$ is equal to 1.0.

The subscripted B's (e.g., B_a) are coefficients which can be easily calculated from the responses, Y, or using a multiple regression computer program.

After the coefficients have been calculated, the response equation [Eq. (16.14)] may be used to predict the response of combinations of the N components in the system. With the aid of a computer, responses may be calculated over the simplex space, and contour diagrams printed (see also Fig. 16.6). The contour plot is a graphic description of the response surface resulting from data derived from experimental designs such as the simplex. For the two-component system (Fig. 16.7), the response surface is simply the solubility curve. With three components, a three-dimensional figure would be necessary to show the response surface. A contour plot is a means of illustrating the response on a two-dimensional surface, as is familiar to those who have been exposed to contour maps. A computer may be programmed to produce two-dimensional figures (commercial programs are also available), which are slices through the three-dimensional figure for a three-component system. The slices are taken at a constant concentration of one of the components. In computer outputs, the regions of equal response are indicated by a common symbol, such as a letter or a figure. An example of a contour plot was shown in Fig. 16.6. The contour plot will be discussed further in the example that follows. Examination of the contour plot(s) allows the experimenter to choose formulations which have predicted responses of some specified magnitude.

When constructing an empirical response equation based on a limited number of experimental observations, one should understand that predicted values based on the equation may be in error for several reasons. For example, the empirical equation (or model, as it is often called) rarely exactly defines the experimental system. The equation is an approximation to the system. To understand this important concept, note that the same problem would exist if we had only two points in the experimental space. The empirical equation derived from the two points could only relate the observations by a straight line. In-between points could only be predicted on the basis of the straight-line relationship (see Figs. 16.3 and 16.4).



If the true relationship of the X, Y variables were curved, the linear interpolation would be in error. In the simplex design, we used a limited number of points to define a relatively large region of response. Even if the model represented by the empirical equation is a reasonable representation of the true surface, other sources of variation can contribute to error in the prediction equation and predicted responses (e.g., error in measuring the response). Thus, in these systems, we have at least two obvious sources of variability: that due to the empirical model and that due to observational errors.

How can we protect ourselves from inadvertantly proceeding with predictions when the derived equation is indeed inaccurate? As insurance against such a possibility, it is a good idea to run one or more extra-design points. These points are not used to estimate the coefficients in the simplex equation [Eq. (16.14)] but will be used as checkpoints. Once the simplex equation is derived, the result at the extra-design checkpoint(s) is predicted based on the equation, and its agreement with the observed value assessed. If the agreement is close, we have increased faith in the predictive power of the response equation (see Sec. 16.2). If we have an estimate of error from replication or other means, we may wish to perform a statistical test to test the adequacy of the model (a statistician may be consulted for this calculation).

The calculation of the simplex equation coefficients is easily accomplished using the following formulas. These formulas are an extension of those discussed previously for the two-component system as applied to a three-component system. The general formulas for calculation of coefficients for an *N*-component system may be found in Ref. 6.

$$
\begin{aligned}
B_1 &= Y_1, \text{ the response at } 100\%\ A \\
B_2 &= Y_2, \text{ the response at } 100\%\ B \\
B_3 &= Y_3, \text{ the response at } 100\%\ C \\
B_{12} &= 4(Y_{12}) - 2(Y_1 + Y_2), \text{ where } Y_{12} \text{ is the response at } 50-50\ AB \\
B_{13} &= 4(Y_{13}) - 2(Y_1 + Y_3), \text{ where } Y_{13} \text{ is the response at } 50-50\ AC \\
B_{23} &= 4(Y_{23}) - 2(Y_2 + Y_3), \text{ where } Y_{23} \text{ is the response at } 50-50\ BC \\
B_{123} &= 27(Y_{123}) - 12(Y_{12} + Y_{13} + Y_{23}) + 3(Y_1 + Y_2 + Y_3), \\
&\quad \text{where } Y_{123} \text{ is the response at } 1/3A\ 1/3B, \text{ and } 1/3C
\end{aligned}
\tag{16.15}
$$

The discussion above has been based on an experimental situation where the components being varied in the simplex design comprise the entire mixture (100%). In pharmaceutical formulations, a more common situation is one in which part of the formulation must remain fixed (e.g., drug concentration in a tablet). The remaining components, which may be varied, therefore do not make up 100% of the mixture. In addition, the lower limit for the varying components is often not equal to 0. For example, some components must be present in some minimal quantity in order that a marketable product can be manufactured. This is known as a design with constraints. For tablets, some minimal amount of a lubricating agent may be necessary in order to obtain an acceptable product. These modifications in the simplex design present no problem, however, because we can restrict the treatment of the simplex to those components which are varied, and with suitable transformations, treat the data in exactly the same way as described above. For example, if the components to be varied make up 60% of the total formulation ingredients, we can appropriately transform the actual percentages of these components so that the transformed percentages total 100%. In a three-component mixture containing 20% of each of three components, each component can be transformed to 33.3% (1/3) for purposes of the simplex analysis. Transformations can also be made where the components have a lower limit greater than 0% and an upper limit less than 100%, as will be explained in the following worked example.

The example presented below is an experiment in which a simplex design was used to obtain a formulation with optimal properties. This example should clarify the concepts and procedures described above. This experiment was prompted by problems with tablet hardness for a large-volume marketed product. Although the reason for the problem was

Table 16.7 Results of a Three-Component Simplex System for Tablet Hardness

Formulation components			Transformed proportion			
A	B	C	A	B	C	Average hardness, *Y*
55	10	10	1.0	0	0	6.1
10	55	10	0	1.0	0	7.5
10	10	55	0	0	1.0	5.3
32.5	32.5	10	0.5	0.5	0	6.6
32.5	10	32.5	0.5	0	0.5	6.4
10	32.5	32.5	0	0.5	0.5	6.9
25	25	25	0.33	0.33	0.33	7.3
32.5[a]	21.25	21.25	0.5	0.25	0.25	7.2

[a] Extra-design checkpoint.

not obvious, the pharmaceutical product development scientists felt that the cause could be traced to three components of the tablets, which we will denote as ingredients A, B, and C. Together, these components consisted of 25% of the original formulation, or 75 mg of the total tablet weight of 300 mg. A careful evaluation of the product ingredients indicated that the three components had to be present in an amount equal to at least 10 mg each in order for the tablet to be satisfactorily compressed. Thus the recommended simplex design to obtain a satisfactory tablet hardness consisted of varying the three components with the constraint that the sum of the components must be 75 mg, and that each component be present in an amount equal to at least 10 mg.

In order to apply the simplex equation to be derived from this experiment in a convenient manner, the actual concentrations used should be *transformed* such that the minimum concentration (10 mg) corresponds to 0% and the highest concentration corresponds to 100%.* In our example, the transformation is the same for all three components because each component is subject to the same restrictions. The minimum quantity is 10 mg and the maximum is 55 mg. (The other two components, each at 10 mg, make up the 20-mg difference, a total of 75 mg.) The transformation is as follows:

$$\text{Transformed proportion} = \frac{\text{Amount used} - \text{minimum}}{\text{maximum} - \text{minimum}} = \frac{\text{Amount used} - 10}{55 - 10} \tag{16.16}$$

Thus a formulation prepared with a $50 - 50$ mixture of components A and B would actually contain 32.5 mg of A, 32.5 mg of B, and 10 mg of C. Note that from Eq. (16.16), if a component is at a concentration of 32.5 mg, the transformed proportion is $(32.5 - 10)/(55 - 10) = 0.5$. A formulation with ''100%'' A would actually contain 55 mg of A, 10 mg of B, and 10 mg of C.

The three-component simplex design was run with one checkpoint, as shown in Table 16.7. The hardness values represent the average hardness of 20 tablets taken at random

* If there are no constraints on the upper and lower limits, the highest concentration would ordinarily be 100% and the lowest 0%.

from the experimental batches. The simplex coefficients are computed as described previously [Eq. (16.15)], resulting in the following equation:

$$Y = 6.1(A) + 7.5(B) + 5.3(C) - 0.8(A)(B) + 2.8(A)(C) + 2.0(B)(C) + 15(A)(B)(C) \quad (16.17)$$

For example, the coefficient B_{123} is calculated as follows:

$$27(7.3) - 12(6.6 + 6.4 + 6.9) + 3(6.1 + 7.5 + 5.3) = 15$$

(*A*), (*B*), and (*C*) in Eq. (16.17) are the transformed proportions. The extra-design checkpoint (the final formulation in Table 16.7) has a response of 7.2. The predicted value based on Eq. (16.17) is 7.09, very close to the observed value, 7.2. This is some confirmation of the adequacy of Eq. (16.17) as a predictor of tablet hardness. Figure 16.9 shows a contour plot of the results of the experiment based on Eq. (16.17). Tablets with high hardness are found in the region with relatively larger amounts of component B. If a tablet hardness of 7 or more is satisfactory, the pharmaceutical scientist has a choice of formulations. The final composition may then be dependent on other factors, such as cost or other tablet properties.

The following example shows data (Table 16.8) and analysis from a replicated simplex design that gives an estimate of experimental error. The design is a basic three-component (A, B, and C) simplex design with a center point consisting of 1/3 of each of the three components. This example is set up for a computer analysis. Note that the interaction term coefficients are the product of the main effect coefficients. For example for Run #7, the ABC interaction is 0.333 × 0.333 × 0.333 = 0.037. The computer analysis gives the

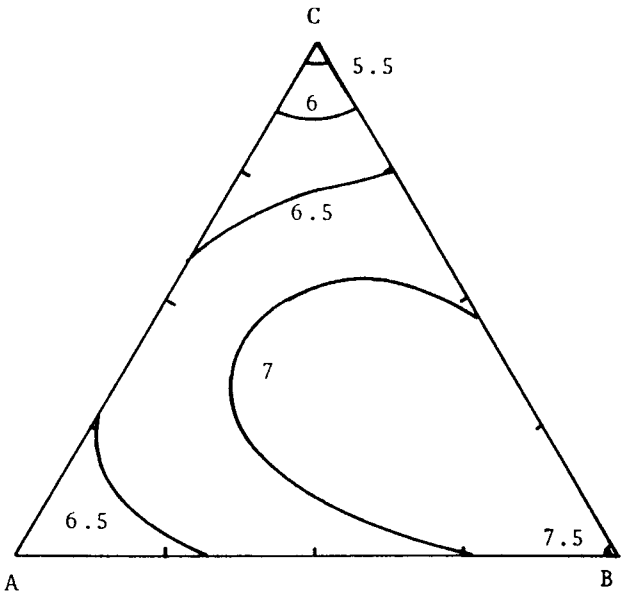


Figure 16.9 Contour plot of three-component simplex system (Table 16.7).

Table 16.8 Example of a Replicated Simplex Design

Run	A	B	C	AB	AC	BC	ABC	Response
1	1	0	0	0	0	0	0	38
2	0	1	0	0	0	0	0	27
3	0	0	1	0	0	0	0	46
4	0.5	0.5	0	0.25	0	0	0	33
5	0.5	0	0.5	0	0.25	0	0	51
6	0	0.5	0.5	0	0	0.25	0	32
7	0.333	0.333	0.333	0.111	0.111	0.111	0.037	48
8	1	0	0	0	0	0	0	42
9	0	1	0	0	0	0	0	28
10	0	0	1	0	0	0	0	41
11	0.5	0.5	0	0.25	0	0	0	35
12	0.5	0	0.5	0	0.25	0	0	47
13	0	0.5	0.5	0	0	0.25	0	32
14	0.333	0.333	0.333	0.111	0.111	0.111	0.037	50

Independent variable	Regression coefficient	Standard error	Lower Upper 95% C.L.	Standardized 95% C.L.
A	40	1.535299	36.36959	43.63041
B	27.5	1.535299	23.86959	31.13041
C	43.5	1.535299	39.86959	47.13041
AB	1	7.521398	−16.78528	18.78528
AC	29	7.521398	11.21472	46.78528
BC	−14	7.521398	−31.78528	3.78528
ABC	277.1	52.90734	151.9937	402.2056

		Analysis of Variance Section			
Source	DF	Sum of squares	Mean square	Prob *F*-Ratio	Level
Intercept	0	0	0		
Model	7	22461	3208.714	680.6364	0.000000
Error	7	33	4.714286		

regression coefficients for the response equation, and an ANOVA to estimate the experimental error. The variance estimate is 4.71.

A check point was run at A = 0.25, B = 0.25, and C = 0.5 with a response of 46. The model predicted 49.2.

In my experience, this approach gives excellent results.

16.5 SEQUENTIAL OPTIMIZATION

Sequential optimization was developed as a means to optimize a process in a stepwise fashion. Evolutionary operation (EVOP) uses factorial type designs and usually requires a large number of experiments [8]. A relatively simple approach to sequential optimization is a stepwise application of the simplex procedure [9,10]. The procedure consists of first

generating data from $n + 1$ experiments where n is the number of independent variables or factors. Based on the $n + 1$ responses and predetermined rules, one result is eliminated from the set and a new experiment is performed. A decision is made as a result of the most recent experiment, generating another new experiment, and so on, eventually terminating the design at an ''optimal'' response. Thus each new experiment leads the researcher on a path towards an optimum. The procedure and rules are illustrated in the following example. For further details and illustrations, the reader is encouraged to study Refs. 9–11.

16.5.1 An Example of Sequential Simplex Optimization

This example is based on the presentation by Shek et al. [11] using the simplex procedure to optimize properties of a capsule formulation. They were interested in optimizing dissolution and compaction rates as a function of the factors (or variables) drug, disintegrant, lubricant, and fill weight. In this synthetic example, we will look at a single response, dissolution at 30 minutes, as a function of 3 variables: disintegrant, lubricant, and fill weight.

We start with 4 experiments (we have 3 variables). There are no firm rules regarding the design of these experiments, but principles of good experimental design should prevail. For example, a 1/2 replicate of a 2^3 factorial design can be used for the initial 4 experiments. This requires setting low $(-)$ and high $(+)$ levels for each factor; see Table 16.9.

Let W = vector of worst response
Let S = vector of second worst response
Let B = vector of best response
Let R_w = worst response
Let R_s = second worst response
Let R_b = best response
Let P = average vector after elimination of worst response among formulations under consideration

Note that since Formula 2 shows the worst response (the longest dissolution time) $\bar{P}$ is the average of experiments 1, 3, and 4 and is equal to (33.3, 0.87, 300). For example, the first vector element refers to the average disintegrant $= (+50 \; -0 \; +50)/3 = 33.3$. Procedure:

Step 1. Eliminate W, the vector of the worst response from the data set and compute R [Eq. (16.18) below], the formulation for the new experiment.

$$R = \bar{P} + (\bar{P} - W)$$
$$(33.3, 0.87, 300) + (33.3, -1.33, 200) = (66.6, -0.46, 500) \tag{16.18}$$

Table 16.9 Initial Four Experiments for Simplex Experiment

Experiment	Disintegrant	Lubricant	Fill weight	Response
1	+(50)*	−(0.2)	−(100)	37
2	−(0)	+(2.2)	−(100)	58
3	−(0)	−(0.2)	+(400)	46
4	+(50)	+(2.2)	+(400)	40

* Parenthetical value is the amount of ingredient in the formulation.

In this example, we need 66.6 of disintegrant, −0.46 of lubricant and a fill weight of 500. We will interpret this result after the rules are specified and we proceed with the optimization.

If the response from experiment R, R_r, is better than the second-worst response, R_s, but worse than the best response, retain R_r and proceed to Step 1, evaluating a new formulation with the new set of 4 formulations.

If the response to R_r is better than the best response, proceed to Step 2.

If the response to R_r is worse than the second-worst response, go to Step 3.

If the response to R_r is worse than the worst response, go to Step 4.

Step 2. Compue E [Eq. (16.19) below] and evaluate R_e.

$$E = \bar{P} + 2(\bar{P} - W) \tag{16.19}$$

If R_r is better than the response to E, R_e, retain R. If R_e is better than R_r, retain E.

Step 3. Compute C_r [Eq. (16.20) below] and evaluate the response to C_r, R_{cr}.

$$C_r = \bar{P} + 0.5(\bar{P} - W) \tag{16.20}$$

Retain C_r. However, if R_{cr} is worse than R_s (the next-to-worst response), then set $R_w = R_s$ and $W = S$. (This means that the worst response is set equal to the next-to-worst response.) Set R_{cr} as the next to worst response, i.e., $S = C_r$ and $R_s = R_{cr}$.

Step 4. Compute C_w [Eq. (16.21) below] and evaluate R_{cw}. Retain C_w. However, if R_{cw} is worse than R_s (the next-to-worst response), then set $R_{cw} = R_s$ and $W = S$. (this means that the worst response is set equal to the next-to-worst response.) Set R_{cw} as the next to worst response, i.e., $S = C_w$ and $R_s = R_{cw}$.

Summary of Calculation of New Formulations

1. $R = \bar{P} + (\bar{P} - W)$ (16.18)

   R_r = The response to Formula R

2. $E = \bar{P} + 2(\bar{P} - W)$ (16.19)

   R_e = The response to Formula E

3. $C_r = \bar{P} + 0.5(\bar{P} - W)$ (16.20)

   R_{cr} = The response to Formula C_r

4. $C_w = \bar{P} - 0.5(\bar{P} - W)$ (16.21)

   R_{cw} = The response to Formula C_w

Although this procedure may appear confusing, if one follows the example, the process will be clarified.

We have already calculated the vector for the first new formulation using Step 1 above: (66.6, −0.46, 500). The response to this formulation will replace the worst formulation, W, which is formulation 2. Unfortunately, we cannot prepare this formulation because of the negative quantity of lubricant. We will make a rule that in such impossible situations we consider the response to this new formulation to be worse than the remaining formulations under consideration (formulations 1, 3, and 4).

This sends us to Step 4 according to our rules. The formulations under consideration are 1, 3, 4, and 5 in Table 16.10. According to Eq. (16.21)

$$C_w = (33.3, 0.87, 300) - 0.5(-33.3, 1.33, -200)$$
$$= (50, 0.20, 400)$$

Table 16.10 Sequential Experiments in Optimization Process

Experiment	Disintegrant	Lubricant	Fill weight	Response
1	50	0.2	100	37
2	0	2.2	100	58 (W_1)[a]
3	0	0.2	400	46 (W_3)
4	50	2.2	400	40
5	66.6	−0.46	500	− (W_2)
6	50	0.20	400	44 (W_4)
7	100	1.54	200	42 (W_6)
8	83.3	2.42	67	43 (W_5)
9	58.4	0.75	316	36
10	8.5	0.07	416	41 (W_7)
11	39	0.56	344	44 (W_8)
12	56.2	0.8	308	35

[a] W_1 means that this result was eliminated after the first evaluation.

The response, R_{cw}, to C_w is 44. According to Step 4 above, we retain this result. This is shown as experiment 6 in Table 16.9. We now operate on experiments 1, 3, 4, and 6; experiment 3 is the new worst result.

We go to Step 1 and compute our new formulation R from Eq. (16.18):

$$R = (50, 0.87, 300) + (50, 0.67, -100) = (100, 1.54, 200)$$

The response R, is 42 (represented by experiment 7 in Table 16.9). This is better than the second worst response (44 for experiment 6) and we retain R_r as directed in Step 1 above. We recompute R for the set of experiments 1, 4, 6, and 7:

$$R = (66.7, 1.31, 233) + (16.7, 1.11, -167) = (83.3, 2.42, 67)$$

The response, R_r, is 43. This is worse than the second-to-worst response, 42. Therefore we go to Step 3:

$$C_r = P + 0.5(\bar{P} - W)$$

$$C_r = (66.7, 1.31, 233) + 0.5(-16.7, -1.11, 167)$$

$$= (58.4, 0.75, 316)$$

The new response (experiment 9) is 36.

According to our rules, we go to Step 2:

$$E = \bar{P} + 2(\bar{P} - W)$$

$$E = (69.5, 1.05, 272) + 2(-30.5, -0.49, 72)$$

$$= (8.5, 0.07, 416)$$

The response to E is 41. According to Step 2, we retain R in lieu of E because R gave the better response. We compute a new R from Step 1:

$$R = (69.5, 1.05, 272) + (-30.5, -0.49, 72)$$

$$= (39, 0.56, 344)$$

The response is 44. Our new set of 4 experiments is numbers 1, 4, 9, and 11, with number 11 the worst.

We go to Step 4 and compute C_w because the value of R is worse than R_w:

$$C_w = (69.5, 1.05, 272) - 0.5(30.5, 0.49, -72)$$
$$= (54.2, 0.8, 308)$$

The response was 35 (see experiment 12).

The experiments may continue as described above until repeated experiments do not show improvement. We are searching for an optimal response in the presence of variability. In the present case, a formula containing approximately 55 of disintegrant and 0.75 of lubricant with a fill weight of 300 mg appeared to show minimal dissolution time; the study was stopped after experiment 12.

As with other optimization procedures presented in this chapter, studying details in the literature references is essential to understand the procedure and calculations [8–11].

16.6 Screening Designs

Usually, we know the factors that we wish to investigate, from our experience. However, in new, unknown, situations, it is possible that we may consider a number of factors to investigate, to see if any of these may affect the response or outcome. If there are only a few such variables (or factors), we may wish to use a factorial or fractional factorial design. If there are many potential factors of interest, screening designs are available that use less runs, but do give us insight into effects of interest. The most popular of such designs are the Plackett-Burman designs.

Screening designs may be useful if little is known of the system. In most cases, one should have a reasonable idea of which variables are important, and their effective ranges. But, we may be surprised. If everything were known, experimentation would not be becessary. Also, one should be careful not to neglect potentially important variables.

Screening designs, in general, are fractional factorials of 2^n designs that estimate main effects, but not interactions. If results of such experiments point to specific factors, one can follow up with more complete designs to evaluate specific interactions.

A twelve-run design is shown in Table 16.11. Note that the − and + signs refer to the low and high levels of the factor, respectively. Thus, for example, Factor 1 in Run 1

Table 16.11 Twelve-Run Plackett-Burman Design

Run	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11
1	+	+	−	+	+	+	−	−	−	+	−
2	+	−	+	+	+	−	−	−	+	−	+
3	−	+	+	+	−	−	−	+	−	+	+
4	+	+	+	−	−	−	+	−	+	+	−
5	+	+	−	−	−	+	−	+	+	−	+
6	+	−	−	−	+	−	+	+	−	+	+
7	−	−	−	+	−	+	+	−	+	+	+
8	−	−	+	−	+	+	−	+	+	+	−
9	−	+	−	+	+	−	+	+	+	−	−
10	+	−	+	+	−	+	+	+	−	−	−
11	−	+	+	−	+	+	+	−	−	−	+
12	−	−	−	−	−	−	−	−	−	−	−

is at the high level. (See Chapter 9 for further explanation of terminology.) For other designs, e.g., higher-order or more complex designs, a statistician should be consulted. In general, variability cannot be estimated without replication (run the design in duplicate, for example) or partial replication. This would increase the size and cost of the experiment. As in other design considerations, the cost and time considerations must be weighed against the information gained from expanded experiments. If less factors than runs are used, an estimate of variability can be provided. This is shown in the following example.

An example of a 12-run Plackett-Burman design is shown in Table 16.12. This design estimates the main effects of six variables. This leaves 5 d.f. for estimating the error. The estimates based on columns 7–11, inclusive, are only used to compute the variability, and are not related to the six factors in the experiment. An example of an experiment using this design could be as follows. The effect of six variables on the dissolution of a tablet is to be investigated. The six factors are (X_1) hardness, (X_2) Level of disintegrant, (X_3) time of mixing granulation, (X_4) level of lubricant, (X_5) type of coating, and (X_6) tablet press pressure. The response is the percent dissolution in 30 min. Each factor is set at a low (-1) and a high ($+1$) level. (Note that ''type of coating'' is arbitrarily set at -1 and $+1$.)

The analysis is most easily accomplished using a multiple regression computer program. When designating values for the model in the computer program, it is convenient to input -1 for the low level and $+1$ for the high level. Table 16.13 shows an example of relevant computer output.

Note that only main effects are estimated. The error term is comprised of the five columns that were not assigned to factors (columns 7–11). If only five factors were investigated, columns 6–11 would be used to estimate error with 6 degrees of freedom. The estimate of error allows us to test the main effects for significance. This is a conservative test because the error will be, if anything, estimated on the high side. That is, if any interactions are present, the error estimate will be too high. This means that we may miss some significant effects if interaction is present. In this example, X_2 just misses significance, and X_3 and X_5 are significant. Again, the six factors are (X_1) hardness, (X_2) Level of disintegrant, (X_3) time of mixing granulation, (X_4) level of lubricant, (X_5) type of coating,

Table 16.12 Example of Twelve-Run Plackett-Burman Design

Dissolution	X1	X2	X3	X4	X5	X6	Error	Error	Error	Error	Error
75	1	1	−1	1	1	1	−1	−1	−1	1	−1
104	1	−1	1	1	1	−1	−1	−1	1	−1	1
57	−1	1	1	1	−1	−1	−1	1	−1	1	1
54	1	1	1	−1	−1	−1	1	−1	1	1	−1
46	1	1	−1	−1	−1	1	−1	1	1	−1	1
58	1	−1	−1	−1	1	−1	1	1	−1	1	1
3	−1	−1	−1	1	−1	1	1	−1	1	1	1
98	−1	−1	1	−1	1	1	−1	1	1	1	−1
80	−1	1	−1	1	1	−1	1	1	1	−1	−1
12	1	−1	1	1	−1	1	1	1	−1	−1	−1
100	−1	1	1	−1	1	1	1	−1	−1	−1	1
13	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1

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Table 16.13 Multiple Regression Computer Output of Data in Table 16.12

Independent variable	Regression coefficient	*T*-Value (Ho: B=0)	Prob. level	Decision (5%)
Intercept	58.33	13.3748	0.000042	Reject Ho
X1	−0.167	−0.0382	0.970996	Accept Ho
X2	10.33	2.3692	0.064013	Accept Ho
X3	12.5	2.8660	0.035158	Reject Ho
X4	−3.167	−0.7261	0.500353	Accept Ho
X5	27.5	6.3052	0.001477	Reject Ho
X6	−2.667	−0.6114	0.567651	Accept Ho

Analysis of Variance

Source	DF	Sum of squares	Mean square	*F*-Ratio	Prob. level
Intercept	1	40833.33	40833.33		
Model	6	12437.33	2072.889	9.0810	0.014
Error	5	1141.33	228.267		
Total	11	13578.67	1234.424		

and (X_6) tablet press pressure. Therefore, we might wish to consider the level of disintegrant, time of mixing, and type of coating if we wish to modify the dissolution. The type of coating seems to have the greatest effect.

KEY TERMS

Checkpoint
Coding
Composite designs
Contour plot
Extra-design points
Factorial designs
Fractional factorial designs
Grid
Independence
Model
Model error
Multiple regression
Optimization
Orthogonality
Plackett–Burman
Polynomial equation
Replication
Response equation
Response surface
Screening designs
Sequential optimization
Simplex design
Simplex space
Transformation

EXERCISES

1. Calculate the predicted response from Eq. (16.6) for
   (a) $X_1 = 1$ mg, $X_2 = 1$ mg, $X_3 = 2.5$ mg
   (b) $X_1 = 2$ mg, $X_2 = 1$ mg, $X_3 = 4$ mg
   [Note that Eq. (16.6) uses coded values; see Eq. (16.4).] For example, the coded value for $X_1 = 1$ mg is $0 = (1 - 1)/1$.

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2. Show that the transformed values of $X_1 = 1$, $X_2 = 0.5$, and $X_3 = 2.5$ are all equal to zero for the three variables in Exercise Problem 1.
3. Calculate the coefficients for the polynomial equation, (16.8). The coefficients are calculated from the data in Table 16.4.
4. Show that decoded values of *A* and *B* equal to 0.5 and 1, respectively, are equal to 8.75 mg of *A* and 100 mg of *B*, for the data of Table 16.4 and Eq. (16.8). Calculate the expected response of this combination of *A* and *B* using Eq. (16.8).
5. A formulation was to be prepared to optimize dissolution time. (The formulation with the dissolution time of approximately 15 min is ''optimal.'') Stearic acid and mixing time were varied according to a 2^2 factorial design with the following results:

		Stearic acid	
		0.25%	1%
Mixing time (min)	15	10	23
	30	21	25

   (a) Construct a polynomial response equation [see Eq. (16.8)].
   (b) What concentration of stearic acid and mixing time would you choose for the final product?

**6. Calculate the maximum solubility based on Eq. (16.13), using procedures of calculus. [Hint: Set the first derivative equal to 0 after substituting (1.00 − A) for B.]

7. A total of 100 mg of three components, stearic acid (*A*), starch (B), and DCP (C), are to be added to a tablet formulation. Dissolution time was measured in a simplex design with the following results:

100% A:	292.0 min
100% B:	5.6 min
100% C:	50.4 min
50% A, 50% B:	25.6 min
50% B, 50% C:	15.6 min
50% A, 50% C:	124.5 min
1/3A, 1/3B, and 1/3C:	37.0 min

   (a) Compute the simplex equation coefficients.
   (b) Give a combination with very fast dissolution.
   (c) Give a combination that has a dissolution time of 90 min.

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** Optional, more advanced problem.

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GLOSSARY

a	calculated intercept in regression
ANCOVA	analysis of covariance
ANOVA	analysis of variance
b	calculated slope in regression
BMS	between mean square
BSS	between sum of squares
C. T.	correction term
CI	confidence interval
CV	coefficient of variation; relative error; relative standard deviation
CXR	column $\times$ row interaction
df	degrees of freedom
E	expected number in chi-square table
F	F value for F distribution
Ha	alternative hypothesis
Ho	null hypothesis
ln	natural log
LSD	least significant difference
O	observed number in chi-square table
p	estimated proportion (binomial)
p (A)	probability that event will occur
p (A|B)	conditional probability of A given B
p_o	true or hypothesized proportion
q	probability of failure in binomial
R	range
r	calculated correlation coefficient
r (Dixon)	computation for outlier analysis
r^2	square of correlation coefficient
RSD	relative standard deviation
S	sample standard deviation

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S^2	sample variance
$S^2y.x$	estimated variance from line fitting
t	*t* value for *t* distribution
Tn	test for outlier
σ	true standard deviation of distribution
w	weight in weighted least squares
WSS	within sum of squares
X_i	*i*th observation
Z	normal standard deviate
χ^2	chi square
Δ	delta, true change or difference
N	sample size
Σ	sum of observations
α	alpha level or error for null hypothesis; error of first kind
β	beta error (1-power)
δ	observed change or difference
μ	true mean of distribution

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ANVENDELSE AV KJEMOMETRI

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The Swedish Chemical Society

1.4 Försöksplanering och optimering för experimentalisten

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1.4.1 Inledning

Försöksplanering, eller experimentell design, och optimering är verktyg som bör användas för att systematiskt undersöka olika typer av problem som uppstår inom t ex forskning, utveckling och produktion. Det är uppenbart att om försök utförs slumpmässigt, så får man också slumpmässiga resultat. Därför är det viktigt att planera sina experiment och att göra detta så att de innehåller den information man är ute efter.

På följande sidor presenteras *försöksplanering och optimering* på ett sätt som ska ge *experimentalisten* verktyg i den praktiska verkligheten tillsammans med den teoretiska bakgrund som behövs för att fatta riktiga beslut i den experimentella situationen.

Klargör målsättningen

Vad är målet?

Denna fråga måste ställas på alla aspekter inom ett forsknings- eller utvecklingsprojekt. Både vad gäller helheten och på alla detaljer inom varje delprojekt. När målsättningen är klar skall problemet analyseras med hjälp av följande frågor:

Vad är känt?
Vad vet vi inte?
Vad behöver vi undersöka?

För att experimenten skall kunna planeras och utföras på ett rationellt sätt så måste problemet konkretiseras.

Vilka experimentella variabler kan undersökas?
Vilka responser kan jag mäta?

När responserna och de experimentella variablerna har definierats, så kan experimenten planeras och utföras på ett sådant sätt att maximal information erhålls ur ett minimalt antal experiment.

Terminologi

För att underlätta kommunikationen och se till att vi menar samma sak, så introduceras redan från början några olika termer. Fler

termer kommer att introduceras efter hand och i sitt sammanhang.

Experimentell domän - det experimentella område, definierat av variabelinställningarna, som undersöks.
Faktorer - de variabler som vi kan ändra oberoende av varandra för att påverka utfallet i ett experiment.
Oberoende variabler - samma som faktorer.
Kontinuerliga variabler - oberoende variabler som kan ändras steglöst.
Diskreta variabler - oberoende variabler som ändras stegvis, exempelvis typ av lösningsmedel.
Beroende variabler - variabler som observeras och är ett resultat av att oberoende variabler förändras.
Responser - samma som beroende variabler.
Residual - avvikelse mellan den beräknade modellen och det experimentella resultatet.

Empiriska modeller

Det är rimligt att anta att resultatet av experiment beror på hur experiment har utförts. Detta betyder då att resultatet kan beskrivas som en funktion av de experimentella variablerna.

$$y = f(x)$$

En sådan funktion kan approximeras till olika typer av polynom och ändå ge en bra beskrivning av sambandet mellan de experimentella variablerna och responsen inom en begränsad experimentell domän. Tre typer av polynomapproximationer kommer att beskrivas.

Den enklaste polynommodellen innehåller enbart linjära termer och förutsätter då linjära samband mellan de experimentella variablerna och responsen. Med två variabler erhålls följande *linjära modell*:

$$y = b_0 + b_1x_1 + b_2x_2 + residual$$

Nästa nivå på modell innehåller även termer som beskriver samspelseffekter mellan olika experimentella variabler detta ger en *andra ordningens interaktionsmodell*:

$$y = b_0 + b_1x_1 + b_2x_2 + b_{12}x_1x_2 + residual$$

De två ovanstående modellerna används för att undersöka experimentella system för exempelvis sållning, robustnesstest och liknande.

För att kunna bestämma ett optimum (maximum eller minimum) introduceras kvadratiska termer i modellen. Dessa termer ger då ett mått på krökningar, dvs icke-linjära samband mellan de experimentella variablerna och responsen. Detta ger ett polynom som beskriver en *kvadratisk modell*:

$$y = b_0 + b_1x_1 + b_2x_2 + b_{11}x_1^2 + b_{22}x_2^2 + b_{12}x_1x_2 + residual$$

Dessa polynom innehåller olika antal okända *parametrar, b*, som skall bestämmas. För de olika modellerna krävs därför olika typer av experimentella designer.

1.4.2 Sållningsförsök ("Screening")

För varje experimentellt förfarande finns en mängd faktorer, experimentella variabler, som kan påverka resultatet. Sållning innebär att man undersöker vilka experimentella variabler och interaktioner mellan dessa som har ett verkligt inflytande på resultatet, mätt som en eller flera responser.

Några allmänna råd för sållningsförsök

Precisera problemet:
Tänk igenom *hela* förfarandet - olika delmoment, kritiska steg, utgångsmaterial, utrustning, m.m. Försök få en helhetsbild av problemet.

Definiera respons:
Vilken respons kan mätas?
Vilka felkällor finns vid mätningen?
Kan förloppet följas över tiden?

Välj variabler:
Vilka experimentella variabler finns?
Tänk igenom och gradera variablerna - viktiga, troligen viktiga, troligen oviktiga?
Vilken variation finns i variablerna och vilken variation kan detta förväntas ge i responsen?
Välj experimentell domän.
Är alla variabler intressanta i den experimentella domänen?
Vilka samspelseffekter är direkt förutsägbara?
Vilka variabler samverkar sannolikt *inte*?

Detta ger en lista över responser, intressanta experimentella variabler och deras eventuella samspelseffekter. Gå igenom denna lista *kritiskt* ytterligare några gånger.

Den tid man ägnar i början av planeringen har man ofta igen mångfaldigt i slutändan.

Man har nu kommit fram till vilka variabler som måste undersökas samt vilka som är av mer marginellt intresse och kan hållas konstanta, dvs "bakas in" i proceduren. Tänk på att det oftast är mer ekonomiskt att ta med en eller flera extra variabler i det första sållningsförsöket än att lägga till en variabel vid ett senare tillfälle.

Tänk därefter igenom *hur* de olika variablerna skall definieras. Det är ibland möjligt att hushålla med experimenten och få önskad information med färre variabler än vad som ursprungligen definierades. Man kan t.ex. definiera mängd/mängd-förhållanden som variabler i stället för att låta koncentrationer av olika ämnen variera:

koncentrationerna [A], [B], [C] : tre variabler ger samma information som förhållandena A/B eller C/B : två variabler

I stället för att undersöka om *bortforslandet av reaktionsvärme* är väsentligt genom att variera reaktorns form o.s.v. bör man tänka efter om inte en signifikant samspelseffekt mellan *omrörarhastighet* och *reaktionstemperatur* egentligen innehåller information även om detta.
När alla aspekter på försöket penetrerats och variabler, responser samt experimentell domän valts är det dags för nästa steg i planeringsprocessen.

Ansätt en modell och planera experimenten:
Med den slutliga listan över vilka variabler som skall undersökas planerar man sedan en serie experiment så att man kan skatta effekterna av de olika variablerna. För sållningsförsök använder man sig av linjära modeller eller andra ordningens interaktionsmodeller. Dessa ger ingen perfekt beskrivning av responsytan (ingen kurvatur) men ger uppskattningar av lutningen, b_i, längs varje variabel-axel samt vridningen, b_{ij}, för att beskriva interaktioner.

Detta är precis vad som behövs för att bestämma variablernas inflytande.

Kompletta faktorförsök på två nivåer

Ett faktorförsök innebär att man undersöker hur en eller flera responser varierar beroende på förändringar av de olika experimentella variablerna, *faktorerna*.

I detta avsnitt skall vi gå igenom uppställning och utvärdering av kompletta faktorförsök på två nivåer.

I ett komplett faktorförsök undersöks samtliga kombinationer av k faktorer på två nivåer. Ett komplett faktorförsök innehåller därför 2^k experiment. I Tabell 1 visas försöksplaner för kompletta faktorförsök med 2, 3 och 4 variabler. Faktorernas nivåer anges med – för låg nivå och + för hög nivå.

Försöksplanerna konstrueras enligt en standardordning:

Kolonn 1: (variabel 1 eller faktor 1) varannan – , varannan +.
Kolonn 2: två – , två + osv.
Kolonn 3: fyra – , fyra + osv.
Kolonn 4: åtta – , åtta + osv.

För större kompletta faktorförsök fortsätter man bara på samma sätt, dvs

Kolonn 5: sexton – , sexton + osv.

Detta kan generaliseras till *k* variabler (faktorer) och täcker då samtliga variationer.
I de kompletta faktorförsöken läggs alltid en centrumpunkt till för att undersöka eventuella krökningar. Försöksplanerna i Tabell 1 kallas också för *designmatriser*.

Tabell 1. Faktorförsöksuppställningar.

Två variabler			Tre variabler				Fyra variabler				
Exp	Variabler		Exp	Variabler			Exp	Variabler			
nr.	x_1	x_2	nr.	x_1	x_2	x_3	nr.	x_1	x_2	x_3	x_4
1	–	–	1	–	–	–	1	–	–	–	–
2	+	–	2	+	–	–	2	+	–	–	–
3	–	+	3	–	+	–	3	–	+	–	–
4	+	+	4	+	+	–	4	+	+	–	–
			5	–	–	+	5	–	–	+	–
			6	+	–	+	6	+	–	+	–
			7	–	+	+	7	–	+	+	–
			8	+	+	+	8	+	+	+	–
							9	–	–	–	+
							10	+	–	–	+
							11	–	+	–	+
							12	+	+	–	+
							13	–	–	+	+
							14	+	–	+	+
							15	–	+	+	+
							16	+	+	+	+

I Tabell 1 anges nivåerna för faktorerna (variablerna) till – eller +. Minus-nivån innebär att variabeln skall hållas på låg nivå medan plus-nivån innebär att variabeln skall hållas på hög nivå. En noll-nivå (centrumpunkt) innebär att variabeln hålls mitt emellan hög och låg nivå. Vad – och + skall motsvara defineras utifrån vad som anses vara en rimlig variation att undersöka. På så sätt har storleken på den experimentella domän som skall undersökas fastställts. För två och tre variabler kan den experimentella domänen och designen illustreras på ett enkelt sätt. För två variabler utgör experimenten i designen hörnen i en kvadrat (Figur 1) medan de i en design med tre variabler blir hörnen i en kub (Figur 2).

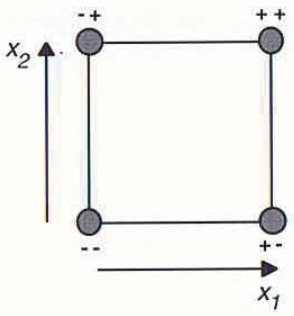


Fig. 1. Experimenten i en design med två variabler.

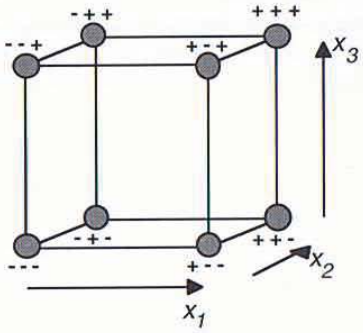


Fig. 2. Experimenten i en design med tre variabler.

Med fyra eller flera variabler utgör designerna s.k. hyperkuber. Dessa fungerar på samma sätt som en vanlig kub, men är omöjlig att grafiskt illustrera.

Tecken för samspelseffekter

Tecknet för en samspelseffekt mellan variabel 1 och variabel 2 definieras som tecknet för produkten av variabel 1 och variabel 2, dvs. minus multiplicerat med minus blir plus, plus multiplicerat med plus blir plus och minus multiplicerat med plus blir minus. Det är nu möjligt att konstruera teckenkolonner för samtliga samspelseffekter vid ett faktorförsök.

Exempel med två variabler.

x_1	x_2	x_1x_2
–	–	+
+	–	–
–	+	–
+	+	+

Ett praktiskt exempel med 3 variabler (faktorer)

Detta exempel illustrerar hur man med hjälp av teckentabellerna kan beräkna huvudeffekter och samspelseffekter i ett fullständigt faktorförsök.

Exemplet är från en organisk syntes där man ville undersöka hur tre olika variabler x_1, x_2 och x_3 påverkade utbytet. I Tabell 2 är variablerna specificerade tillsammans med den experimentella domän man valt.

Tabell 2. Variabler och nivåer.

Variabler	Experimentell domän (-)-nivå	0-nivå	(+)-nivå
x_1: Katalysatorkoncentration (%)	0.1	0.2	0.3
x_2: Reaktionstemperatur (°C)	60	70	80
x_3: Reaktionstid (min)	20	30	40

En teckentabell (designmatris) för att variera 3 variabler på två nivåer konstrueras enligt Tabell 3.

Tabell 3. Design och respons (utbyte).

Exp.	x_1	x_2	x_3	Utbyte (%)
1	–	–	–	73
2	+	–	–	71
3	–	+	–	79
4	+	+	–	82
5	–	–	+	78
6	+	–	+	89
7	–	+	+	83
8	+	+	+	93
9	0	0	0	81

Försöken utvärderas därefter för att anpassa responsen till en modell, i detta fall en andra ordningens interaktionsmodell:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{123}x_1x_2x_3$$

Tabellen ovan användes för att beräkna huvudeffekterna och medelvärdet, b_0. Centrumpunkten använder man för att kontrollera om ytan är krökt. Ligger responsen för centrumpunkten långt ifrån medelvärdet b_0 är ytan krökt och då får man utöka modellen med kvadratiska termer.

$b_0 = 1/8 \cdot (73+71+79+82+78+89+83+93)$
$= 81$

Huvudeffekterna beräknas genom att använda tecknet i respektive kolonn för att antingen addera eller subtrahera värdet för responsen, y. Därefter divideras med antalet experiment.

$b_1 = 1/8(-73+71-79+82-78+89-83+93) = 2.8$
$b_2 = 1/8(-73-71+79+82-78-89+83+93) = 3.2$
$b_3 = 1/8(-73-71-78-82+78+89+83+93) = 4.9$

För att sedan kunna beräkna samspelseffekterna konstruerar man teckenkolonner för respektive effekt. I detta fallet blir det: x_1x_2, x_1x_3, x_2x_3 och $x_1x_2x_3$. Tecknen i dessa får man genom att multiplicera tecknet för respektive huvudeffekt i varje experiment, Tabell 4. Denna typ av matris kallas *modellmatris* eller *beräkningsmatris*. Nollexperimentet har tagits bort i denna matris eftersom detta inte används för att beräkna effekterna, utan endast används för jämförelse med medelvärdet.

Tabell 4. Modellmatris och respons.

Exp.	I	x_1	x_2	x_3	x_1x_2	x_1x_3	x_2x_3	$x_1x_2x_3$	Utbyte (%)
1	+	–	–	–	+	+	+	–	73
2	+	+	–	–	–	–	+	+	71
3	+	–	+	–	–	+	–	+	79
4	+	+	+	–	+	–	–	–	82
5	+	–	–	+	+	–	–	+	78
6	+	+	–	+	–	+	–	–	89
7	+	–	+	+	–	–	+	–	83
8	+	+	+	+	+	+	+	+	93

Beräkningarna för samspelseffekterna utförs sedan på samma sätt som för huvudeffekterna.

$b_{12} = 1/8(73-71-79+82+78-89-83+93) = 0.5$
$b_{13} = 1/8(73-71+79-82-78+89-83+93) = 2.5$
$b_{23} = 1/8(73+71-79-82-78-89+83+93) = -1.0$
$b_{123} = 1/8(-73+71+79-82+78-89-83+93)$
$= -0.8$

Effekterna för variablerna sättes därefter in i modellen som beskriver responsens beroende av de olika variablerna:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{123}x_1x_2x_3$$

$$y = 81 + 2.8x_1 + 3.2x_2 + 4.9x_3 + 0.5x_1x_2 + 2.5x_1x_3 - 1.0x_2x_3 - 0.8x_1x_2x_3$$

Modellen ovan beskriver nu hur responsen y påverkas av de olika experimentella variablerna samt dess samspelseffekter. I modellen kan vi se att variabel x_3 påverkar utbytet mest, en höjning av variabel 3 med en skalad enhet (från t.ex. 0 till +1) ger en ökning av utbytet med 4.9 %. Detta innebär i reella variabler en ökning av reaktionstiden med 10 minuter.

För att effekterna ska anses som signifikanta bör deras bidrag vara större än det experimentella felet.

Facit till övningsexemplen finns på den medföljande disketten till boken.

ÖVNINGSEXEMPEL 1

Faktorförsök 2^3: Farmaci, kärntillverkning

Detta exempel är hämtat från ett projekt inom Pharmacia. Processen går ut på att tillverka koffeinkärnor av en viss storlek (0.71-1.4 mm).

Tre variabler undersöktes: Mängd vatten vid granulering (Granv), sfäroniseringshastighet (Sfhast) och sfäroniseringstid (Tidsfpl).

Ett fullständigt faktorförsök 2^3 gjordes för att undersöka robustheten hos processen. Variablernas nivåer och designen var som följer:

	Experimentell domän	
	-	+
x_1: Granv	25.37	25.93
x_2: Sfhast	650	950
x_3: Tidsfpl	60	180

Design och responser.

Exp nr.	x_1	x_2	x_3	Utbyte (0.71-1.4mm) (%)
1	-	-	-	97.4
2	+	-	-	98.1
3	-	+	-	97.1
4	+	+	-	97.8
5	-	-	+	98.6
6	+	-	+	98.2
7	-	+	+	98.3
8	+	+	+	98.3

Uppgift:

Utred hur de experimentella variablerna påverkar utbytet enligt följande responsfunktion:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{123}x_1x_2x_3$$

ÖVNINGSEXEMPEL 2

Faktorförsök 2^3: Farmaci, tablettillverkning

Vid tablettillverkning ansågs tre variabler kunna påverka tablettens tjocklek. Dessa variabler undersöktes vid tablettslagningen med ett faktorförsök. De olika variablerna var mängden stearat (smörjmedel), mängden aktiv substans och mängden stärkelse (sprängmedel). Den experimentella domänen tillsammans med försöksplan och erhållna resultat visas nedan.

Variabler och experimentell domän vid tablettslagning (övningsexempel 2).

Variabler		Experimentell domän (–)-nivå	0-nivå	(+)-nivå
x_1 :	Mängd stearat (mg)	0.5	1.0	1.5
x_2 :	Mängd aktiv substans (mg)	60	90	120
x_3 :	Mängd stärkelse (mg)	30	40	50

Försöksplan och erhållna resultat (övningsexempel 2).

Exp nr.	Variabler x_1	x_2	x_3	Tjocklek (mm) y
1	–	–	–	4.75
2	+	–	–	4.87
3	–	+	–	4.21
4	+	+	–	4.26
5	–	–	+	5.25
6	+	–	+	5.46
7	–	+	+	4.72
8	+	+	+	5.22
9	0	0	0	4.86

Uppgifter:

a) Utred hur de experimentella variablerna påverkar tablettens tjocklek. Finns det anledning att förmoda att samspelseffekter är viktiga?
b) Bestäm en polynomisk responsfunktion som innehåller endast sannolikt signifikanta termer. Använd sedan denna modell för att beräkna hur mycket stärkelse som skall användas med 100 mg aktiv substans, så att man erhåller tabletter som är 5.00 mm tjocka.

ÖVNINGSEXEMPEL 3

Sållning: Godistillverkning - sega råttor

Antag att du är utvecklingsingenjör vid ett mindre godisföretag. Företaget vill förbättra tillverkningen av sega råttor så att de blir bättre i konsistens, lättare att tillverka, få bättre lagringsegenskaper och bli billigare att tillverka. Du får till hjälp nedanstående recept och detta skall nu förbättras.

Recept: 90 g socker
50 g glukos
25 ml vatten
40 g gelatinlösning
(14 g gelatinpulver + 26 ml vatten)

Utförande: Gelatinpulver och vatten blandas och värmes svagt tills allt gelatinet löst sig. Gelatinlösningen får sedan svalna! Socker, glukos och vatten kokas till 114°C. Låt blandningen svalna till högst 100°C och tillsätt gelatinlösningen. Ev. färg tillsätts. Nu kan även smakämnen tillsättas, t.ex. några droppar essens och/eller lite 50%-ig citronsyra.
Låt blandningen stå i vattenbad vid 80°C i 15 minuter. Obs! Det är viktigt att temperaturen hålls! Skumma under tiden.

Förbered gjutningen, dvs häll potatismjöl på en bricka och jämna av ytan med en linjal. Gör sedan fördjupningar i mjölet att gjuta massan i. Strö lite potatismjöl även ovanpå råttorna.

Uppgifter:

a) Läs igenom receptet och identifiera experimentella variabler. Fundera kritiskt över listan och välj ut de variabler som du anser viktigast. Ange även möjliga responser.
b) Lägg upp en försöksdesign.

1.4.3 Reducerade faktorförsök

Om man vill undersöka effekterna av k variabler har vi sett att man med ett komplett faktorförsök (2^k experiment) kan bestämma samtliga huvudeffekter och samtliga samspelseffekter. För 7 variabler krävs 128 experiment; 10 variabler 1024 experiment; 15 variabler 32,768 experiment. Det är uppenbart att man ganska snabbt passerar gränsen för vad som är möjligt att genomföra. För många problemsituationer är det oftast rimligt att anta att samspelseffekter mellan tre eller flera variabler är försvinnande små. Dessa kan då försummas i jämförelse med huvudeffekter och två-variabelinteraktioner. Detta innebär att 128 experiment är onödigt många för att bestämma medelutbyte, sju huvudeffekter och 21 tvåfaktors samspelseffekter, d.v.s. 29 parametrar. För detta skulle precis 29 experiment kunna vara tillräckliga. Vi skall nu se hur man kan definiera designmatriser så att man med en fraktion (1/2, 1/4, 1/8, 1/16 ..... $1/2^p$) av ett komplett faktorförsök kan skatta huvudeffekter och samspelseffekter med 2^{k-p} experiment, där k är antalet variabler och p definieras av storleken på fraktionen.

Om man till att börja med endast vill skatta huvudeffekter kan man undersöka 3 variabler i 4 experiment; upp till 7 variabler i 8 experiment; upp till 15 variabler i 16 experiment etc. Detta svarar mot en *responsfunktion*:

$$y = \beta_o + \Sigma\beta_i x_i + \varepsilon$$

Det är sedan möjligt att *komplettera* experimenten så att eventuella samspelseffekter kan skiljas ut om det skulle finnas anledning att misstänka att sådana är viktiga. Detta innebär att man undersöker en *utökad responsfunktion*:

$$y = \beta_o + \Sigma\beta_i x_i + \Sigma\Sigma\beta_{ij} x_i x_j + \varepsilon$$

I många fall behöver man inte undersöka samspelseffekter mellan *alla* variabler som man *inledningsvis* tagit med. Efter en första serie experiment och en utvärdering av en linjär modell visar det sig ofta att några variabler har ett litet, eller rent av försumbart, linjärt inflytande. Sådana variabler visar mycket sällan starka samspelseffekter med andra variabler. Samspelseffekter förekommer oftare mellan variabler som också har starkt linjärt inflytande. Naturligtvis är detta resonemang inte alltid sant, men ger en möjlighet att förenkla problemet och kommer därför att reducera antalet försök som behövs för att komplettera med.

Detta betyder att det är en fördel att ta med alla potentiellt intressanta variabler i en inledande serie sållningsexperiment ("screening"). Detta kan göras utan att antalet experiment blir orimligt stort. En analys av dessa experiment kommer att identifiera *vilka* variabler som bör ingå i en mer detaljerad studie.

Princip för konstruktion av reducerade faktorförsök

Det är *modellmatriser* från ett komplett faktorförsök som används för att definiera *designmatriser* till reducerade faktorförsök. Detta betyder att vi låter kolonnerna i modellmatrisen **X** till ett faktorförsök definiera inställningen av variablerna i en serie experiment. Vi kan ta med så många variabler som det finns kolonner i **X**. Vi kommer i

detta avsnitt att använda arabiska siffror med fet stil för att beteckna variabelkolonner i matriserna, samt **I** för att beteckna kolonnen för den konstanta termen i modellen.

Exempel: Ett faktorförsök för två variabler har följande kompletta modellmatris

I	**1**	**2**	**12**
1	-1	-1	1
1	1	-1	-1
1	-1	1	-1
1	1	1	1

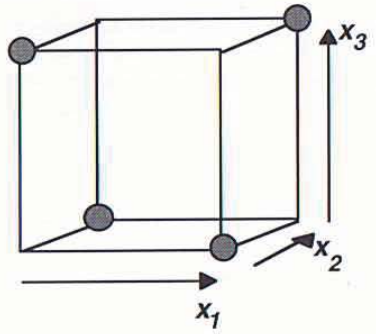
Kolonnen **12** skall tolkas som produkten av **1·2**. Vi kan använda kolonnerna **1**, **2** och **12** för att definiera variationen hos tre variabler, x_1, x_2 och x_3 i fyra försök och detta ger designmatrisen

Exp no	x_1	x_2	x_3
1	-1	-1	1
2	1	-1	-1
3	-1	1	-1
4	1	1	1

Detta är en halv fraktion av ett komplett faktorförsök för tre variabler och man kan se att experimenten motsvarar Exp.nr. 5, 2, 3 och 8 i det kompletta faktorförsöket.

Exp no	x_1	x_2	x_3
1	-1	-1	-1
2	1	-1	-1
3	-1	1	-1
4	1	1	-1
5	-1	-1	1
6	1	-1	1
7	-1	1	1
8	1	1	1

Figur 3 visar hur experimenten för den halva fraktionen fördelar sig i den rymd som spänns upp av de tre variablerna. Man kan också se att experimentpunkterna utgör en tetraeder, vilket är den största volymen som kan spännas ut av fyra punkter. Detta visar



Figur 3. Fördelning av experimenten i ett 2^{3-1} reducerat faktorförsök.

på en viktig egenskap hos reducerade faktorförsök - experimenten täcker så stor del som möjligt av den rymd som spänns upp av variablerna. Hela rymden kan inte täckas av ett begränsat antal punkter, men ett reducerat faktorförsök väljer ut experiment som är så långt ifrån varandra som möjligt och därför täcker en maximal variation i den experimentella rymden.

Ett annat exempel:
Vi kan studera 7 variabler i ett 2^{7-4} reducerat faktorförsök.
Designen definieras utifrån modellmatrisen till ett 2^3 faktorförsök.

Designmatris 2^{7-4}:

		x_1	x_2	x_3	x_4	x_5	x_6	x_7
Exp no	**I**	**1**	**2**	**3**	**12**	**13**	**23**	**123**
1	1	-1	-1	-1	1	1	1	-1
2	1	1	-1	-1	-1	-1	1	1
3	1	-1	1	-1	-1	1	-1	1
4	1	1	1	-1	1	-1	-1	-1
5	1	-1	-1	1	1	-1	-1	1
6	1	1	-1	1	-1	1	-1	-1
7	1	-1	1	1	-1	-1	1	-1
8	1	1	1	1	1	1	1	1

Ett komplett faktorförsök för sju variabler ger 128 försök. Designens experimentella punkter definierar "hörnen" i en hyperkub i den sju-dimensionella rymden som spänns upp

av de sju variablerna. Den reducerade designen 2^{7-4} är 1/16 av den kompletta designen och väljer ut åtta av de 128 hörnen på sådant sätt att de är så långt ifrån varandra som möjligt och således täcker en maximal variation i den experimentella rymden.
I det reducerade faktorförsöket har vi alltså definierat variablerna x_4 - x_7 som kolonnerna med samspelseffekterna för variablerna i kolonn **1**, **2** och **3**. Kolonnerna i det reducerade faktorförsöket är då *ortogonala* och därför är det möjligt att skatta medelvärde och samtliga huvudeffekter *oberoende av varandra och med maximal precision*.

På samma sätt kan kolonnerna i en 2^4 faktoriell design användas för att definiera variationerna av upp till 15 variabler i 16 försök, dvs ett 2^{15-11} reducerat faktorförsök. Detta är 1/2048 av ett 2^{15} faktorförsök.

Designerna kan användas för att anpassa linjära modeller

$$y = \beta_0 + \Sigma \beta_i x_i + \varepsilon$$

till experimentella data. Beräkningarna görs på samma sätt som tidigare beskrivits för faktorförsök.

Naturligtvis kan man inte få lika mycket information om varje variabel i reducerade faktorförsök som man kan i kompletta faktorförsök. Priset man får betala är att huvudeffekter och samspelseffekter "förorenar varandra", dvs de är *sammansmälta* (eng. *confounded*).

Sammansmältning och alias

För att visa vad som menas med sammansmältning ska vi först se på ett enkelt exempel: tre variabler ska undersökas i ett 2^{3-1} reducerat faktorförsök. Man kan då se att för experimenten så varierar varje variabel alltid som produkten av de övriga två variablerna och kolonnen **I** som produkten av alla tre variablerna, $x_1 \cdot x_2 \cdot x_3$.

Exp no	$x_1x_2x_3$ **I**	x_2x_3 x_1	x_1x_3 x_2	x_1x_2 x_3
1	1	-1	-1	1
2	1	1	-1	-1
3	1	-1	1	-1
4	1	1	1	1

Om ett komplett faktorförsök med tre variabler valts, så skulle följande modell kunnat ansättas:

$$y = \beta_0+\beta_1x_1+\beta_2x_2+\beta_3x_3+\beta_{12}x_1x_2+\beta_{13}x_1x_3 +\beta_{23}x_2x_3+\beta_{123}\,x_1x_2x_3+\varepsilon$$

Eftersom nu bara fyra experiment görs kan man endast skatta fyra parametrar. I den reducerade designen gäller följande samband:

$$\begin{aligned} x_1 &= x_2x_3 \\ x_2 &= x_1x_3 \\ x_3 &= x_1x_2 \\ \mathbf{I} &= x_1x_2x_3 \end{aligned}$$

Om dessa samband sätts in i modellen ovan får vi

$$y = \beta_0 + \beta_{123} + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_3 + \beta_{13} x_2 + \beta_{23} x_1 + \varepsilon$$

Detta kan reduceras till

$$y = (\beta_0 + \beta_{123}) + (\beta_1 + \beta_{23})\, x_1 + (\beta_2 + \beta_{13})\, x_2 + (\beta_3 + \beta_{12})\, x_3 + \varepsilon$$

vilket är en linjär modell med fyra parametrar

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + \varepsilon$$

där parametrarna är linjärkombinationer av de "sanna" effekterna

$$\begin{aligned} b_0 &= \beta_0 + \beta_{123} \\ b_1 &= \beta_1 + \beta_{23} \\ b_2 &= \beta_2 + \beta_{13} \\ b_3 &= \beta_3 + \beta_{12} \end{aligned}$$

Parametern b_1 är ett estimat av den "sanna" parametern β_1, men detta estimat är förorenat av ett bidrag från den "sanna" två-varia-

belinteraktionen β_{23}. Man säger att β_1 är *sammansmält* med β_{23} och att b_1 är en *alias* av de sammansmälta effekterna.

Detta är alltså priset man betalar då man använder reducerade designer. Det är möjligt att erhålla estimat av effekterna, men dessa estimat kommer att vara linjärkombinationer av sanna effekter.

Generatorer till reducerade faktorförsök

För att analysera vilka effekter som kommer att vara sammansmälta ska vi introducera ett nytt begrepp, *generatorn* till ett reducerat faktorförsök. Som exempel använder vi den reducerade faktoriella designen 2^{3-1}. För att förstå varför generatorer är praktiska att använda skriver vi ner hela matrisen för en 2^3 faktoriell design.

	I	1	2	3	12	13	23	123
	1	-1	-1	-1	1	1	1	-1
*	1	1	-1	-1	-1	-1	1	1
*	1	-1	1	-1	-1	1	-1	1
	1	1	1	-1	1	-1	-1	-1
*	1	-1	-1	1	1	-1	-1	1
	1	1	-1	1	-1	1	-1	-1
	1	-1	1	1	-1	-1	1	-1
*	1	1	1	1	1	1	1	1

Bland dessa experiment finns precis fyra där variabel **3** varierar på samma sätt som produkten mellan **1** och **2**. Dessa experiment är markerade med *. För dessa experiment kan man även se att **1=23**, **2=13** och **I=123**. Det är ganska arbetsamt att identifiera sådana samband genom att titta på variabelmatriser från ett komplett faktorförsök, särskilt om matriserna blir lite större. De fyra experiment som uppfyller sambandet **3=12** är hälften av det kompletta faktorförsöket. Fyra experiment motsvarar ett komplett faktorförsök 2^2. Detta betyder att vi kan använda modellmatrisen från en 2^2-design för att definiera variationen för den extra variabeln **3**, och därmed få den reducerade faktoriella designen 2^{3-1}. Den halva fraktion där **3=12** fås genom att låta kolonn **12** i modellmatrisen till 2^2-designen definiera variationen för variabel **3**.

			3
I	**1**	**2**	**12**
1	-1	-1	1
1	1	-1	-1
1	-1	1	-1
1	1	1	1

Dessa är nu samma experiment som valdes från det kompletta faktorförsöket, de som markerats med *.
Matrisen ovan har vissa speciella matematiska egenskaper. Om kolonnerna multipliceras med varandra får man alltid en annan kolonn i matrisen.
Man kan också se att

I·1 = 1 dvs. **I1 = 1**
I·2 = 2 dvs. **I2 = 2**
I·12 = 12 dvs. **I12 = 12**

Multiplikation med **I** ändrar inget eftersom vi multiplicerar elementen i den andra kolonnen med (+1). Multiplikation av en kolonn med sig själv innebär att (-1) multipliceras med (-1) och (+1) med (+1). Detta ger alltid kolonn **I**.

$$1 \cdot 1 = 1^2 = I$$
$$2 \cdot 2 = 2^2 = I$$
$$12 \cdot 12 = 1^2 \cdot 2^2 = I$$

etc.

I det reducerade faktorförsöket ovan varierade vi **3** som produkten **12**. Därmed har vi också avsiktligt sammansmält β_3 med β_{12}. Andra effekter kommer emellertid också att vara sammansmälta.

Vi har *genererat* den reducerade faktoriella designen från det lilla kompletta faktorförsöket genom att sätta **3=12**. Genom att utnyttja räknereglerna, som definierats ovan, och multiplicera båda sidor i sambandet **3=12** med **3** får man följande:

$$3 \cdot 3 = 12 \cdot 3$$

vilket ger

$$I = 123$$

Detta samband utgör *generatorn* och visar hur de olika kolonnerna kan multipliceras samman för att ge kolonn **I**. Generatorn kan nu användas för att identifiera sammansmältningsmönstret på följande sätt:

Om vi multiplicerar varje kolonn i modellmatrisen med generatorn för det reducerade faktorförsöket, så kan vi identifiera vilka kolonnmultiplikationer som ger kolonnerna i designmatrisen, dvs. multiplikation av **I = 123** med

I 1 2 12

ger

I · (I = 123) dvs. **I = 123**
1 · (I = 123) dvs. **1 = 23**
2 · (I = 123) dvs. **2 = 13**
12 · (I = 123) dvs. **12 = 3**

Dessa resultat syns tydligare om de skrivs som extra kolonnrubriker i modellmatrisen:

123	**23**	**13**	**3**
I	**1**	**2**	**12**
1	-1	-1	1
1	1	-1	-1
1	-1	1	-1
1	1	1	1

När dessa kolonner används för att beräkna effekterna får man följande:

Från kolonn	estimatet	
I		$\beta_0 + \beta_{123}$
1		$\beta_1 + \beta_{23}$
2		$\beta_2 + \beta_{13}$
12		$\beta_{12} + \beta_3$

Ytterligare generatorer

Antag att man vill undersöka fem variabler. För att göra detta i ett komplett faktorförsök behövs 32 experiment. Om man vill få en grov uppskattning om i vilken utsträckning variablerna påverkar resultatet, så räcker en 2^{5-2} reducerad faktoriell design med åtta experiment. För att definiera en sådan design kan vi använda den nu bekanta trevariabelmatrisen från ett 2^3 faktorförsök. Denna innehåller följande kolonner

I 1 2 3 12 13 23 123

För att definiera de extra variablerna **4** och **5** kan någon av de fyra interaktions-kolonnerna användas. Vi väljer att definiera dessa variabler som

4 = 23 dvs. $\mathbf{4^2 = 234}$
5 = 123 dvs. $\mathbf{5^2 = 1235}$

vilket ger följande *oberoende generatorer*:

I = 234 = 1235

Här gäller att all multiplikation av de oberoende generatorererna också kommer att ge kolonn **I** som resultat. Detta betyder att

I = 234 · 1235

vilket kan förenklas till

I = 145

Vi måste därför lägga denna *härledda generator* till de oberoende generatorerna för att få en komplett uppsättning generatorer

I = 234 = 1235 = 145

Denna uppsättning generatorer innehåller fyra "strängar". Antalet "strängar" bestäms av att en 2^{5-2}-design är 1/4 av en komplett design. Åtta rader och 32 kolonner i denna matris måste "vikas samman" för att ge en 8 x 8-matris. Som ett resultat av detta sammanfaller fyra av de 32 kolonnerna på varje kolonn i 8 x 8-matrisen. Från denna uppsättning av generatorer får man följande sammansmältningsmönster:

I	**1**	**2**	**3**	**12**	**13**	**23**	**123**
234	**1234**	**34**	**24**	**134**	**124**	**4**	**14**
1235	**235**	**135**	**125**	**35**	**25**	**15**	**5**
145	**45**	**1245**	**1345**	**245**	**345**	**12345**	**2345**

Tillsammans med antagandet att interaktionseffekter mellan tre eller fler variabler är små jämfört med huvudeffekterna och interaktionseffekter mellan två variabler, så ger experimenten ovan följande estimat av modellparametrarna:

Från kolonn	estimatet β_0
1	$\beta_1 + \beta_{45}$
2	$\beta_2 + \beta_{34}$
3	$\beta_3 + \beta_{24}$
12	$\beta_{12} + \beta_{35}$
13	$\beta_{13} + \beta_{25}$
23	$\beta_4 + \beta_{23} + \beta_{15}$
123	$\beta_5 + \beta_{14}$

Vi hade kunnat låta variablerna **4** och **5** definieras av andra kolonner. Vi kunde ha valt t.ex.

4 = 12 och **5 = 13**, vilket ger följande uppsättning generatorer

I = 124 = 135 = 2345

och följaktligen ett annorlunda sammansmältningsmönster än det föregående.

Upplösningen hos ett reducerat faktorförsök

Upplösningen (eng. "*resolution*") hos en reducerad faktoriell design definieras av längden av den kortaste "strängen" i uppsättningen av generatorer. Upplösningen anges vanligen med romerska siffror.
I en design med

upplösning III är huvudeffekter sammansmälta med två-variabelinteraktioner;

upplösning IV är huvudeffekter sammansmälta med tre-variabelinteraktioner och två-variabelinteraktioner är sammansmälta med varandra;

upplösning V är huvudeffekter sammansmälta med fyr-variabelinteraktioner och två-variabelinteraktioner är sammansmälta med tre-variabelinteraktioner.

Reducerade faktoriella designer med högre upplösning än *V* används sällan i sållningsförsök.

Hur man separerar sammansmälta effekter

I exemplet ovan utgjorde den 2^{5-2} faktoriella designen 1/4 av ett komplett faktorförsök. De "extra" variablerna definierades som **4=23** och **5=123** vilket gav de oberoende generatorerna

I = 234 = 1235

Designen har upplösningen III, dvs huvudeffekterna är sammansmälta med två-variabeleffekter.

En ny fjärdedel fås genom att byta tecken på kolonn **4 = -23**. Ännu en ny genom att byta tecken på kolonn **5 = -123**. Den återstående fjärdedelen fås genom att byta tecken på båda kolonnerna, **4 = -23** och **5 = -123**. Dessa sätt att definiera de "extra" variablerna motsvarar olika uppsättningar generatorer:

Design A: **I = 234 = 1235 = 145**
Design B: **I = -234 = 1235 = -145**
Design C: **I = 234 = -1235 = -145**
Design D: **I = -234 = -1235 = 145**

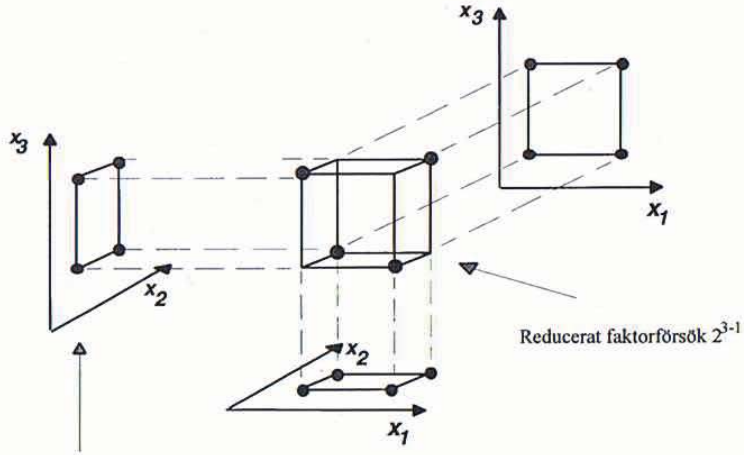
De två första är alltså oberoende generatorer och **±145** har erhållits genom att multiplicera de oberoende generatorerna. Eftersom uppsättningen av generatorer är olika, så blir även sammansmältningsmönstret olika. För att få överblick har endast huvudeffekter samt två-variabeleffekter tagits med:

Design A	Design B	Design C	Design D
β_0	β_0	β_0	β_0
$\beta_1 + \beta_{45}$	$\beta_1 - \beta_{45}$	$\beta_1 - \beta_{45}$	$\beta_1 + \beta_{45}$
$\beta_2 + \beta_{34}$	$\beta_2 - \beta_{34}$	$\beta_2 + \beta_{34}$	$\beta_2 - \beta_{34}$
$\beta_3 + \beta_{24}$	$\beta_3 - \beta_{24}$	$\beta_3 + \beta_{24}$	$\beta_3 - \beta_{24}$
$\beta_{12} + \beta_{35}$	$\beta_{12} + \beta_{35}$	$\beta_{12} - \beta_{35}$	$\beta_{12} - \beta_{35}$
$\beta_{13} + \beta_{25}$	$\beta_{13} + \beta_{25}$	$\beta_{13} - \beta_{25}$	$\beta_{13} - \beta_{25}$
$\beta_4 + \beta_{23} + \beta_{15}$	$\beta_4 - \beta_{23} - \beta_{15}$	$\beta_4 + \beta_{23} - \beta_{15}$	$\beta_4 - \beta_{23} + \beta_{15}$
$\beta_5 + \beta_{14}$	$\beta_5 - \beta_{14}$	$\beta_5 - \beta_{14}$	$\beta_5 + \beta_{14}$

Antag nu att en serie experiment har gjorts i enlighet med fraktionen A. Vi kan då göra en ny serie experiment enligt någon av de andra fraktionerna. I uppställningen ovan kan man se att fraktion B är komplementär till fraktion A på ett sådant sätt att alla huvudeffekter kan separeras från sammansmältning med två-variabeleffekter. Två-variabeleffekterna kommer emellertid att vara sammansmälta med varandra.
När ett reducerat faktorförsök gjorts, så finns det alltid möjlighet att göra experimenten i en komplementär fraktion för att eliminera sammansmälta effekter.

Alla experiment är "nyttiga"

Om man skulle komma till slutsatsen att någon variabel förmodligen inte har någon effekt på responsen, så ökar informationen om de kvarvarande variablerna. Hela experimentet blir då en större fraktion med färre variabler. De gjorda experimenten kan nu användas för att skatta tidigare ej upplösta interaktioner. För tre variabler kan detta illustreras som i Figur 4.



Figur 4. Effekten av att en variabel stryks i ett 2^{3-1} reducerat faktorförsök.

Om det skulle visa sig att en eller flera variabler saknar inflytande, så kan aliaserna bestämmas med hjälp av generatorerna för den ursprungliga designen genom att alla element som innehåller den "ointressanta" variabeln stryks.

Utvärdering av faktorförsök och reducerade faktorförsök

Hur ska man tolka de skattade modellparametrarna vid ett faktorförsök och när ska man anse att en variabel har ett verkligt inflytande?

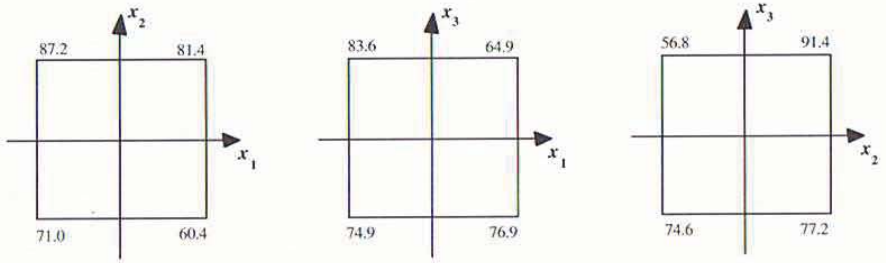
Vid faktorförsök testar man varje variabel på två nivåer, -1 och +1. För kontinuerliga variabler innebär detta att man projicerar, *skalar*, den naturliga variabeln så att den experimentella domänen beskrivs av intervallet - 1 till +1. Eftersom alla variabler är normerade på detta sätt blir den relativa betydelsen av ändringen i varje variabel direkt relaterad till storleken på regressionskoefficienterna i modellen. Detta betyder att viktiga variabler har stora, positiva eller negativa, modellparametrar.

Om en responsmodell innehåller interaktionstermer, $\beta_{ij}\, x_i\, x_j$, kan utvärderingen av olika variablers påverkan underlättas om man gör en enkel projektion av responsytan i (x_i, x_j)-planet, dvs man beräknar responsvärdet för $x_1 = \pm 1$ och $x_2 = \pm 1$ medan andra variabler hålles konstant, –1, 0 eller +1.

Exempel: Antag att utbytet, y (%), beskrivs av tre variabler x_1, x_2 och x_3 enligt responsytan

$$y = 75.0 - 4.1x_1 + 9.3x_2 - 0.9x_3 + 1.2x_1 x_2 - 5.1x_1 x_3 + 8.0x_2 x_3$$

Projektionerna har gjorts så att den tredje variabeln är satt lika med 0.



Om: $x_3 = 0$ $x_2 = 0$ $x_1 = 0$

Gynnsamma (gunstige) betingelser:

$x_1 =$ -1	$x_1 =$ -1	$x_2 = 1$
$x_2 =$ 1	$x_3 =$ 1	$x_3 = 1$

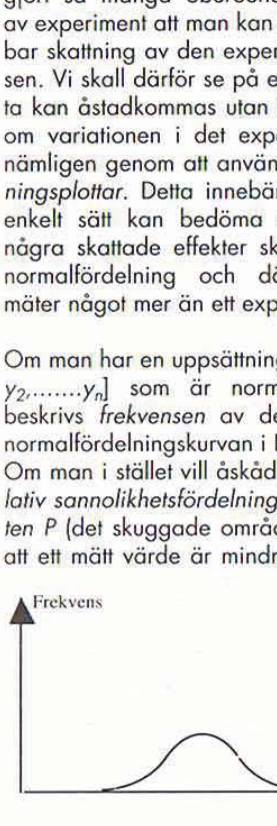
Sammanfattningsvis bör ett gott utbyte, $y = 99.4\%$, kunna erhållas med $x_1 = -1$, $x_2 = 1$ och $x_3 = 1$.

Normalfördelningsplottar för att urskilja signifikanta effekter

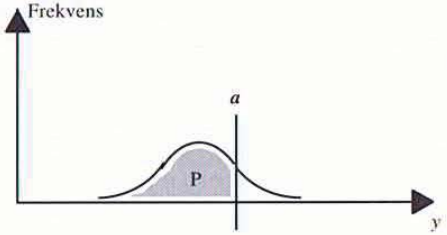
Om man har en oberoende skattning, s^2, av det experimentella felet, t ex genom att man har gjort något experiment flera gånger (och på ett sådant sätt att felen kan antas vara oberoende), kan man använda kända statistiska fördelningar och jämföra skattade effekter med det experimentella felet. Sådana jämförelser blir emellertid ofta inte speciellt upplysande om det experimentella felet är skattat med få frihetsgrader, eftersom det då krävs ganska stora effekter för att de skall anses vara signifikanta.

Vid sållningsförsök har man i allmänhet inte gjort så många oberoende upprepningar av experiment att man kan göra en användbar skattning av den experimentella variansen. Vi skall därför se på en metod där detta kan åstadkommas utan tidigare kunskap om variationen i det experimentella felet, nämligen genom att använda *normalfördelningsplottar*. Detta innebär att man på ett enkelt sätt kan bedöma om någon eller några skattade effekter skiljer sig från en normalfördelning och därmed sannolikt mäter något mer än ett experimentellt fel.

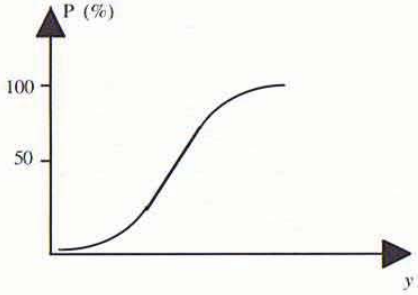
Om man har en uppsättning mätta data [y_1, y_2,.....y_n] som är normalfördelade, så beskrivs *frekvensen* av den klockformade normalfördelningskurvan i Figur 5.
Om man i stället vill åskådliggöra en *kumulativ sannolikhetsfördelning*, dvs *sannolikheten P* (det skuggade området i Figur 6) för att ett mätt värde är mindre än $y = a$, kan man göra det med en plot som visar P mot y. För normalfördelningen beskriver detta en S-formad kurva, Figur 7.



Figur 5. Klockformad normalfördelning.

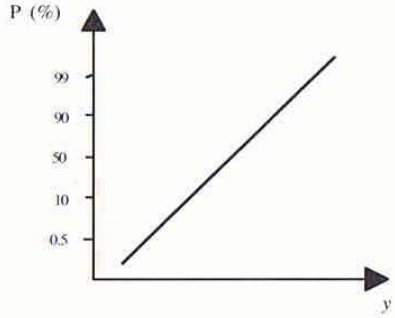


Figur 6. Sannolikheten P (y ≤ a).



Figur 7. Kumulativ sannolikhetsfördelning.

Man kan sedan justera indelningen på P-axeln, så att krökarna rätas ut och fördelningsfunktionen beskrivs av en rät linje (Figur 8). Papper med sådan gradering kallas normalfördelningspapper.



Figur 8. Plot på normal fördelningspapper.

Om man nu har skattat "effekter" vid ett faktorförsök kan man, genom att plotta dessa på normalfördelningspapper, undersöka om dessa "effekter" i själva verket beskriver ett normalfördelat brus, det experimentella felet, i stället för att vara mått på verkliga effekter.

För att göra en normalfördelningsplot ordnar man de skattade effekterna i storleksordning (ta inte med medelvärdet b_0):

minst, näst minst, ...störst
1 2 q

Om experimenten gjorts i slumpmässig ordning kan vi uppfatta de *q* effekterna som ett slumpmässigt stickprov av en *normalfördelad* population av experimentella fel. Detta gäller om variablerna *inte* har någon effekt. Den första (minsta) effekten kan då antas representera *P(1/q · 100)*% av den kumulativa fördelningsfunktionen. Den andra effekten representerar *P(2/q · 100)*% osv till den sista effekten som antas representera *P(q/q · 100)*%.

Dela sedan in intervallet [0 - 100%] på *P*-axeln i *q* lika stora intervall. Varje intervall blir då 100/q. Den minsta effekten plottas sedan i mittpunkten på det första intervallet, näst minsta effekten i mittpunkten på det andra intervallet, osv.

Placeringen i mittpunkten på varje intervall kan sammanfattas med en formel:

$$P(\%) = \frac{100\ (Q - \frac{1}{2})}{n}$$

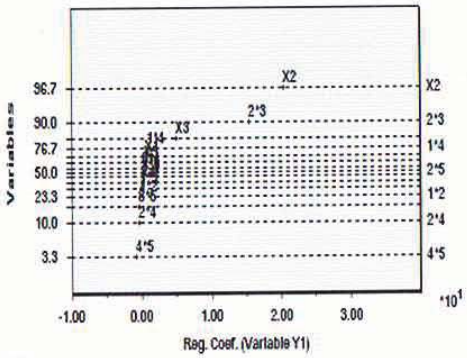
där *Q* är effektens ordningsnummer och *n* är antalet parametrar.
Om en normalfördelningsplot ska visa distributionen av det experimentella felet, så bör den gå genom punkten (0, 50%). Det förväntade medelvärdet av det experimentella felet är noll, och det bör därför vara möjligt att "förankra" linjen i punkten (0, 50%) för att erhålla en maximal anpassning.

Exempel: I Tabell 5 har parametrarna rangordnats efter effekternas storlek.

Denna tabell har sedan omsatts till en normalfördelningsplot, Figur 9.

Tabell 5. Rangordning av effekter och motsvarande sannolikhetskoordinat P.

Ordn.nr	Effekt	Parameter	P(%)
1	-0.124	b_{45}	3.3
2	-0.067	b_{24}	10.0
3	-0.067	b_{35}	16.7
4	-0.029	b_{12}	23.3
5	-0.010	b_{13}	30.0
6	0.010	b_4	36.7
7	0.029	b_{15}	43.3
8	0.029	b_{25}	50.0
9	0.029	b_{34}	56.7
10	0.067	b_5	63.3
11	0.086	b_1	70.0
12	0.124	b_{14}	76.7
13	0.503	b_3	83.3
14	1.504	b_{23}	90.0
15	2.007	b_2	96.7



Figur 9. Normalfördelningsplot av parametrarna i Tabell 5.

Två linjära koefficienter, b_2 och b_3 samt en samspelskoefficient, b_{23} avviker från en tänkt rät linje i Figur 9. Linjen går genom (0, 50%) vilket stödjer antagandet att den representerar distributionen av det experimentella felet. Alla koefficienter som hör till variablerna x_1, x_4 och x_5, projiceras på linjen. Detta kan tolkas som att dessa variabler inte påverkar resultatet (y_1) inom den experimentella domänen. Ytterligare experiment för att optimera resultatet behöver nu endast beakta variablerna x_2 och x_3. Sållningsförsöket har med andra ord sorterat ut de variabler som är signifikanta.

ÖVNINGSEXEMPEL 4

Reducerat faktorförsök 2^{5-2}: Konstruktion av försöksplan

Du har bestämt dig för att göra ett sållningsförsök där fem (5) experimentella variabler skall undersökas. Du har också gjort bedömningen att ett reducerat tvånivåers faktorförsök är en lämplig första ansats.

Uppgifter:
(a) Ställ upp en försöksplan för fem variabler i ett reducerat faktorförsök 2^{5-2}. Det finns flera möjligheter.

(b) Undersök hur huvudeffekter och tvåsamspelseffekter är kopplade (confounded) i de skattningar du kan göra med den försöksplan du valt under (a).

(c) Ställ upp ytterligare en försöksplan med vars hjälp kompletterande information kan erhållas. (*Tips*: teckenbyte på lämpliga kolonner eller fold-over design.) Resultatet av den kompletterande försöksplanen tillsammans med försöksplanen under (a) ska göra det möjligt att skatta samtliga huvudeffekter fria från tvåfaktor-interaktioner. Visa att detta är möjligt med den valda försöksplanen.

ÖVNINGSEXEMPEL 5

Reducerat faktorförsök 2^{5-1}: Farmaci, preformulering

Exemplet är från Leuenberger & Becher (1975). Syftet med den här studien var att hitta de bästa hjälpämnena att blanda med den aktiva substansen med hänsyn till stabiliteten. Därför blandades den aktiva substansen med olika hjälpämnen. Analyser gjordes för att kontrollera om några nedbrytningsprodukter bildades.

Uppgifter:
a) Konstruera ett reducerat faktorförsök med 16 experiment. Använd den osannolikt

Variabler och experimentell domän vid preformuleringsförsök (övningsexempel 5).

Variabler		Experimentell domän (-)-nivå	(+)-nivå
x_1:	Fyllnadsmedel	Laktos	mannitol
x_2:	Smörjmedel	stearinsyra	magnesiumstearat
x_3:	Sprängmedel	majsstärkelse	mikrokristallin cellulosa (Avicel®)
x_4:	Bindemedel	polyvinylpyrrolidon(PVP)	gelatin
x_5:	Fuktighet	inget vatten tillsatt	vatten tillsatt

signifikanta interaktionstermen $x_1 \cdot x_2 \cdot x_3 \cdot x_4$ för att konstruera kolumnen för x_5. Börja den första kolumnen x_1 med – + – + etc.

Responsen y, består av % intakt aktiv substans:

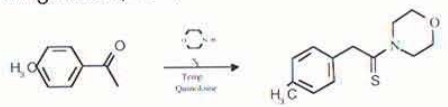
Exp	y (%)	Exp	y (%)
1	59.6	9	54.1
2	86.4	10	45.8
3	95.0	11	92.8
4	97.0	12	96.1
5	83.4	13	53.6
6	53.8	14	64.7
7	93.7	15	94.0
8	99.7	16	96.3

b) Skatta effekterna (koefficienterna) av de experimentella variablerna och utvärdera deras inflytande.
c) Rekommendera ett recept!

ÖVNINGSEXEMPEL 6

Reducerat faktorförsök 2^{5-1}: Willgerodt-Kindler-reaktionen

Exemplet är taget från Carlson *m.fl.* (1986). En organisk syntetisk reaktion, Willgerodt-Kindler-reaktionen, studerades. Fem experimentella variabler undersöktes i ett sållningsförsök, 2^{5-1}.



De experimentella variablerna samt design och utbyten ges i tabellerna nedan.

Variabler och experimentell domän för sållningsförsök med Willgerodt-Kindler reaktionen.

Variabler		Experimentell domän (–)-nivå	(+)-nivå
x_1:	Mängd svavel/keton (mol/mol)	5	11
x_2:	Mängd amin/keton (mol/mol)	6	10
x_3:	Reaktionstemperatur (°C)	100	140
x_4:	Partikelstorlek på svavel (mesh)	240	120
x_5:	Omrörarhastighet (varv/min)	300	700

Försöksplan och utbyten i sållningsförsöket (övningsexempel 6).

Exp nr	Variabler x_1	x_2	x_3	x_4	x_5	Utbyte (%) y
1	–	–	–	–	+	11.5
2	+	–	–	–	–	55.8
3	–	+	–	–	–	55.8
4	+	+	–	–	+	75.1
5	–	–	+	–	–	78.1
6	+	–	+	–	+	88.9
7	–	+	+	–	+	77.6
8	+	+	+	–	–	84.5
9	–	–	–	+	–	16.5
10	+	–	–	+	+	43.7
11	–	+	–	+	+	38.0
12	+	+	–	+	–	72.6
13	–	–	+	+	+	79.5
14	+	–	+	+	–	91.4
15	–	+	+	+	–	86.2
16	+	+	+	+	+	78.6

Försöksplan baserat på ett reducerat faktorförsök 2^{5-1} (**I = 12345**) användes i sållningsförsöket (se tabellen ovan).

Uppgifter:

Bestäm en andra ordningens interaktionsmodell för att beskriva utbytet som en funktion av de experimentella variablerna. Identifiera sannolikt signifikanta variabler med hjälp av normalfördelningsplot.

1.4.4 Optimering

En experimentell undersökning som utförts etappvis leder så småningom fram till ett behov att optimera den studerade processen. I detta avsnitt kommer följande tre optimeringsstrategier att beröras:

Gradientmetoden
Simplexoptimering
Responsytemetodik

Av dessa är det enbart med responsytemetodik som ett exakt optimum kan bestämmas. De andra två metoderna används för att lokalisera en ny bättre domän nära ett optimum eller för att "ringa in" ett optimum.

Gradientmetoden

I gradientmetoden används den linjära polynomfunktion som beräknats från ett reducerat faktorförsök. Denna funktion innehåller endast förstagradstermer och approximerar responsytan med ett plan/hyperplan. Om man t.ex. vill hitta ett maximum för en respons bestämmer man den riktning utefter planet som ger störst ökning av responsen. Koefficienterna i modellen ger information om planets lutning och beskriver på så sätt hur mycket varje experimentell variabel påverkar resultatet, dvs hur snabbt man närmar sig ett optimum genom förändringar av de experimentella variablerna.

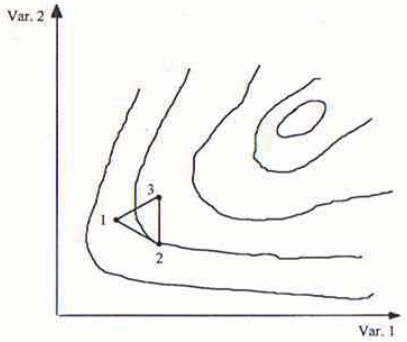
Med ledning av detta gör man en serie experiment längs denna riktningsvektor och följer den så länge responsen förbättras. När inte responsen längre blir bättre gör man ett nytt faktorförsök i en ny domän och bestämmer en ny förstagradsmodell. Med denna tar man ut en ny riktning mot optimum.

När detta förfarande inte längre förmår ge en tydlig förbättring av responsen har man kommit till en *stationär domän*, dvs responsytan har inte någon lutning. Detta kan vara nära ett optimum, men även en sadelpunkt eller stationär ås.

För att undersöka hur responsytan ser ut i den stationära domänen får man bestämma en mer fullständig responsfunktion med interaktions- och andragradstermer (se nedan om responsytemetodik). Gradientmetoden är följaktligen enbart användbar för att leta upp en ny experimentell domän.

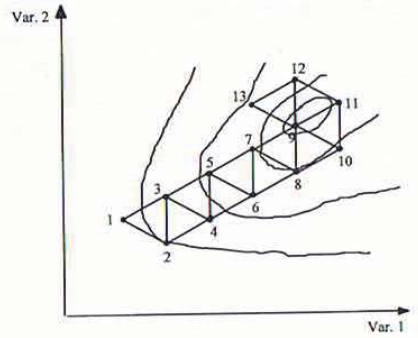
Simplex-optimering

En simplex är en geometrisk figur, en $(k+1)$-hörning där k är antalet variabler i en k-dimensionell rymd som ska undersökas. När antalet variabler är två är simplexen en triangel (Figur 10).



Figur 10. Simplex i två variabler.

Simplexoptimering är en stegvis optimeringsstrategi. Detta innebär att experimenten görs ett och ett, undantaget startsimplexen där alla experimenten kan göras parallellt. Principen för en simplexoptimering är mycket enkel och kan illustreras i Figur 11. För att maximera t.ex ett utbyte i en kemisk syntes gör man först $k+1$ experiment för att erhålla en startsimplex. Utbytet i varje hörn i simplexen undersöks och det "sämsta" hörnet speglas geometriskt genom tyngdpunkten för de övriga hörnen i simplexen. På detta sätt erhålles en ny simplex. För det nya hörnen beräknas koordinaterna (dvs. de experimentella inställningarna) och experimentet genomförs samt utbytet bestäms. I den nya simplexen speglas nu det nya "sämsta" hörnet geometriskt på samma sätt som tidigare så att ytterligare en ny simplex erhålles, osv. På detta sätt kan man fortsätta tills simplexen roterat ett varv (Figur 11) och då har ett optimum ringats in. En helt roterad simplex kan användas för att beräkna en responsyta och denna design kallas Doehlert-design eller uniform shell design. Dessa beskrivs längre fram i texten.



Figur 11. Illustration av en simplex-optimering med två variabler.

Regler för simplex optimering

Start: För *k* variabler görs *k+1* experiment på ett sådant sätt att variablerna bestäms av koordinaterna i en simplex. För två variabler skall de beskriva en triangel. För tre variabler är det en god idé att startsimplexen designas som ett 2^{3-1} reducerat faktorförsök. (I övriga fall bör designmatriserna som ges i Appendix III användas.) Responsen för experimenten i simplexens hörn mäts.

Regel 1: Spegla koordinaterna för det "sämsta hörnet" i linjen/planet som bildas av de återstående *k* hörnen och gör ett nytt experiment med koordinaterna för denna punkt som variabelinställningar. På så sätt fås en ny simplex av de *k* återstående hörnen tillsammans med den nya punkten. *Fortsätt på detta sätt tills responsen ej förbättras.*

Regel 2: Om reponsen i ett nytt experiment blir det sämsta, så ger regel 1 att nästa experiment skall utföras i den sämsta punkten i den föregående simplexen. I stället skall då den *näst sämsta* punkten speglas genom de övrigas geometriska tyngdpunkt.

Regel 3: Om en spegling ger att nästa experiment skall utföras utanför den möjliga experimentella domänen, så tilldelas responsen i denna punkt ett omöjligt lågt värde och därefter följs regel 2.

Beräkning av experimentella inställningar för ett nytt experiment i simplexen

När de *k+1* experimenten i startsimplexen utförts undersöks responsen i de olika hörnen. I en simplex för två variabler betecknas det sämsta hörnet *S*, det bästa hörnet *B* och det näst sämsta *N*. *M* är tyngdpunkten i det hyperplan (med två variabler mittpunkten på en linje) som spänns upp av de återstående hörnen då *S* tagits bort (Figur 12).

Förenklat kan speglingen beskrivas enligt följande:

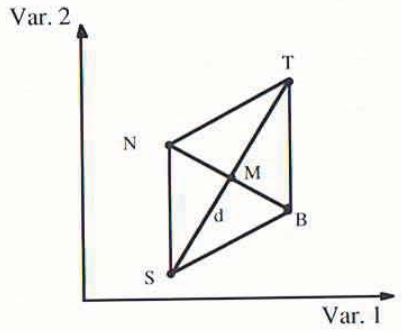
tyngdpunkten $M = \frac{N+B}{2}$

avståndet $d = M - S$

den nya punkten $T = M + d$

detta ger

$T = M + M - S = \frac{2(N+B)}{2} - S$



Figur 12. Spegling av det "sämsta" hörnet i en simplex.

Generellt för *k* variabler betyder detta att koordinaterna x_{iT} i det nya hörnet *T* kan beräknas enligt följande formel

$$x_{iT} = \frac{2}{k}\left(\sum_{\substack{\text{alla } j \\ \text{utom } S}} x_{ij}\right) - x_{iS}$$

Responsytemetodik

Responsytor är ett verktyg för att "exakt" bestämma ett optimum och ett bra sätt att grafiskt åskådliggöra olika experimentella variablers inflytande på responserna. För att kunna bestämma ett optimum hos en responsyta, så måste polynomfunktionen innehålla kavdratiska termer.

Här kommer två typer av designer, som gör det möjligt att anpassa det experimentella resultatet till en kvadratisk polynommodell, att beskrivas.

$$y = \beta_0 + \sum_1^k \beta_i x_i + \sum_1^k \beta_{ii} x_i^2 + \sum_{i<j} \sum \beta_{ij} x_i x_j + \varepsilon$$

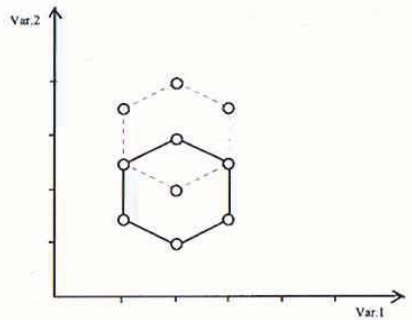
Exempel på designer där resultatet kan anpassas till modellen ovan är

Doehlert-design (uniform shell design)
Sammansatta försöksplaner (composite design)

Det finns även andra typer av designer, där en del är varianter på samma tema, t ex Box-Behnken- och D-optimal design samt olika typer av blandningsdesigner. För dessa hänvisas till de textböcker som finns i referenslistan.

Doehlert design

Som nämnts tidigare bildar en simplex-optimering i två variabler, när den roterar kring ett optimum, en hexagon. En sådan design kallas Doehlert-design och tillåter beräkning av en responsyta med ett minimum av experiment, vilket gör den tilltalande. En annan attraktiv egenskap hos designen är att den lätt kan byggas ut för att utforska en intilliggande domän genom att lägga till ett fåtal experiment (se Figur 13).



Figur 13. Doehlert design i två variabler. Streckad design är kompletterad med tre experiment för att undersöka en intilliggande domän.

En Doehlert-design i två variabler får då en designmatris som visas i Tabell 6. Motsvarande designmatriser för tre till sju variabler beskrivs i Appendix IV.

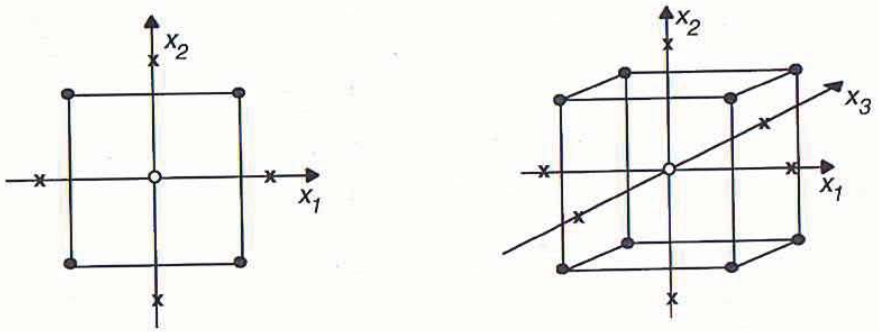
Tabell 6. Doehlert-design för responsytemodellering med två variabler (skalade).

Exp nr	x_1	x_2
1	0.000	0.000
2	-1.000	0.000
3	-0.500	-0.866
4	1.000	0.000
5	0.500	0.866
6	-0.500	0.866
7	0.500	-0.866

Sammansatta försöksplaner

En sammansatt försöksplan med centrumpunkt består av följande delar:

a) Ett faktorförsök (eller reducerat faktorförsök) med variablerna x_i på nivåerna ± 1.
b) Experiment i centrumpunkten, dvs. $x_i = 0$ för alla *i*.
c) Experiment där $x_i = \pm \alpha$ med $x_j \neq x_i = 0$. Dessa punkter ligger i ett koordinatsystem på variabelaxlarna och med ett avstånd ± α från origo; de kallas *axelpunkter.*



Figur 14. Sammansatta försöksplaner med centrumpunkt för två resp. tre variabler. De olika markeringarna betyder • faktorförsök, ○ centrumpunkt och x axelpunkter.

Om experimenten beskrivs som punkter i ett koordinatsystem definierat av x_i-axlarna, så kan designen i två och tre variabler grafiskt beskrivas enligt Figur 14.

Designmatriserna för en Central Composite Design med två resp. tre variabler finns i Tabellerna 7 och 8.

Värdet på α varierar med antalet variabler. Värden som gäller för upp till sex variabler finns i Tabell 9.

Tabell 7. Central Composite Design för två variabler.

Två variabler

x1	*x2*	
−1	−1	*Faktorförsök*
1	−1	
−1	1	
1	1	
0	0	*Centrumpunkt*
−α	0	*Axelpunkter*
α	0	
0	−α	
0	α	

Tabell 8. Central Composite Design för tre variabler.

Tre variabler

x1	*x2*	*x3*	
−1	−1	−1	*Faktorförsök*
1	−1	−1	
−1	1	−1	
1	1	−1	
−1	−1	1	
1	−1	1	
−1	1	1	
0	0	0	*Centrumpunkt*
−α	0	0	*Axelpunkter*
α	0	0	
0	−α	0	
0	α	0	
0	0	−α	
0	0	α	

Tabell 9. Sammansatta försöksplaner med centrumpunkt (Central Composite Designs).

Antal variabler	2	3	4	5	5	6	6
						2^{5-1}	2^{6-1}
Antal experiment i faktorförsöket	4	8	16	32	16	64	32
Antal experiment i axelpunkter	4	6	8	10	10	12	12
Värde på α	1.414	1.682	2.000	2.378	2.000	2.828	2.378

ÖVNINGSEXEMPEL 7

Simplexoptimering

I en process studerades en reaktion med avseende på två experimentella variabler, temperatur och pH. I tabellen nedan utgör de tre första experimenten startsimplexen.

	pH	temp	Yield (%)
Exp 1	6.90	25	29
Exp 2	7.05	26	38
Exp 3	6.95	28	41
Exp 4	7.10	29	68
Exp 5	7.00	31	45
Exp 6	7.15	32	56
Exp 7	7.25	30	63
Exp 8	7.20	27	41

Uppgifter:

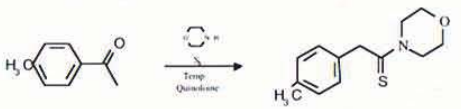
a) Räkna ut hur nästa experiment ska utföras och använd sedan utbytet i tabellen (Exp 4, etc) för att identifiera nästa simplex. För få variabler, som i detta fall, kan experimenten också utvärderas grafiskt på millimeterpapper.

b) Använd de sju experiment som ringar in optimum (dessa beskriver en Doehlert-design) för att beräkna en responsytemodell och bestäm de optimala betingelserna.

ÖVNINGSEXEMPEL 8

Responsyta: Willgerodt-Kindler-reaktionen

Willgerodt-Kindler-reaktionen, som studerades i ett sållningsförsök 2^{5-1} i övningsexempel 6 undersöktes vidare. De signifikanta variablerna visade sig vara mängderna svavel resp. amin samt reaktionstemperaturen.



För att undersöka vilka reaktionsbetingelser som ger maximalt utbyte gjordes en responsytestudie.

Den experimentella domänen, designmatris och utbyten ges i tabellerna nedan.

Variabler och experimentell domän för responsytemodelleringav Willgerodt-Kindler-reaktionen (övningsexempel 8).

Variabler		Experimentell domän				
		-1.682	-1	0	1	1.682
x_1:	Mängd svavel/keton (mol/mol)	2.95	5.0	8.0	11.0	13.05
x_2:	Mängd amin/keton (mol/mol)	4.63	6.0	8.0	10.0	11.37
x_3:	Reaktionstemperatur (°C)	86	100	120	140	154

Designmatris och utbyten vid Willgerodt-Kindler-syntes (övningsexempel 8).

Exp nr.	x_1	x_2	x_3	Utbyte (%)
1	-1	-1	-1	11.5
2	1	-1	-1	43.7
3	-1	1	-1	38.0
4	1	1	-1	75.1
5	-1	-1	1	79.5
6	1	-1	1	88.9
7	-1	1	1	77.6
8	1	1	1	78.6
9	-1.682	0	0	48.5
10	1.682	0	0	91.5
11	0	-1.682	0	58.8
12	0	1.682	0	94.7
13	0	0	-1.682	14.4
14	0	0	1.682	94.1
15	0	0	0	83.9
16	0	0	0	84.2
17	0	0	0	85.6
18	0	0	0	82.6
19	0	0	0	83.2
20	0	0	0	84.9

Uppgifter:

Anpassa med hjälp av ett datorprogram en kvadratisk responsytemodell till data.

Utvärdera modellen, samt gör en kanonisk analys för att bestämma de optimala betingelserna. Gör projektioner av responsytan.

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1.5 Blandnings-designer

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1.5.1 Introduktion

Blandningar torde vara den absolut vanligaste leveransformen för de flesta typer av kemiska produkter. Läkemedel, plaster, målarfärger, många typer av livsmedel samt ett stort antal andra områden skulle kunna användas som exempel på hur blandningar används för att leverera den avsedda funktionen. Antalet ingående komponenter är minst två men kan vara i princip hur stort som helst, även om man i praktiken sällan stöter på produkter som är uppbyggda av fler än 10 komponenter. Karakteristiskt för en blandning är också att de ingående komponenterna i allmänhet inte har utsatts för kemiska reaktioner efter blandningen. Varje komponent i en blandning kan i sin tur bestå av ett stort antal kemiska föreningar vilket naturligtvis ofta ställer till svårigheter vid tolkning av försöksresultaten.

Detta kapitel visar på några sätt att effektivisera arbetet med att utveckla nya formuleringar i form av blandningar. Olika typer av blandningsdesigner beskrivs översiktligt tillsammans med exempel som beskriver arbetsgången. Behandlingen av de teoretiska detaljerna får den intresserade läsaren söka vidare efter i referenserna.

1.5.2. Varför blandningsdesigner ?

Inom många industrier som formulerar blandningar kan följande beskrivning av utvecklingsarbetet kanske verka välbekant: Den vanligaste formen av försöksplanering innebär att formuleraren delar in sin nya produkt i ett antal grundkomponenter. En emulsion, till exempel, består i allmänhet av en oljefas med oljelösliga substanser, en vattenfas med vattenlösliga substanser samt en tredje fas med aktiva substanser såsom arom, antioxidant, färg, parfym eller dylikt. Till varje fas väljer formuleraren de substanser som ska ge de önskade funktionerna. Den billigaste råvaran väljs till baskomponent och de övriga betraktas som additiv. Sedan följer försöksplaneringen: förhållandet mellan vattenfas och oljefas väljs på grundval av erfarenhet eller modellrecept i avsikt att ge produkten den konsistens och funktionalitet som eftersträvas. Sedan varieras typen av additiv i respektive fas, varje additiv ofta på 3 - 5 nivåer, för att optimera stabilitet och konsistens. Ofta avslutar man formuleringsarbetet med att variera typen av baskomponent samt förhållandet mellan olje- och vattenfas för att kompensera för de förändringar i systemets beteende som orsakats av valet av additivtyp och -halt. Antalet försök som görs är oftast stort: i många fall behövs 30 - 40 försök för att hitta rätt. På grund av det stora antalet försök är replikat

inte heller att tänka på så möjligheterna att dra slutsatser om formuleringens robusthet är små.

Denna typ av försöksplanering börjar i ökande utsträckning ersättas med användning av statistiska metoder och matematisk modellering. De vanliga metoderna för försöksplanering som bygger på ortogonala försöksplaner och responsyteanpassning fungerar dock otillfredsställande för blandningsdesigner. Orsaken till detta är att summan av alla komponenter i en blandning måste uppgå till 100 %: vi kan inte arbeta med negativa koncentrationer eller koncentrationer över 100 %. Lyckligtvis finns det teoretiska modeller som klarar av de begränsningar som blandningarna på detta sätt automatiskt ger upphov till.

Det finns naturligtvis situationer där statistiskt baserad försöksplanering och modellering inte är lämpad. Den ovan beskrivna emulsionsformuleringssituationen är ett typexempel på när den statistiska försöksplaneringen måste kombineras med kunskap om fysikalisk kemi. System som på ett eller annat sätt riskerar att innehålla diskontinuiteter lämpar sig inte för en okritisk användning av blandningsdesigner. Exempelvis kan den ovan beskrivna emulsionen plötsligt omvandlas till en flytande kristall eller till en lösning med helt andra egenskaper beroende på att man i sin design har hamnat utanför emulsionsområdet.

Det finns också tillfällen när man kan använda en vanlig ortogonal design för att lösa sitt problem. Om försöksdomänen är liten i förhållande till det möjliga försöksområdet och nära en kant kan man ofta utan vidare lägga ut en ortogonal design i denna domän. Det förutsätter att bulkkomponentens koncentrationsändring kan anses vara försumbar i förhållande till responsytan, det vill säga att det är tillsatserna som har störst responsfaktorer. Ett typiskt fall är när man vill undersöka effekten av ett eller additiv som tillsätts i låga halter (0-2 %) och där bulkkomponenten i första hand fungerar som bärare av additivet.

1.5.3. Allmän teori för blandningsdesigner

Statistisk försöksplanering bygger på att man kan skapa *modeller* som beskriver beteendet för ett system i en begränsad *försöksdomän*. Modellen beskriver en *respons*, η, som funktion av *variabler* (*komponenter* i blandningsdesign), $\mathbf{x}$:

$$\eta = f(\mathbf{x}) \qquad (1)$$

I praktiken är den sanna responsen η inte åtkomlig för experimentalisten, det finns alltid ett slumpmässigt eller systematiskt fel behäftad med mätproceduren. Vi får istället nöja oss med att studera en verklig respons $y = \eta + \varepsilon$ och istället använda oss av olika typer av förenklingar för att skapa oss en modell att arbeta med:

$$\mathbf{y} = f(\mathbf{X}) + \mathbf{e} \qquad (2)$$

där $\mathbf{y}$ är en vektor av responser, $\mathbf{X}$ är en matris som innehåller våra försökspunkter (halter av komponenter) och $\mathbf{e}$ är en vektor av residualer. Ofta kan man approximera responsytan $f(\mathbf{X})$ med en Taylorutveckling och på så sätt få en enkel polynom-modell att arbeta med:

$$y = \beta_0 + \Sigma \beta_i^* x_i + \text{högre ordningens termer} \qquad (3)$$

Koefficienterna β anpassas till försöksdata $\mathbf{y}$ på ett sådant sätt att $\mathbf{e}$ minimeras, exempelvis genom minsta kvadratmetoden. För att kunna bestämma β på effektivaste sätt bör man använda en lämplig *försöksplan* som bestämmer i vilka punkter man skall mäta sin respons.

En grundlig teoretisk behandling av de olika typerna av modeller samt blandningsdesigner för dessa ges av Cornell (1981). Allmänna riktlinjer för utvärdering av modeller ges också av Draper & Smith (1966) och Snee (1971). Nedan ges endast kortfattade sammanfattningar av de teoretiska aspekterna för de olika modellerna och den intresserade läsaren får forska vidare i den tekniska litteraturen. Exempel på användningar av blandningsdesigner från olika tillämpningsområden ges i referenserna 6-11.

En väsentlig följd av att variablerna i en blandningsdesign inte är oberoende av varandra kommer fram vid studien av modellerna nedan. I det generella fallet ges modellen av ekvation 3. Men i en blandning med q komponenter kan man alltid uttrycka halten av en komponent i termer av de övriga:

$$x_q = 1 - \Sigma x_i \qquad (4)$$

Om man substituerar uttrycket för komponent q i ekvation 3 med relationen i ekvation 4 eliminerar man visserligen problemet med att komponenterna i blandningen är beroende av varandra men man tappar samtidigt möjligheter att dra slutsatser om modellens beroende av komponent q. Därför använder man sig istället av följande knep: β_0 i ekvation 3 multipliceras med Σx_i vilket inte ändrar uttryckets värde eftersom $\Sigma x_i = 1$. Man får då för en linjär modell med två komponenter följande utseende på ekvation 3:

$$y = \beta_0 + \Sigma \beta_i^* x_i \qquad (5a)$$

$$y = \beta_0 * \Sigma x_i + \Sigma \beta_i^* x_i \qquad (5b)$$

Detta ger efter förenkling det s k kanoniska polynomet

$$y = \Sigma \beta^*_i * x_i \quad i=1,2 \qquad (5c)$$

I allmänhet bryr man sig inte om att markera att man använder sig av den kanoniska formen av polynomet i blandningsdesigner och utesluter därför asterisken efter koefficienten.

Avsnitt 5 presenterar de ekvationer som används för olika typer av modeller för blandningar med två eller tre komponenter. Ekvationerna gäller generellt för ett utökat antal komponenter men blir naturligtvis mer oöverskådliga ju fler komponenter som ingår.

1.5.4 Arbetsgång vid försöksplanering med hjälp av blandningsdesigner

Följande steg används vanligen vid arbete med blandningsdesigner:

1. Definiera problemet
   - vad vill jag veta ?
   - är problemet ett blandningsproblem ?
   - är responserna mätbara med tillräcklig precision ?
2. Välj variabler
   - vilka variabler finns det att välja bland ?
   - vad vet jag sedan tidigare om deras betydelse ?
   - är variablerna inställbara med tillräcklig precision ?
3. Bestäm begränsningar i variablerna
   - kan alla variabler variera mellan 0 och 100 % ?
   - är variationen i någon variabel begränsad ?
4. Välj initial modell
   - vad vet/tror jag om responsytans utseende ?
   - vill jag optimera eller sålla ?
   - hur många försök kan jag göra ?
5. Välj design i förhållande till modell
   - utvärdera design med avseende på standard error, leverage och kollinearitet
6. Utarbeta försöksplan
   - randomisera försöksordning
   - vid behov: gör en blockning av försöken
7. Utför experimenten
   - notera alla avvikelser från försöksplan och använd sedan de verkliga värdena vid bearbetning av försöksutfallet
8. Anpassa försöksdata till modell
   - verifiera modell via variansanalys och analys av residualer
   - eventuellt: transformera data för att förbättra normalitet
   - eventuellt: revidera modellen
9. Validera modellen
   - kontrollförsök eller rimlighetsstudier
10. Prediktioner/optimering utifrån modell
    - använd modellen till att undersöka frågeställningen

Dessa punkter utförs standardmässigt vid arbete med blandningsdesigner. Vissa punkter bör man lägga ner mer möda på att få rätt från början, speciellt om försöken är dyra och besvärliga att utföra. Det lönar sig oftast att lägga ner extra arbete på punkterna 1-2 och 4-5.

I nedanstående avsnitt beskrivs vissa av ovanstående punkter i detalj.

1.5.5 Val av modell

Allmänna kriterier för val av modell

Vid val av modell bör man först ställa sig frågan: Vad vill jag veta ? Om avsikten med försöket är att få en ungefärlig uppfattning av responsytans utseende kan man hålla sig till enkla linjära modeller. Om man vill optimera en respons bör man istället utveckla modeller som väl beskriver responsytan i det aktuella området, det vill säga kvadratiska eller kubiska modeller. Valet av modell påverkas också av antalet försök som man kan eller vill göra. Det går självfallet åt fler försök att undersöka en kvadratisk eller kubisk än för en linjär modell. Om antalet försök som man kan göra är begränsat bör man i första skedet välja en linjär modell och använda de extra försöken man kan göra till replikat och till kontrollpunkter. Om modelleringen i första skedet misslyckats kan man alltid komplettera med försök för att utvidga modellen.

Fysikaliska/kemiska och empiriska modeller

En vanlig situation i försökshänseende är att man har en fysikalisk eller kemisk teori eller modell som man vill undersöka eller verifiera. Exempel på sådana modeller eller teorier är den välbekanta allmänna gaslagen pV=nRT som beskriver hur tryck, temperatur, volym och materiemängd i en gasblandning är relaterade till varandra. Användning av blandnings-designer eller andra typer av statistiska försöksplaner lönar sig dåligt i sådana fall. Det är istället bättre att utgå ifrån teorin och låta den bestämma i vilka punkter man bör utföra experimenten och sedan använda hypotesprövning och variansanalys för att avgöra om modellen stämmer.

Om man inte har en fysikalisk eller kemisk modell till sitt förfogande kan man försöka beskriva beteendet för sitt system i en begränsad experimentell domän med en empirisk modell. Denna empiriska modell är sällan tolkningsbar i fysikaliska eller kemiska termer och skall ses endast som en beskrivning av responsytan i den aktuella försöksdomänen. Detta är speciellt viktigt i blandningsdesigner där kvadratiska, kubiska och interaktionstermer snabbt kan förlora sin mening om man försöker tolka dem som fysikaliska eller kemiska egenskaper.

Modeller för blandningsdesigner

Linjära modeller

Den enklaste modellen för blandningsdesigner är den linjära modellen som för två komponenter ges av ekvation 6:

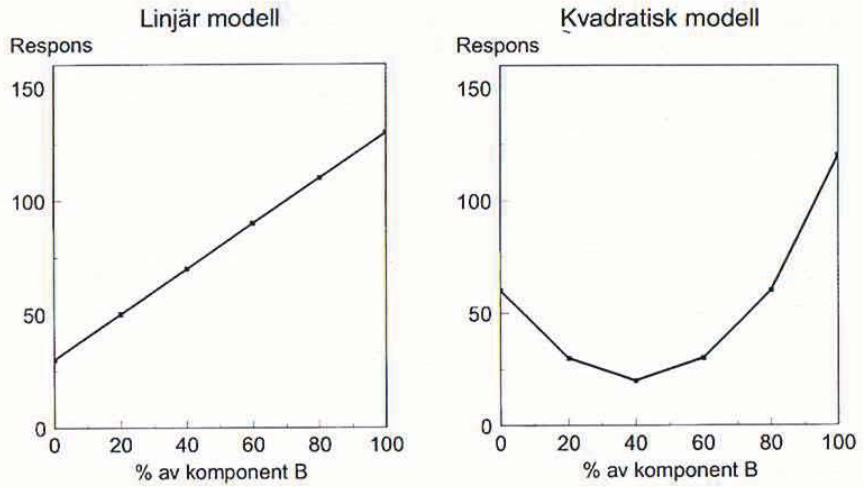
$$y = \beta_1 * x_1 + \beta_2 * x_2 + \varepsilon \qquad (6)$$

där $x_1 + x_2 = 1$, $0 \le x_i \le 1$ *och* ε *är* $N(0, \sigma^2)$

Denna ekvation ger med två komponenter en rät linje som förbinder ytterpunkterna av försöksdomänen enligt figur 1a. Om man istället har tre komponenter ger ekvation 6 ett plan.

Kvadratiska modeller

I blandningsdesigner är den kvadratiska modellen är egentligen en linjär modell med interaktionstermer. Interaktionstermen har, som tidigare påpekats, inte nödvändigtvis en fysikalisk/kemisk innebörd utan betraktas som ett uttryck för avvikelse från den linjära modellen. Ekvation 7a ger exempel på en sådan modell för en tvåkomponentblandning:



Figur 1: Modeller i tvåkomponentsystem.

$$y = \beta_1 * x_1 + \beta_2 * x_2 + \beta_{12} * x_1 * x_2 + \varepsilon \qquad (7a)$$

där $x_1 + x_2 = 1$, $0 \le x_i \le 1$ *och* ε *är* $N(0,\sigma^2)$

Om relationen $x_1 + x_2 = 1$ sätts in i ekvationen fås istället ekvation 7b som tydligt visar modellen kvadratiska struktur:

$$y = \beta_1 * x_1 + \beta_2 * (1-x_1) + \beta_{12} * x_1 * (1-x_1) + \varepsilon \qquad (7b)$$

Utseendet på den kvadratiska modellen är förstås den sedvanliga parabolen som förbinder ändpunkterna i domänen (figur 1b).

Kubiska modeller

Kubiska modeller kan behövas om responsytan uppvisar en stark kurvatur eller asymmetri.

Reducerad kubisk

Den reducerade kubiska modellen i tre komponenter består av en kvadratisk modell utökad med en trekomponentsinteraktion:

$$y = \beta_1 * x_1 + \beta_2 * x_2 + \beta_3 * x_3 + \beta_{12} * x_1 * x_2 + \beta_{13} * x_1 * x_3 + \beta_{23} * x_2 * x_3 + \beta_{123} * x_1 * x_2 * x_3 + \varepsilon \qquad (8)$$

där $x_1 + x_2 + x_3 = 1$, $0 \le x_i \le 1$ *och* ε *är* $N(0,\sigma^2)$

Fullständisk kubisk

Den fullständiga kubiska modellen är sällan användbar eftersom antalet försök för att bestämma koefficienterna för denna modell växer lavinartat vid ökat antal komponenter. För fullständighetens skull redovisas dock utseendet för denna modell för en trekomponentblandning i ekvation 9:

$$y = \beta_1 * x_1 + \beta_2 * x_2 + \beta_3 * x_3 + \beta_{12} * x_1 * x_2 + \beta_{13} * x_1 * x_3 + \beta_{23} * x_2 * x_3 + \delta_{12} * x_1 * x_2 * (x_1 - x_2) + \delta_{13} * x_1 * x_3 * (x_1 - x_3) + \delta_{23} * x_2 * x_3 * (x_2 - x_3) + \beta_{123} * x_1 * x_2 * x_3 + \varepsilon \qquad (9)$$

där $x_1 + x_2 + x_3 = 1$, $0 \le x_i \le 1$ *och* ε *är* $N(0,\sigma^2)$

Reducering av modeller

Om utvärderingen av försöksdata visar att den modell som man föreslagit från början ger en del koefficienter som är icke signifikant skilda från 0 kan modellen med fördel reduceras genom att motsvarande termer elimineras. De överflödiga datapunkter som man på detta sätt åstadkommer används istället för att uppskatta modellens lack-of-fit. Man kan också om man har kännedom om interaktionerna i systemet redan från början välja att utföra färre försök än vad som krävs för att bestämma alla koefficienter och sedan utvärdera den reducerade modellen istället. Detta tillvägagångssätt kan dock vara farligt om man inte validerar att de antaganden man gjorde om interaktioner verkligen stämmer.

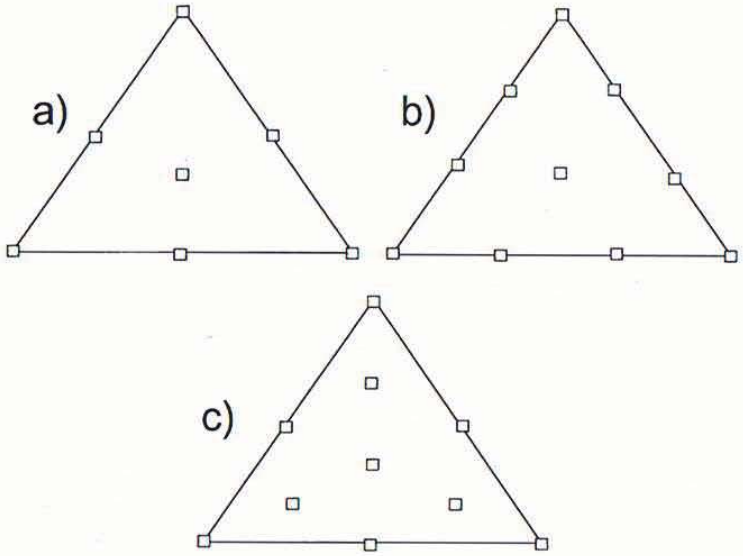
1.5.6 Val av design

Allmänna kriterier för val av design

Vid val av lämplig blandningsdesign börjar man i vanlig ordning med att sammanställa de komponenter som skall ingå i försöket. Man bestämmer de begränsningar som finns på halten av de olika komponenterna samt tänker efter om det finns olika typer av kombinerade begränsningar av typen *Halt av A + Halt av B* skall vara max 50 %. Man bör också fundera över om en av komponenterna skall hållas konstant i försöksserien så att summan av de övriga komponenterna inte blir 100 %. I vissa fall är variationen i en komponent liten i förhållande till de övriga: exempelvis kan halten av en aktiv substans varieras mellan 0.1 och 0.5 % medan övriga komponenter varierar mellan 10 och 50 % i blandningen. I sådana fall bör den aktiva halten hållas konstant i första omgången av försöket och sedan varieras i ett nytt försök där man koncentrerar sig på denna komponent.

Simplex-baserade designer

Om man inte har några övre begränsningar på försöksdomänen kan man i regel använda en simplex-design. En simplex är en regelbunden geometrisk figur av formen $x_1 + x_2 + \ldots + x_q = 1$ som avgränsas av linjer och plan i den q-dimensionella rymden. Figur 2 visar de vanligaste simplex-designerna för ett trekomponentsystem utan begränsningar i variablerna. Figur 2a visar en enkel design med försök i 3 hörnpunkter, med 3 binära (1/2, 1/2) blandningar samt med en ternär (1/3, 1/3, 1/3) blandning. Med denna design kan en kvadratisk modell för responsytan bestämmas. Den ternära blandningen bör replikeras minst 3 gånger för att kunna bestämma modellens medelfel. Om en modell med högre ordning (reducerad kubisk) eller om man vill bestämma modellens "lack-of-fit" bör den enklaste designen i figur 2a utökas med några punkter. Detta kan göras genom att punkterna läggs som i figur 2b med 6 binära blandningar (1/3, 2/3) eller som i figur 2c där ternära blandningar används istället. Dessa designer är likvärdiga ur effektivitetssynpunkt i många fall.

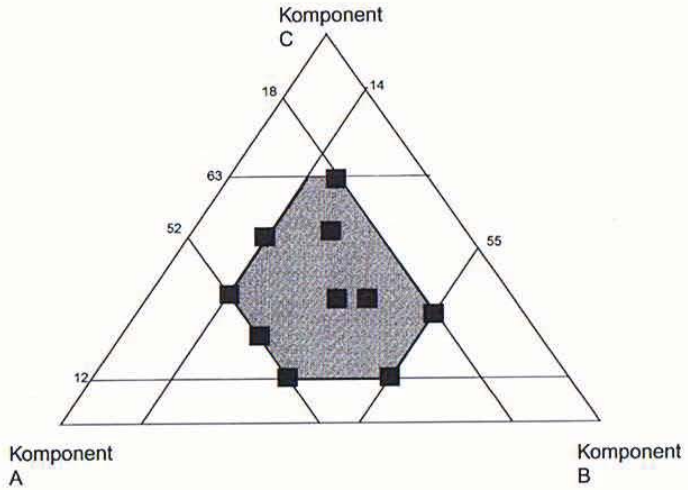


Figur 2: Olika typer av simplex-designer

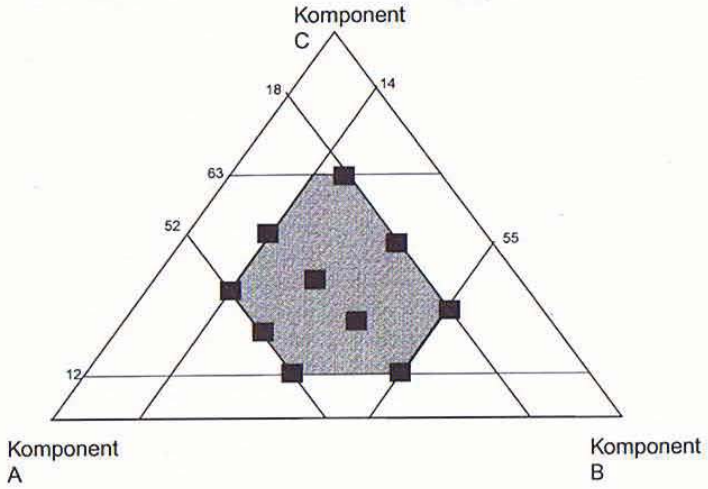
Avståndsbaserade designer

I en avståndsbaserad design läggs punkterna ut sekventiellt på så sätt att avståndet mellan punkterna maximeras. Ett antal kandidatpunkter i domänen genereras och läggs sedan ut en i taget tills tillräckligt antal punkter genererats för att bestämma modell samt "lack-of-fit". I en variant ("modified distance") utvärderas varje punkt med avseende på dess förmåga att bidraga till modellen innan den läggs ut. Endast punkter som på ett signifikant sätt bidrar till bestämningen av koefficienterna i responsytemodellen används. Figur 3 visar begränsningar samt designpunkter för en modifierad avståndsbaserad design i tre variabler med begränsningar både uppåt och neråt (komponent A får variera mellan 18 och 52 %, komponent B mellan 14 och 55 % och komponent C mellan 12 och 63 %). Försöksplanen är utarbetad för en reducerad kubisk modell. Observera att 4 punkter utförs i replikat det vill säga att totalt 14 försök krävs i detta fall.

Avståndsbaserade modeller används när simplexbaserade modeller inte fungerar det vill säga när det finns en övre begränsning på halten av en eller flera komponenter. Avståndsbaserade designer måste utvärderas med avseende på kollinearitet innan



Figur 3: Avståndsbaserad design



Figur 4: D-optimal design

användning för att användbara uppskattningar av koefficienterna skall kunna göras.

D-optimal design

En D-optimal design minimerar variansen i bestämningen av koefficienterna. Detta görs genom att ett antal kandidatpunkter genereras som sedan läggs ut om de motsvarar urvalskriterierna. Den D-optimala designen är modellberoende och kan kräva en optimering av antalet replikat och kontrollpunkter för att passa till den valda modellen. Även den D-optimala designen har sin användning i lägen där variablerna har övre begränsningar. Figur 4 visar designpunkter enligt den D-optimala designen för samma situation som i avsnittet om simplexbaserade designer. Även här krävs 14 försök inklusive replikaten.

Antal designpunkter och replikat

Som framgår av ovanstående figurer och designförslag bör man göra upprepningar av försöken i flera designpunkter för att det skall gå att bestämma modellens medelfel. Man bör dessutom lägga ut kontrollpunkter om man vill räkna ut modellens avvikelse från det sanna beteendet ("lack-of-fit"). Om antalet försök blir alltför stort för att man skall kunna utföra alla föreslagna experiment bör man fundera på att reducera modellen. Man kan också minska på antalet kontrollpunkter även om det medför att möjligheten att bestämma "lack-of-fit" försvinner.

1.5.7 Utvärdering av modell

Utvärdering av modeller

När man väl utfört sina försök och ställt samman sina data, kontrollerat rimligheten i resultaten samt i största möjliga mån eliminerat rena slarvfel kan man börja det roliga arbetet med att utvärdera sina modeller. Under förutsättning att man följt försöksplanen, gjort tillräckligt många replikat samt lagt ut de rätta kontrollpunkterna bör utvärderingen av modellen inte ge några problem.

För att välja bästa möjliga modell för de betingelser som gällt för försöken kan flera olika statistiska tekniker användas:

* variansanalys
* sekventiell reducering eller utökning av modellen
* residualplottar
* normalfördelningsplottar
* plottar av prediktioner mot observationer

Steg 1 i utvärderingen är att uppskatta modellens koefficienter med minsta kvadratmetoden. De koefficienter som inte är signifikanta skilda från 0 kan med fördel elimineras ur modellen.

Steg 2 innebär att med hjälp av variansanalys uppskatta modellens precision med bland annat lack-of-fit. Variansanalysen visar hur väl den uppmätta responsen beskrivs av modellen.

I *steg 3* används residualplottar och normalfördelningsplottar för att kontrollera att de kriterier som gäller för minsta kvadratmetoden är uppfyllda: normalfördelade slumpfelstermer med konstant varians. Om normalfördelningsplottarna visar stora icke-linearieteter eller om residualplottarna uppvisar några trender måste modellen korrigeras, exempelvis genom transformation av responserna. Vanliga transformationer i detta fall är logaritmering eller kvadratrot (för värden >= 0). Residualplottar, normalfördelningsplottar samt plottar av predikterade mot observerade värden kan också avslöja systematiska fel och avvikande mätpunkter (outliers). Orsaken till outliers skall alltid undersökas och vara bakgrunden till behandling av dessa. Man skall ta ställning till om observationen skall uteslutas och modellen korrigeras.

Steg 1 - 3 upprepas tills experimentalisten är nöjd med modellen och dess precision.
I en del design-program finns dessutom möjligheten att använda sekventiella regressionsmetoder som utifrån vissa fastställda kriterier reducerar eller utökar modellen. Det är dock viktigt att även här genomföra modellkontrollen för att verifiera modellens prognosförmåga.

1.5.8 Användning av modell

Om modelleringen enligt ovan varit framgångsrik kan man börja använda sin modell i det fortsatta arbetet. Om avsikten med försöksplanen varit att sålla bland variablerna kan modellen ha visat vilka variabler som är intressanta för fortsatt optimering. Man kan också vara intresserad av responsytans utseende och brukar då rita ut två- eller tredimensionella avbildningar av denna. Ofta vill man bestämma en blandning där två eller flera responser har de önskade värdena. I sådana fall är konturplottar av responsytorna ovärderliga som hjälpmedel eftersom de kan läggas på varandra (antingen med hjälp av datorn eller rent mekaniskt efter överföring till overheadfilm).

I alla dessa fall bör man ha förvissat sig om att de modeller man arbetar med uppfyller de grundläggande kraven:

- framtagna med hjälp av blandningsdesign
- verifierade med hjälp av olika statistiska test och plottar
- verifierade kemiskt/fysikaliskt: stämmer detta med verkligheten ?

Om man har gjort detta så kommer de modeller man utvecklat att effektivisera utvecklingsarbetet samtidigt som en ökad kunskap om systemen man jobbar med utvecklas.

1.5.9 Programvaror för blandningsdesign

Blandningsdesigner finns implementerade i endast ett fåtal kommersiella programpaket för experimentell design. Följande två program är de enda som anges ha moduler för blandningsdesigner i en nyligen publicerad

sammanställning av Arteaga *et al.* (1994):
* Design-Expert, Stat-Ease, USA
* Mixture Design, Statistical Programs, USA
Exemplen i detta kapitel är konstruerade och utvärderade med Design-Expert version 4.0.

1.5.10. Exempel: Smältpunkter för fettblandningar

Definition av problemet

Man behöver en modell för att uppskatta smältpunkterna för blandningar av olika fetter som används som bakfett vid tillverkning av småkakor. De ingående fetterna har smältpunkterna 33 C (K33), 50 C (S50) samt 59 C (R59). Dessa fetter blandas med 40 % av en fjärde komponent. Tidigare screeningförsök har gett följande lämpliga intervall för de olika komponenterna; K33: 5-20 %, S50: 15-30 % samt R59: 10-25 %. All tidigare erfarenhet visar att den här typen av system inte visar enbart linjära effekter.

Problemet är helt tydligt ett blandningsproblem. Smältpunkterna bestäms med hjälp av en standardmetod (DSC) och precisionen i x-variablerna bestäms av noggrannheten vid invägning.

Val av modell

Tidigare erfarenhet visar att en linjär modell inte är tillräckligt bra för att beskriva responsytan. En kvadratisk modell ansätts för att klara hela försöksdomänen.

Val av design

Eftersom begränsningar i variablerna finns används en D-optimal design utökad med några kontrollpunkter. Dessutom görs ett replikat i fyra av designpunkterna för bestämning av pure error.

Resultat

Resultaten tillsammans med designpunkterna redovisas i Tabell 1.

Utvärdering av modellen

Utvärderingen gjord med Design-Expert visas nedan.

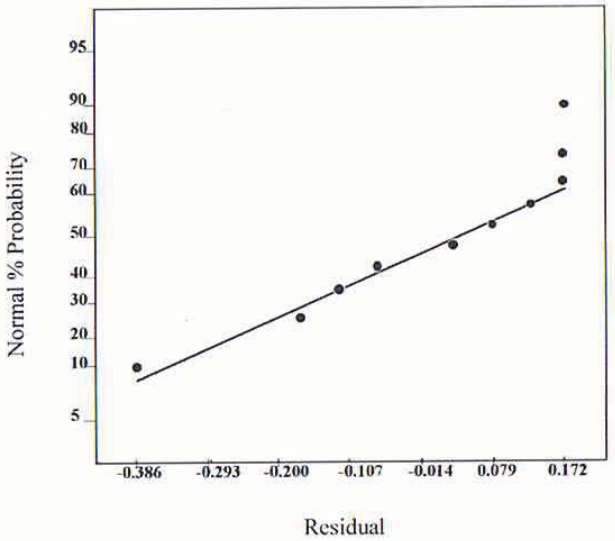
En sekventiell modellanpassning visar att den linjära och den kvadratiska modellen ger signifikant förbättring av precisionen (höga värden på "mean square"). Den kvadratiska modellen uppvisar dessutom ett någorlunda bra värde på "lack-of-fit" testet, "mean square" = 0.20 vilket dock är betydligt högre än "mean square" för "pure error" (<0.01). R^2 och adj-R^2 är också tillräckligt bra för den kvadratiska modellen varför denna väljs som bästa möjliga alternativ.

En stegvis regressions-analys ("stepwise regression") användes sedan för att uppskatta koefficienterna för modellen. Denna analys visar att alla interaktionstermerna (*AB, AC* och *BC*) är signifikanta och väljs in i modellen. Variansanalysen visar att den reducerade kvadratiska modellen är signifikant men att signifikant "lack-of-fit" föreligger "mean square"(l-o-f) = 0.24 mot "mean square(pure error)" = 0.02).
Koefficienterna visar att interaktionstermerna sänker smältpunkten för blandningar av komponent *B* och *C* medan i blandningar av *A* och *C* samt *A* och *B* fås en höjning.

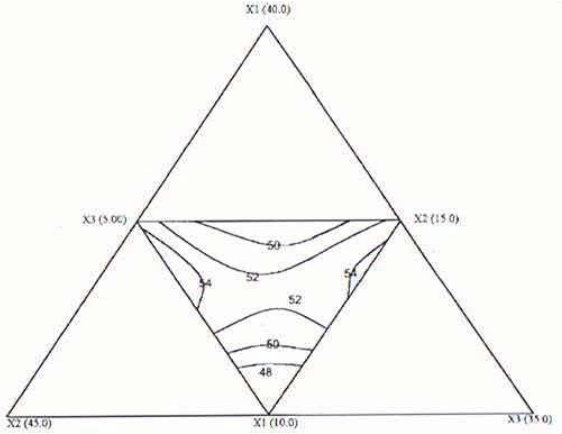
Residualerna är små i förhållande till mätprecisionen samt den praktiska användningen (det räcker att ange smältpunkten i hela grader).

Normalfördelningsplotten av residualerna (figur 5) uppvisar en jämn spridning. De tre kontrollpunkterna har en förhållandevis hög leverage och påverkar därigenom starkt modellens utseende.

Responsytan visas i en konturplott i figur 6 .



Figur 5: Normalfördelningsplott för residualer. Model: Quadratic.



Figur 6: Responsyteplott för smältpunkt.
Model: Quadratic. Actual components: X1 = R59 X2 = S50 X3 = K33

Tabell 1. Resultat från test.

Sequential Model Sum of Squares

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F VALUE	PROB > F
MEAN	24900.1	1	24900.1		
Linear	238.8	2	119.4	25.16	< 0.001
Quadratic	32.9	3	11.0	141.38	< 0.001
SpecCubic	0.2	1	0.2	10.30	0.049
FullCubic	0.0	0			
RESIDUAL	0.1	3	0.0		
TOTAL	25172.1	10			

Lack of Fit Tests

MODEL	SUM OF SQUARES	DF	MEAN SQUARE	F VALUE	PROB > F
Linear	33.1	4	8.3	355.12	< 0.001
Quadratic	0.2	1	0.2	10.30	0.049
SpecCubic	0.0	0			
FullCubic	0.0	0			
PURE ERR	0.1	3	0.0		

ANOVA for Mix Quadratic Model

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F VALUE	PROB > F
MODEL	271.7	5	54.34	700.49	< 0.001
RESIDUAL	0.3	4	0.08		
Lack Of Fit	0.2	1	0.24	10.30	0.049
Pure Error	0.1	3	0.02		
COR TOTAL	272.0	9			

Final Equation in Terms of Pseudo Components:

M-Peak =

-	7.07	* A
+	60.63	* B
+	57.93	* C
+	106.38	* AB
+	109.98	* AC
-	78.42	* BC

Obs order	Actual	Predicted	RESIDUAL	LEVERAGE	COMPOSITION R59 A	S50 B	K33 C	Run order
1	39.60	39.67	-0.07	0.498	10	30	20	5
2	39.70	39.67	0.03	0.498	10	30	20	4
3	53.00	52.92	0.08	0.498	25	15	20	7
4	52.80	52.92	-0.12	0.498	25	15	20	6
5	53.50	53.37	0.13	0.498	25	30	5	10
6	53.20	53.37	-0.17	0.498	25	30	5	3
7	52.10	52.49	0.39	0.379	20	25	15	9
8	49.80	49.63	0.17	0.877	15	27.5	17.5	1
9	52.50	52.33	0.17	0.877	22.5	20	17.5	8
10	52.80	52.63	0.17	0.877	22.5	27.5	10	2

1.5.11 Referenser

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APPENDIX II: Grunnleggende statistikk

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AII.1 Innledning

Kjemometri og statistikk kan betraktes som to adskilte vitenskaper, "fundamentalt forskjellige" vil kanskje rene statistikere og kjemometrikere si. Faktum er at de stadig knyttes tettere sammen. Selv om statistikeren i utgangspunktet baserer seg på hypotesetesting og statistiske fordelinger, mens kjemometrikeren gjerne anvender hypotesegenerering og latente informasjonsbærere, vil begge angrepsvinkler og metodesett med fordel anvendes innen forskning og industri. For brukerne spiller det ingen rolle om metodene kalles statistiske eller kjemometriske. Disse kan altså integreres i en helhetlig filosofi, hvor de mest anvendelige metoder i øyeblikket "plukkes fra verktøykassen".

I kjemometrisk sammenheng er det således nyttig å supplere med statistiske tester, samt å kjenne til forutsetningene og antagelsene om fordelinger som ligger bak. Når man skal anvende enkle, sammenlignende tester, blir ofte distribusjonsparametrene gjennomsnitt og varians (se nedenfor) sammenlignet enten mot en standardverdi eller mot den korresponderende parameter i en annen fordeling. Gjennomsnitt $\bar{X}$ og varians (s^2) er altså nøkkelparametre i ulike fordelinger:

$$\bar{X} = \frac{1}{n}\sum_{i=1}^{n} X_i \quad og \quad s = \sqrt{\frac{\sum_{i=1}^{n}(X_i - \bar{X})^2}{n-1}}$$

der X_i er prøve nr. i sin verdi i en serie av n prøver.

Som eksempler på nær integrering mellom statistikk og kjemometri kan nevnes at kjemometrikere gjerne nytter *F*-testen til kartlegging av "outliere" (ekstreme prøver) i et datasett. Likeså er det svært nyttig å anvende konfidensintervall rundt predikterte responser ($\hat{y}$-verdier). I det følgende presenteres således endel grunnleggende statistisk teori som kan komme til nytte for eksperimentalisten. For dypere innsikt i disse metodene henvises til Bhattacharyya and Johnson (1977), Sokal & Rohlf (1981), Montgomery (1984), Cohen (1988) og Lea *og medarbeidere* (1991).

AII.2 Normalfordelingen

Normalfordelingen (ofte omtalt som Gauss-fordelingen) legges til grunn for en rekke tester. Dersom man kan anta at prøvegjennomsnittet X er tilnærmet normalfordelt (som oftest når n >20)[1], kan man nytte de såkalte parametriske tester. Alternativt må man enten transfomere dataene for å få dem normalfordelt eller man må benytte de ikke fullt så robuste ikke-parametriske statistiske testene. Normalfordelingen har en sannsynlighetsfordeling gitt ved:

$$P(z) = \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{(x-\mu)^2}{2\sigma^2}} \quad , \quad -\infty < x < \infty$$

med gjennomsnitt μ og standardavvik σ
Notasjon: N (μ, σ), *P* = sannsynlighet, *X* = primærvariabel

$$P[\mu - \sigma < X < \mu + \sigma] = 0.685$$
$$P[\mu - 2\sigma < X < \mu + 2\sigma] = 0.954$$
$$P[\mu - 3\sigma < X < \mu + 3\sigma] = 0.997$$

Den standardiserte normalfordelingen (Z) er et spesialtilfelle med $\mu = 0$ og $\sigma = 1$, *N* (0,1).

Verdier for *P* [a ≤ Z ≤ b] finnes i statistiske tabellsamlinger. Tabellen angir vanligvis arealet til venstre for en bestemt verdi. Når variablene ikke har *m* = 0 og *s* = 1, finnes sannsynligheter for disse fordelingene ved å *standardisere* variabelen.

Hvis X er *N* (μ, σ), så er

$$Z = \frac{x - \mu}{\sigma}$$ standard normalfordelt; *N* (0,1)

Eksempel på standardisering:

Bestem *P* [X > -3] når X er *N* (1,4):
Ovenfor har vi brukt greske tegn for å definere populasjonsparametre og romerske tegn

$$P[X > -3] = P[X - 1 > -3 - 1]$$
$$= P\left[\frac{X-1}{4} > \frac{-3-1}{4}\right] = P[Z > -1] = 0.8413$$

("vanlige" bokstaver) for å definere prøveparametre. En prøveserie (utvalg, *engelsk:* "samples") er ikke identisk med populasjonen (virkeligheten), men danner et bilde av denne. Som oftest er det egentlig populasjonen, hvor prøvene tas fra, vi er interessert i. Det er derfor vanlig å bruke forskjellige symboler for populasjons- og utvalgsparametre.

1 *Sentralgrenseteoremet* sier at når prøvetakingsstørrelsen *n* øker, vil fordelingen til prøvegjennomsnittet $\bar{x}$ fra en *tilfeldig* prøvetakingsserie hos praktisk talt enhver populasjon nærme seg en normalfordeling.

Fordelingen til gjennomsnittet av en variabel:

Ofte prøver vi å estimere (anslå) parameteren i en matematisk modell av virkeligheten, ved å finne et sannsynlig mål på gjennomsnittet fra en avgrenset prøvetakingsserie.

Hvis primærvariabelen X er *N* (μ,σ), vet vi at gjennomsnittet $\bar{X} \sim N(\mu, \frac{\sigma}{\sqrt{n}})$

AII.3 Student's t-fordelingen

Hvis X_1,.....X_n er et tilfeldig utvalg fra en normal populasjon *N* (μ, σ),

så er fordelingen til

$$t = \frac{(\bar{X} - \mu)}{s/\sqrt{n}}$$

en såkalt Student's *t* - fordeling med n-1 frihetsgrader

Forskjellen mellom Z og *t* er at *t* er basert på *s* (estimert standardavvik, som er en tilfeldig variabel) der Z er basert på σ. Vi forventer da at *t* - fordelingen ligner på standard normalfordelingen. Fordi det er en tilfeldig variabel i nevneren i uttrykket for *t*, vil variansen bli >1. Når *n* blir stor, vil *t* - fordelingen nærme seg standard normalfordelingen. Det er altså en *t* - fordeling for hver *n* (eller *n-1* som er antall frihetsgrader). For *n* > 30 (i statistisk litteratur ofte betegnet som "stort utvalg") er fordelingene tilnærmet like.

AII.4 *F*-fordelingen

I mange statistiske analyser inngår sammenligning mellom variansen for to populasjoner, X og Y, for eksempel innen variansanalyse ANOVA (ANalysis Of VAriance) (se avsnitt AII.9).

Antagelser:

1. X_1,.....X_n er et tilfeldig utvalg fra en normal populasjon, *N* (μ_1, σ_1)
2. Y_1,.....Y_n er et tilfeldig utvalg fra en normal populasjon, *N* (μ_2, σ_2)
3. De to utvalgene er uavhengige

Analysene baserer seg på forholdene mellom utvalgsvariansene

$$s_1^2 = \frac{\sum_{i=1}^{n_1}(X_i - \bar{X})^2}{n_1 - 1}, \qquad s_2^2 = \frac{\sum_{i=1}^{n_2}(Y_i - \bar{Y})^2}{n_2 - 1}$$

og spørsmålet er om forholdet s_1^2 / s_2^2 mellom dem er så forskjellig fra 1 at det ikke er noen tilfeldighet, og at de dermed er signifikant forskjellige.

Ut fra ovenstående antakelser er da fordelingen

$$F = \frac{s_1^2 / \sigma_1^2}{s_2^2 / \sigma_2^2} = \frac{\sum_{i=1}^{n_1}(X_i - \bar{X})^2 / (n_1 - 1)\sigma_1^2}{\sum_{i=1}^{n_2}(Y_i - \bar{Y})^2 / (n_2 - 1)\sigma_2^2}$$

F-fordelt med ($v_1 = n_1 - 1$, $v_2 = n_2 - 1$) frihetsgrader

Notasjonen $F_{\alpha(v1,v2)}$ angir et øvre 100(1-α) % kritisk punkt for en *F*-fordeling med $_{v1,v2}$ frihetsgrader, der nullhypotesen om likhet blir forkastet over dette punktet.

Som tidligere nevnt, kan F-testen nyttes til kartlegging av "outliere" (ekstremverdier), som ikke naturlig faller inn i etablerte klasser i datasettet. *Klassifisering* av data er nærmere omtalt i kapittel 1.9. Etter å ha bestemt dimensjonen (sylinder, kule, etc.) til klassens modell, kan man finne distansen (residual standardavviket, RSD) fra en prøve til modellen. RSD fra prøvene kan samles i en distansevektor **s**, som igjen nyttes til å definere det *gjennomsnittlige* residual standardavvik til klassen, s_c. Øvre grense for residual standardavviket s_{max} for prøver som tilhører en klasse kan uttrykkes som:

$$s_{max}^2 = s_c^2 \cdot F_{\alpha(M\text{-}A,\ (M\text{-}A)\cdot(N\text{-}A\text{-}1))}$$

der *N* = antall prøver, *M* = antall variabler og *A* = antall prinsipale komponenter. Signifikansnivået bestemmer hvor grensen blir satt for å definere "outliere" utenfor klassen. Lav α senker risiko for å forkaste prøver med positiv tilhørighet, men øker samtidig sjansen for å akseptere prøver med falsk tilhørighet (se avsnitt AII.6 om hypotesetesting).

AII.5 Estimering

Det er ofte vanskelig å finne den virkelige verdien av for eksempel gjennomsnittet, μ i en populasjon. Derfor må vi *estimere*, og finne et *estimat*. Estimeringen foregår ved at vi måler et utvalg fra populasjonen.

Det finnes to typer av estimering:

- punktestimering
- intervallestimering

Punktestimering

Et punktestimat er et anslag for en ukjent parameter, der anslaget er basert på observasjoner av en tilfeldig fordelt variabel. Det finnes flere estimatorer å velge mellom når vi skal løse et estimeringsproblem. For sentrum i populasjonens fordeling kan vi bruke for eksempel gjennomsnitt, median eller modalverdi. Medianen representerer den midterste verdien i en frekvensfordeling, hvilket vil si den verdi hvor 50 % av prøvene har lavere verdi. Modalverdien er den hyppigst forekommende verdi i en frekvensfordeling. Vi vil at forventningen til estimatoren skal være lik forventningen til den ukjente parameteren. Da er estimatoren *forventningsrett*.

En forventningsrett estimator vil over en lang periode gjennomsnittlig gi riktig resultat (en enkelt estimering kan gi et feil estimat).

Eks. Estimering av μ og σ i $N(\mu, \sigma)$ ved $\bar{x}$ og *s*

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}, \quad \text{og} \quad s = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}}$$

Intervallestimering

I forbindelse med punktestimering foretar man også ofte en intervallestimering ved at man først velger en sannsynlighet, 1- α, som kalles konfidenssannsynligheten. Deretter konstruerer man et intervall på tall-linjen på en slik måte at sannsynligheten for at intervallet skal inneholde den ukjente parameteren blir lik konfidenssannsynligheten. Et *konfidensintervall for en ukjent parameter* er et intervall på tall-linjen som har den egenskapen at det er en på forhånd valgt sannsynlighet, kalt *konfidenssannsynlighet*, for at intervallet inneholder den ukjente parameteren.

Framgangsmåte for å beregne et konfidensintervall:

1. Ta utgangspunkt i en punktestimator for θ; $\hat{\theta}$, for eksempel $\bar{x}$
2. Finn en funksjon av $\hat{\theta}$ og θ med kjent fordeling (i dette tilfellet normalfordeling)
3. Finn α/2 og 1- α/2 kvantilene i fordelingen (i z-tabellen i statistisk tabellsamling)
4. Løs ulikheten med hensyn på θ (her: μ)

Konfidensintervall for μ når σ er kjent:

$$(\overline{X} - z_{\frac{\alpha}{2}} \frac{\sigma}{\sqrt{n}}, \overline{X} + z_{\frac{\alpha}{2}} \frac{\sigma}{\sqrt{n}})$$

der $z_{\frac{\alpha}{2}}$ finnes i en tabell over den standardiserte normalfordeling

Eksempel:

pH er målt 6 ganger, med gjennomsnitt = 5.63. Variansen er kjent: 0.01
X ~ *N* (μ, 0.01). Standardiserer:

$$\frac{\overline{X} - \mu}{\sqrt{\frac{0.01}{6}}} \sim N(0,1)$$

Et 95% konfidensintervall beregnes slik:

P[5.63 - 1.96·0.04 < μ < 5.63 + 1.96·0.04] = 0.95, der 1.96 finnes i tabell for z

95% konf. intervall: [5.55 , 5.71]

Konfidensintervall for μ når σ er ukjent:

Når σ er ukjent, må utvalgsvariansen s^2 beregnes, slik at vi kan estimere σ med *s*, og vi må da benytte *t* - fordelingen til å beregne konfidensintervallet. Fra *t* - fordelingen kan vi da sette opp uttrykket

$$P\left[-t_{\alpha/2} < \frac{\overline{X} - \mu}{s/\sqrt{n}} \leq t_{\alpha/2}\right] = 1 - \alpha$$

Reorganiserer innenfor parentesen:

$$P\left[\overline{X} - t_{\alpha/2} \cdot \frac{s}{\sqrt{n}} < \mu < \overline{X} + t_{\alpha/2} \cdot \frac{s}{\sqrt{n}}\right] = 1 - \alpha$$

Et 100(1-α)% konfidensintervall for μ blir da:

$$\overline{X} \pm t_{\alpha/2} \cdot \frac{s}{\sqrt{n}}$$

I *t* - tabellen bruker vi fortsatt *n-1* frihetsgrader, selv om vi i ovenstående uttrykk dividerer med roten av *n*. For en intuitiv forståelse av dette kan man tenke seg at man i en total populasjon har *n* friheter (frihetsgrader) til å kalkulere standardavviket σ. I en begrenset prøvetakingsserie kalkulerer man imidlertid *s* ved hjelp av differansene (X_i - $\overline{X}$), og når man har kalkulert de første *n-1* differansene er den siste differansen gitt ved verdien til de øvrige i et slikt "lukket" datasett. Da har vi mistet en av de opprinnelige *n* frihetsgrader. Det viser seg at bruken av *n-1* frihetsgrader ved få prøver gir et mer forventningsrett estimat av σ enn bruk av *n* frihetsgrader.

AII.6 Hypotesetesting

En hypotese fremsettes som en *påstand*, og kan aldri bekreftes; derimot kan den *forkastes*. Hypotesen som vi vil "vise" settes opp som H_1 (eller alternativ hypotese) mens det motsatte settes opp som nullhypotesen (H_0). Dersom H_0 forkastes ($p < \alpha$) har vi da stor tro på at H_1 er riktig. Hvis testen ikke gir grunnlag for å forkaste H_0, kan vi ikke påstå noe som helst!

Altså: Ønsker å bekrefte et utsagn:

H_0: "Utsagnet er galt"

H_1: "Utsagnet er riktig"

Ønsker å avkrefte et utsagn:

H_0: "Utsagnet er riktig"

H_1: "Utsagnet er galt"

Framgangsmåte:

1. Still opp en forskningshypotese
2. Overfør denne til en nullhypotese
3. Bruk en statistisk test for å fastslå om resultatet er signifikant (ikke kun tilfeldighet)

Det er mulig å begå to typer feil ved denne fremgangsmåten:

Type 1 feil (α): Forkaste H_0 når H_0 i virkeligheten er riktig.

Type 2 feil (β): Ikke forkaste H_0 når H_0 i virkeligheten er feil.

Å begå "Type 1 feil" (produsentens risiko) betraktes ofte som å være langt mer alvorlig (se nedenfor) ennå begå "Type 2 feil" (konsumentens risiko), men som vi skal se neste side, er ikke dette alltid riktig. Valg av α og β påvirker hverandre. Ved å sette signifikansnivået $\alpha = 0.05$ (altså: 1 - 0.95), godtar man en "kalkulert risiko" på å begå "Type 1 feil" i ett av 20 tilfeller. Dette kan eksempelvis forekomme ved prøvetakingsfeil.

Følgende framgangsmåte velges tradisjonelt:

Lag testmetode slik at:

1. P(Forkaste H_0 når H_0 er riktig) $\leq \alpha$
2. P(Forkaste H_0 når H_1 er riktig) skal være så stor som mulig (1 - β)

1. kalles *nivåkravet* til metoden
2. kalles *styrkekravet* til metoden, der (1 - β) kalles *statistisk styrke*

Eksempel:

To blodprøver er målt for å avgjøre om promillen er ≥ 0.5:
$X_1 = 0.55$, $X_2 = 0.59$.
X_1 og X_2 er uavhengig, $N(\mu, 0.05)$

Vi formulerer først nullhypotesen:

H_0: $\mu < 0.5$ mot H_1: $\mu \geq 0.5$

Vi baserer testen på $\overline{X} \pm t_{\alpha/2} \cdot \frac{s}{\sqrt{n}}$, og forkaster H_0 når $\overline{X} \geq k$

Dersom vi antar signifikansnivå 0.01:

$$P\left(\frac{\overline{x} - 0.5}{0.05/\sqrt{2}} \geq \frac{k - 0.5}{0.05/\sqrt{2}}\right) = 0.01$$

$$\frac{k - 0.5}{0.05/\sqrt{2}} = 2.326 \text{ (fra tabell for Z)}$$

$k = 0.58$, dvs. forkast H_0 når $\overline{x} \geq 0.58$

Her er $\overline{x} = 0.57$, dvs. ingen forkastning

(kan ikke avkrefte at promillen er ≥ 0.50)

Dette er altså en mye brukt metode (Fisher 1949), men ved gitte problemstillinger kan det også være risikalbelt å begå "Type II feil" (Neyman & Pearson 1928, 1939). Vi husker fra starten av dette avsnittet at hvis testen ikke gir grunnlag for å forkaste H_0, kan vi ikke påstå noe som helst! Det kan altså være at testen ikke fanger opp reelle forskjeller mellom populasjoner på grunn av måten testen er formulert, eller ved at prøvetakingsstørrelsen n er for liten. Eksempelvis kan man ønske å få kartlagt om der er relle endringer i klima fra 1995 til år 2000. H_0 blir da:

H0: der er ingen klimaendringer

Klimaendringer går imidlertid gjerne sent, så det skal svært store prøvemengder til for å stadfeste eventuelle forskjeller mellom prøver over tid, med så smale konfidensintervall at disse kan skilles fra hverandre. Dette problemet forsterkes ved stor *naturlig* variasjon. Her foreligger det altså en fare for ikke å forkaste H_0 når denne egentlig er feil. Dette kan være dramatisk for verdens befolkning fordi man ønsker å detektere klimaendringer så snart som mulig.

For å sikre oss mot denne "Type II feil", må vi derfor beregne den statistiske styrken (1 - β). Dette gjøres ved å spesifisere den alternative hypotesen H_1, og deretter se hvilken andel av H_1 sin fordeling som overlapper aksepteringsregionen til H_0. Denne andelen representerer sannsynligheten for β. Størrelsen til β vil avhenge av både α, n og av *effektstørrelsen* (*ES*). *ES* representerer den ønskelige (eller reelle) detekterbare forskjellen mellom de to gjennomsnittene μ_0 og μ_1. Dersom vi ønsker å detektere en gitt effektstørrelse (klimaendring) med lav mulighet for "Type I feil" (liten α) og med høy sikkerhet (høy statistisk styrke), vil dette kreve en høy prøvetakingsstørrelse n.

De valgte nivåene til α og (1-β) settes ofte subjektivt til henholdsvis 0.05 og 0.8. Styrken til ikke-parametriske tester er mindre påvirket av feile antagelser enn tilsvarende hos parametriske metoder, men de har generelt lavere styrke hvis forutsetningene er oppfylt. Eksperimentell design vil generelt bedre den statistiske styrken.

AII.7 Hvor mange prøver (n) er det nødvendig å ta?

Valg av hvor store prøvetakingsserier det er nødvendig å ta blir ofte en vurdering mellom på den ene siden, hvor presist og sikkert man ønsker å bestemme en størrelse og på den andre siden, hvilke ressurser som må settes inn (tid, innsats, tilgjengelig prøvemateriale, penger). Disse to forhold kommer gjerne i konflikt med hverandre. Det er da nyttig med en objektiv vurdering som sikrer ønsket presisjon og sikkerhet, samtidig som n minimaliseres for å spare ressurser. Man kan nytte følgende formel for å komme frem til et forsvarlig prøveantall, der man både tar hensyn til effektstørrelsen (*ES*, i % av gjennomsnitt), signifikansnivå (α), variasjonskoeffisienten (*CV* = standardavvik / gjennomsnitt * 100) og den statistiske styrken (1 - β) (Sokal & Rohlf 1981):

$n \geq 2 \cdot (CV/ES)^2 \cdot (t_{\alpha[v]} + t_{2\beta[v]})^2$, der v = antall frihetsgrader og β kommer fra den statistiske styrken $(1 - \beta)$.

Fremgangsmåten er da:

1. foreta en kvalifisert gjetning for hvor stor *n* må være
2. bruk denne *n* for å finne de to t-verdiene i formelen fra en Student's *t* - tabell
3. sett inn disse verdiene, samt verdiene for *CV* og *ES* i formelen og regn ut
4. avrund tallet du finner opp til neste heltall *n*
5. bruk dette tallet *n* og gjenta punkt 1 - 4 to ganger med de suksessivt nye *n*-verdiene

Nå har tallet stabilisert seg på en gitt *n*-verdi. Metoden er mest følsom for variasjon i parametrene *CV* og *ES*. Dersom man skal teste forskjellen mellom to prosentverdier, må man nytte en annen formel, som man kan finne i Sokal & Rohlf (1981).

AII.8 Regresjon

Formålet med regresjon er å finne en mulig sammenheng mellom *x* (uavhengig variabel) og *y* (avhengig variabel, respons) for senere *prediksjon* av *y* fra *x*. Her vises prinsippet for én *x*- og én *y*- variabel. I kjemometrien har vi generelt mange x- og ofte også mange y-variabler.

Formler for beregning av kvadratsummer:

$$\bar{x} = \frac{1}{n}\sum x_i, \qquad \bar{y} = \frac{1}{n}\sum y_i$$

$$S_x^2 = \sum (x_i - \bar{x})^2 = \sum x_i^2 - n\bar{x}^2$$

$$S_y^2 = \sum (y_i - \bar{y})^2 = \sum y_i^2 - n\bar{y}^2$$

$$S_{xy} = \sum (x_i - \bar{x})(y_i - \bar{y}) = \sum x_i y_i - n\bar{x}\bar{y}$$

Statistisk modell for lineær regresjon:

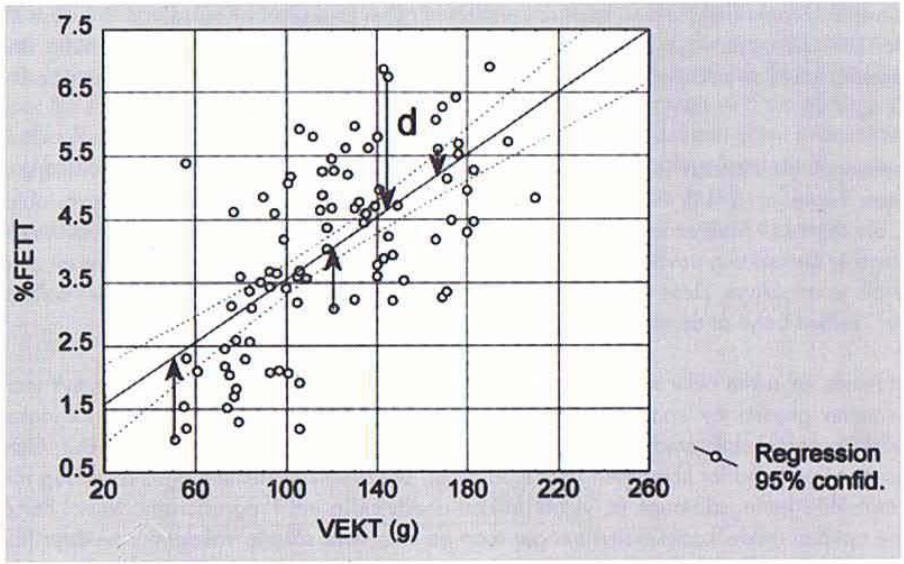
$$y = \alpha + \beta x + \varepsilon$$

Vi skal bestemme α og β ved hjelp av *minste kvadraters metode:*

Kriteriet er å minimalisere D, som er

$$\sum_1^n d_i^2 = \sum_1^n (y_i - a - bx_i)^2$$

En annen måte å formulere det på er : D=Σ(observert respons - prediktert respons)^2. Man legger altså en rett linje gjennom punktskyen av observasjoner på en slik måte at denne minimaliserer de kvadrerte avvikene mellom observasjoner og beregnet modell (Figur 1). Dette oppnås ved å derivere denne D med hensyn på α og β og derved finne verdiene av α og β som gir lavest D. I figur 1 er det etablert en regresjonsmodell mellom fettinnhold i fiskefilet (y) og fiskens vekt (x).



Figur 1. Regresjon mellom fettinnhold i fiskefilet (y) og fiskens vekt (x). Avstanden (d) fra hvert punkt til den tilpassede regresjonslinjen er markert noen steder med piler. De buede linjene indikerer at prediksjonsfeilen øker jo lenger vekk man kommer fra gjennomsnittsvekten.

Prediksjonsfeilen for y, når en ny prøve (x*) brukes til å prediktere y, er gitt ved:

$$\text{estimert standardfeil} = s\sqrt{1 + \frac{1}{n} + \frac{(x^* - \bar{x})^2}{s_x^2}}$$

Prediksjonsfeilen øker altså når avstanden fra sentrum i punktskyen til den nye prøven $(x^* - \bar{x})$ øker. Der etableres derfor gjerne et 95 % konfidensintervall rundt regresjonslinjen (Figur 1).

Ved praktisk bruk av regresjon kan man merke seg at det kan opptre målefeil både i x-retningen og i y-retningen for ethvert punkt. Residualene rundt linjen kan derfor bli videre enn ønskelig, og linjens retning gjennom punktskyen blir beheftet med feil. Forutsetningen for tradisjonell lineær regresjon er opprinnelig at x ikke er beheftet med målefeil. Det er derfor prinsipielt ikke korrekt å nytte denne typen regresjoner i andre tilfeller enn der hvor en har

problemstillingen "mengde målt (y) som funksjon av mengde tilsatt (x)". I praksis er det imidlertid ikke avgjørende om x er beheftet med målefeil (når x antas korrekt målt) så lenge det er en klar sammenheng mellom x og y, og formålet er fremtidig prediksjon av y.

I kjemiske undersøkelser er det som oftest aktuelt å bruke mer enn én x-variabel i regresjon. Et eksempel på dette er multivariabel kalibrering, der ulike målevariabler x_j (for eksempel absorbanser ved ulike bølgelengder) brukes sammen i kalibreringen for en egenskap y (for eksempel konsentrasjon av en kjemisk konstituent), som beskrevet i Kapittel 1.10. I visse tilfeller kan det også være aktuelt å modellere mer enn en enkelt y-variabel samtidig, for eksempel når man studerer sammenhengen mellom sensoriske og kjemiske data (se Kapittel 3.3 og 4.2).

Tradisjonelt ble statistisk regresjon med flere x-variabler utført ved hjelp av såkalt Multippel Lineær Regresjon (MLR). Når to eller flere y-variabler ble modellert samtidig fra x-variablene, ble dette kalt Multivariabel Lineær Regresjon. Imidlertid krever denne teknikken at x-variablene er tilstrekkelig uavhengige av hverandre til at det er klart hvilken effekt hver av dem har på y-variablene. Dette er sjeldent tilfelle i kjemi, der x-variablene som oftest er "kollineære", hvilket betyr at de til en viss grad virker i samme retning.

Det finnes en rekke ulike regresjonsteknikker for kollineære data. I slike metoder må man bestemme graden av kompleksitet i regresjonsmodellen. Gjør man modellen for enkel (undertilpasning), blir prediksjonsevnen for dårlig på grunn av umodellert x-y struktur. Gjør man den imidlertid for komplisert (overtilpasning), blir prediksjonsevnen også for dårlig fordi målefeil i læringsdataene (x, y) blir trukket unødvendig inn i regresjonsmodellen. For å finne optimal modellkompleksitet trenger man en eller flere såkalte valideringsmetoder (for eksempel kryssvalidering).

Delvis minste kvadraters metode (PLS, se kapittel 1.6) er den mest anvendte regresjonsmetoden innen kjemometri, både av statistiske og grafiske grunner. I PLS regresjon, hvor man har flere x_j og ofte flere y_j, legges residualene (avstand mellom punkt og linje) vinkelrett på den rette linjen, slik at man oppnår mer stringente modeller enn for tradisjonell lineær regresjon. For en prediktert verdi $\hat{y}$ kan da $\pm$ 2 · SEP ("standard error of prediction") representere en god tilnærmimg til et 95 % konfidensintervall.

Den mest ønskede egenskap ved en PLS modell er gjerne dens predikterende evne. Vi ønsker altså at modellen skal kunne brukes til å si noe om y når vi har målt noen x-variable. Et problem vil oppstå dersom vi inkluderer for mange komponenter i modellen og derved begynner å modellere støy. Vanlig PLS regresjon er følsom for slik overtilpasning. Dette problemet kan løses ved kryssvalidering, som vil si å først dele prøvene inn i en rekke undergrupper (eventuelt én prøve i hver gruppe ved små matriser), for deretter å prediktere resultatet i den utelatte gruppen fra modellen som er utledet fra resten av datasettet. Dette gir et mål på predikterende evne, den såkalte PRESS verdi ("predictive residual error sum of squares"). PRESS beregnes som summen av kvadrerte avvik mellom prediksjonen og observasjonen av den avhengige variabel for hver prøve (når denne utelates fra modellen). Et alternativt mål er RMSEP ("root mean square error of prediction"), som gir verdier som kan sammenlignes med variablenes måleenhet (se kap. 3.3). Dette er gunstig for å kunne vurdere modellen i forhold til måleusikkerheten for den avhengige variabelen.

AII.9 Variansanalyse (ANOVA)

Variansanalyse brukes ofte til å teste om flere (enn to) prøvegjennomsnitt stammer fra samme populasjon eller om de er forskjellige fra hverandre. Dette kan eksempelvis være aktuelt ved vurdering av veksteffekten til en rekke forskjellige fiskefôr, testet på flere fiskegrupper fra samme populasjon. Fôrets kjemiske sammensetning, som man vil finne effekten av, kalles for *faktor*. Slike faktorer kan ha flere nivåer og kan være definert både kvantitativt og kvalitativt. Kombinasjoner av *faktorer* og *nivåer* kalles *behandling* (treatment).

En-veis ANOVA: En faktor (A) med k nivåer

	A_1	A_2	.	.	A_k
	y_{11}	y_{12}	·	·	y_{1k}
	y_{21}	y_{22}	·	·	y_{2k}
	.	.	.	.	.
	.	.	.	.	.
	y_{n1}	y_{2n}	·	·	y_{nk}
Gj. snitt	$\bar{y}_1$	$\bar{y}_2$	·	·	$\bar{y}_k$

y_{ij}, i = 1,2,.....,n
j = 1,2,.....,k

Observasjon = total gjennomsnitt + effekt av behandling + residual

$$y_{ij} = \bar{y} + (\bar{y}_j - \bar{y}) + (y_{ij} - \bar{y}_j)$$

Ordner og kvadrerer:

$$\sum_{j=1}^{k}\sum_{i=1}^{n}(y_{ij} - \bar{y})^2 = \sum_{j=1}^{k} n(\bar{y}_j - \bar{y})^2 + \sum_{j=1}^{k}\sum_{i=1}^{n}(y_{ij} - \bar{y}_j)^2$$

Total SS_{tot} = Behandling SS_T + Residual SSE

En ANOVA tabell for 1-veis tilfelle:

Kilde	Sum of squares	Frihetsgrader	Mean square
Behandlingseffekt mellom grupper	$SS_T = \sum_{j=1}^{k} n(\bar{y}_j - \bar{y})^2$	k-1	$MS_T = \frac{SS_T}{k-1}$
Variasjon innen grupper (såkalt "residual")	$SSE = \sum_{i=1}^{k}\sum_{i=1}^{n}(y_{ij} - \bar{y}_j)^2$	k(n-1)	$MSE = \frac{SSE}{k(n-1)}$
Total variasjon	$\sum_{j=1}^{k}\sum_{i=1}^{n}(y_{ij} - \bar{y})^2$	ak-1	

Modell:

$y_{ij} = \mu + \beta_j + e_{ij}$, der

$\mu = \bar{y} = \frac{1}{n}\sum_{i=1}^{n}\sum_{j=1}^{k} y_{ij}$ og β = behandlingseffekt

$\sum_{j=1}^{k}\beta_j = 0$

$e_{ij} \sim N(0, \sigma)$

Formulering av hypotesetest:

$$H_0: \beta_1 = \beta_2 = \ldots\ldots = \beta_k = 0$$
$$H_1: \text{En eller flere } \beta_j \neq 0$$

Ut fra betraktningen at SS_T (mellom grupper) vil bli stor dersom en eller flere β har effekt, og at SSE er et estimat for variansen innen grupper, er det naturlig å se på forholdet mellom disse. En *F*-test nyttes her for å teste nullhypotesen om at variasjon mellom grupper er lik variasjon innen grupper, altså at dette forholdet skal bli tilnærmet lik 1. Dette formuleres da slik:

$$F = \frac{\text{Behandling mean square}}{\text{Residual mean square}} = \frac{SS_T / (k-1)}{SSE / (k(n-1))}$$

der uttrykket over er *F*-fordelt med *(k-1, k(n-1))* frihetsgrader.

Forkastningsregelen blir da:

$$\text{Forkast } H_0: \beta_1 = \beta_2 = \ldots\ldots = \beta_k = 0 \quad \text{hvis}$$
$$\frac{\text{Behandling SS / (k - 1)}}{\text{Residual SS / (k(n - 1))}} > F_\alpha(k-1, k(n-1))$$

Man forkaster da eventuelt nullhypotesen dersom verdien man regner ut overstiger den kritiske verdi for *F*, som finnes i en *F*-tabell i statistiske håndbøker. Ved forkastning av nullhypotesen er der altså en eller flere behandlingseffekter. Der opprettes så et konfidensintervall rundt differansen mellom to ulike grupper, og man sjekker om dette intervallet inkluderer 0, hvilket betyr at differansen eventuelt ikke er signifikant.

1-veis ANOVA kan enkelt utvides til 2-veis og flerveis, hvor man også får innført et kovariansledd, som skyldes samspillseffekter mellom behandlingene. Hvis vi viderefører det innledende eksemplet i dette avsnittet, kan vi tenke oss at fiskegrupper både tilbys forskjellig fôr og oppdrettes ved forskjellig temperatur (to faktorer, eventuelt på flere nivå hver). Vi kan i en 2-veis ANOVA da både kartlegge effekten av fôrets kjemiske sammensetning, temperaturen og vekselvirkningen (samspillseffekten) mellom disse. Den sistnevnte kombinasjonen av faktorer kan gi en ekstra positiv (eller negativ) effekt som tilsier at summen av de to enkeltstående effektene ikke kan forklare hele variasjonen mellom fiskegruppene. Vi refererer til Sokal og Rohlf (1981) for utvidet formelsamling.

AII.10 Referanser

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