Computer Aided Product Design and Development for Peroxide Based Disinfectants

by

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A thesis
presented to the University of Waterloo
in fulfillment of the
thesis requirement for the degree of
Doctor of Philosophy
in
Chemical Engineering

Waterloo, Ontario, Canada, 2010

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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ABSTRACT

Disinfectants are antimicrobial chemicals that are commonly used in health care facilities to prevent or reduce the spread of pathogenic microorganisms. These products are under national regulations for the claims they make and have to be tested for their microbial activity against different microorganisms. They have to also be tested for product stability, corrosion and toxicity. These tests, especially the microbial efficacy tests, are very expensive and take a long time to perform (anywhere from two days to four months). Disinfectant formulations have to have a balance between their microbial activity, corrosivity, and safety. The more active ingredients in the formulation, the stronger the product, but the higher the corrosivity and toxicity. Therefore, it is desirable to use as low concentrations of ingredients as possible in the formulation to achieve the acceptable antimicrobial activity. The final product has to also be chemically and physically stable for at least one year. Consequently, the product development process takes at least six months and sometimes even up to two years. The cost might also reach hundreds of thousands of dollars.

The objective of this project was to design a systematic way to take advantage of the historical data, augment them with some experimental trials, perform a regression analysis using the best possible methods available such as least squares or neural networks, invert the models, and finally use optimization techniques to develop the new products in the shortest period of time. The formulation predicted by this model will be much closer to the final formulation resulting in significant reductions in time and cost of the product development process. Furthermore, the model can be updated with the newly generated data to improve its predictive capability. Lastly, the disinfectant formulation can be viewed as a case study for a broader problem, formulation product design, and can be implemented in similar cases where the formulation of a new product should pass certain interfering criteria, such as adhesives, pharmaceutical drugs, agriculture pesticides, detergents, etc.

ACKNOWLEDGEMENTS

I would like to express my deep gratitude and appreciation to my supervisors, Professor Ali Elkamel, Professor Thomas A. Duever, and Professor Park M. Reilly for their valuable guidance, support and encouragement in my academic life. I consider myself fortunate to have had the chance to learn from such excellent mentors.

I would also like to thank Professor Hector Budman, Professor William Anderson, and Professor Monika Emelko for serving as my committee members and providing me with constructive comments for this thesis.

My appreciations go to external examiner Professor Marc Dubé for his guidance and valuable comments.

My sincere gratitude goes to Virox Technologies, and specifically Mr. Randy Pilon to fund my PhD program. Randy has also been a great friend to me and encouraged me in program, work and life. Without Randy, it was not possible for me to achieve to this goal. I also greatly appreciate all my colleagues at Virox Technologies for their continuous support.

My appreciations go to Professor Syed Sattar, from the University of Ottawa for his continuous support and guidance.

My sincere gratitude goes to those friends I have made during the course of my studies in Waterloo, many thanks to Drs. Mazda Biglari, and Keyvan Nowruzi, for their advice, comments and moral support.

I would like to express my deepest gratitude and love to my parents for their love and constant encouragement throughout my life. I am extremely grateful for the support of my wife, Dr. Neda Arjomandi for her love and patience. Finally, I would like to extend my special love to my sister and brother.

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CHAPTER 1

INTRODUCTION

1.1 Disinfectant Products

A disinfectant is a chemical formulation that inactivates pathogenic microorganisms including bacteria, mycobacteria, fungi, and viruses. The use of disinfectants is widespread with the increasing consumer awareness of the dangers of microbes. Consumers are learning more about microbial risks and this has resulted in the growth of the disinfectant market. Disinfectants are widely used in healthcare settings, in various industries and in homes for disinfecting their surroundings. The market has been driven by growing awareness, media participation and a sharp rise in infections. Along with the increasing use of disinfectants, consumers are demanding safe, effective, convenient, easy to use products. By understanding consumer demand, manufacturers are committed to providing easy to use and application specific products and this has led to increased consumption. The total sales of the disinfectants have been reported to be more than three billion U.S. dollars worldwide in 2008 (Dewan 2009).

There are usually at least a few constraints in developing a disinfectant formulation. These constraints include antimicrobial activity, product shelf life, toxicity, skin/eye irritation, cost and corrosion. Ideally, it is desired to design a product with broad spectrum and fast antimicrobial activity, no corrosivity, lower toxicity, low product cost, high shelf life and no skin/eye irritation. In practice, it is very difficult to achieve such an objective since most of these constraints conflict (Omidbakhsh and Sattar 2006). For example, by using high levels of active ingredients in the formulation, it is possible to achieve a broad-spectrum and fast-acting product; however such an action will result in high product toxicity, high corrosivity, high cost, and most likely skin/eye irritation. On the other hand, designing a very low toxic and low skin/eye irritant product will require using low levels of active ingredients which may result in insufficient antimicrobial activity.

Furthermore, designing a disinfecting product requires considerable experimentation which is typically conducted through trial and error. The required tests include antimicrobial, stability and corrosion tests. Antimicrobial tests are typically expensive to run, cumbersome, take a long time to perform and have typically high error. Traditionally such tests are performed based on “One Factor At-a-Time” (OFAT) experimentation which is proven to be inefficient (Czitrom 1999) often requiring a large number of trials. Here it is desired to run as few numbers of trials as possible to reduce product development cost and time.

1.2 Research Objective

Traditional disinfectant product design methods are based on a trial and error approach using OFAT methodology which is very costly and time consuming. The scientists have to develop a product which inactivates all the desired microorganisms in a specified contact time and has a reasonable shelf life. Furthermore, the product should have the lowest possible concentration of raw materials to be safe for the end users and to have low corrosivity. It is necessary to run a number of tests such as antimicrobial, stability, and corrosion to find the optimum formulation. These tests, especially microbial tests, are very expensive, cumbersome and sometimes very slow. This causes the product design process to take from a few months up to two or three years, and cost hundreds of thousands of dollars. Furthermore, the formulation which is developed in this way is often not optimal.

The objective of this thesis was to develop a computer-aided product development methodology that uses the existing database of microbial efficacy, stability, and corrosion test results, augments them with designed experiments, and analyzes them by either multiple linear regression or neural networks, and then inverting the models can lead to the final desired formulation. This formulation was then tested for its antimicrobial activity, stability and corrosion to confirm the expected properties. The current dataset can be used for future product development and can be easily augmented by further experiments, and the models can be re-analyzed. It can also be adopted in other areas of the formulation development such as detergents, adhesives, agricultural products and so on.

1.3 Research Approach

In this section the various steps taken to develop the required model, design new formulations, and verify these is described. Figure 1 shows the outline of this research.

1.3.1 Experimental Design for Antimicrobial Activity

1.3.1.1 Antimicrobial Activity Modelling

Historical data for bactericidal activity of several disinfectant formulations were available in Virox Technologies, Oakville, ON, Canada, R&D binders. These data were analyzed using linear regression analysis. The data were then augmented using a fractional factorial design. The augmented design was tested and the results were added to the historical data. Then the whole dataset was analyzed to develop a model using regression analysis and neural networks and a model was obtained for each case.

1.3.1.2 Optimization

Before including other constraints such as stability and corrosion resistance, a new product was designed taking into account the antimicrobial activity model only to see if this approach had merit. New product specifications were defined and the optimal product formulation was obtained. In the optimization

program, the objective was set to minimize the product toxicity. The constraints were the minimum required antimicrobial activity, and the ingredients range in the formulation. This process was repeated for both models obtained by least-squares regression analysis and artificial neural networks. Different, but close formulations were obtained from optimization for the two models. The two formulations were made in the lab and were tested for their antimicrobial activity, and both passed the design criteria.

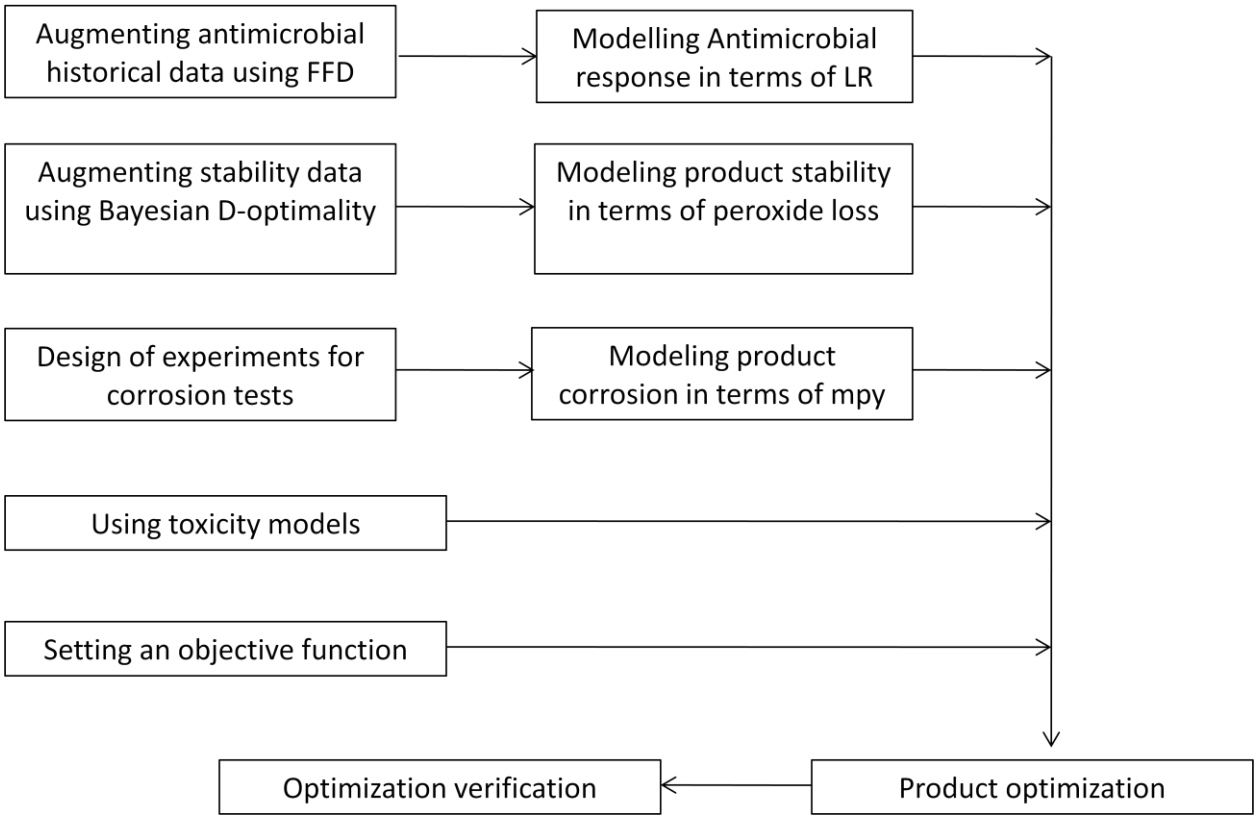

Figure 1.1- Outline of the research

1.3.2 Experimental Design for Stability of Hydrogen Peroxide

1.3.2.1 Hydrogen Peroxide Loss Modelling

Similar to antimicrobial tests, historical data were analyzed; however since the number of historical data were very few, the analysis results were not accurate enough. Therefore, the historical data were augmented using a Bayesian *D*-optimality method. The Bayesian technique took advantage of the prior knowledge; therefore the optimal design tailored specifically for the historical data, and made the further analysis more accurate. The samples based on the designed experiments were made and accelerated

stability tests were conducted and the results were analyzed and a model for hydrogen peroxide loss was obtained. In parallel, the historical data were also augmented using fractional factorial design, and the results were compared to those of the Bayesian *D*-optimality method.

1.3.3 Experimental Design for Corrosion

No useful historical data were found as the starting point. The historical data details revealed that the previous tests were not consistent and they could not be analyzed. Therefore, no historical data were used and instead a fractional factorial design (Resolution IV) followed by a Box Behnken were used. The samples were made and tested for their brass corrosion. The results were analysed and a model was obtained for the brass corrosion.

1.3.4 Product Formulation Optimization

A new product was designed based on the several constraints namely maximum allowed peroxide loss, minimum antimicrobial activity, maximum allowed corrosion, maximum allowed toxicity and ingredients range. The objective function was to minimize the cost of the solution. A formulation was obtained from this optimization method.

1.3.5 Experimental Verification

The optimal formulation obtained earlier was made and tested for its antimicrobial activity, peroxide loss, and brass corrosion and the test results showed that the formulation met all the desired criteria.

1.4 Thesis Outline

This thesis is described in 9 Chapters and is organized as follows:

Chapter 1 presents the introduction, objective of the research and the research approach.

Chapter 2 includes the literature review which contains a description of chemical product design, introduction to experimental design, regression analysis, optimization, review of disinfectant chemicals, microbiology of the organism studied, hydrogen peroxide decomposition, and corrosion.

Chapter 3 provides the materials and methods which are applied in this research including stock culture preparation, hydrogen peroxide measurement method, hydrogen peroxide stability test method, and corrosion coupon test method.

Chapter 4 compares different optimality criteria from three perspectives namely model dependency, prediction power and parameter estimates.

Chapter 5 presents the experimental design for antimicrobial activity, and finds models based on linear regression analysis and artificial neural networks. It then uses each model to optimize a formulation for some given desired product criteria.

Chapter 6 describes the stability data analysis, augmenting the historical data using Bayesian *D*-optimality and comparing it to regular fractional factorial design. It then presents an empirical model for the stability.

Chapter 7 presents the corrosion experimental designs based on a fractional 2^k factorial design followed by a Box-Behnken design. It then provides a model for the corrosion.

Chapter 8 describes the final optimization, using all obtained models, and desired product characteristics, and presents the final formulation. It then compares the estimated product characteristics versus the actual test results.

Finally, Chapter 9 summarizes the significant findings of this work and makes a few recommendations for future research.

CHAPTER 2

LITERATURE REVIEW

2.1 Chemical Product Design

Chemical product design is the procedure consisting of defining product specifications based on market needs, generating ideas to meet these specifications, screening, and finally, deciding what the product should look like and how it should be manufactured (Cussler and Moggridge 2001). In defining needs, the expectations of the customers should be emphasized. A system of ranking is often employed such as essential, desirable and useful. The essential needs are those without which the product cannot succeed, and these cannot be neglected. Desirable and useful needs differentiate the product from competitor products, and can have more marketing value. Next, the qualitative list of needs must be converted into specifications, including as much quantitative and chemical detail as possible. The final step in the needs stage is to specify a benchmark. This can be an already existing or idealized product against which to measure the new design. If the benchmark cannot be beaten, the product is not worth developing. Once the specifications for the target product formulation are chosen, good product ideas must be generated. In the case of a formulation design, these ideas are usually based on using an appropriate mix of raw materials which leads to the desired specifications. Some candidate raw materials from a previous product design project might be already available. This is the case with most formulation development projects, unless a revolutionary product is targeted. The previous projects are usually reviewed to see what useful information can be derived and used in the new project. For example, to design a new disinfectant, this might be achieved using a combination of two or three active ingredients that have been used in an earlier product, or a surfactant blend to increase the cleaning activity of the formulation. Generally speaking, previous information cannot fulfill the requirements of a new product completely, and therefore new concepts need to be investigated. Patents, books, and papers are useful resources to find new concepts for formulation development. They can give very good ideas about some candidate ingredients that were not previously employed and which might be worth trying. The candidate ingredients can be numerous, and it is not realistic or even possible to test them all. Therefore, a few of them must be selected for further investigation. The selection can be done based on some criteria such as candidate ingredients toxicity profile, regulatory limitations, compatibility with other ingredients in the formulation, patent rights, shipping and storage hazards and manufacturing feasibility.

After the selection of some candidate ingredients, prototypes have to be prepared and tested. By fully taking advantage of a historical data base; the final formulation can be obtained by augmenting this database using approximate experimental design techniques in order to maximize information about the new ingredients at the least possible cost.

As an illustration of the above discussion, consider the case of a disinfectant formulation. The needs here can be defined as cleaning performance, level of disinfection kill, product toxicity, materials compatibility and finally product stability. First, brainstorming and research is required to identify possible candidate ingredients. In the case of a revolutionary product, a comprehensive literature search is needed to trigger some valid ideas about new ingredients. Usually, the list of ingredients that contribute to achieve the product specifications is limited, and for each product design, a selection of ingredients from a known list of chemicals that involves antimicrobial active ingredients, builders, surfactants, solvents, and pH buffers is made. Depending on the desired disinfectant specifications, a combination of each of these chemicals might be used. A schematic of the methodology is shown in Figure 2.1.

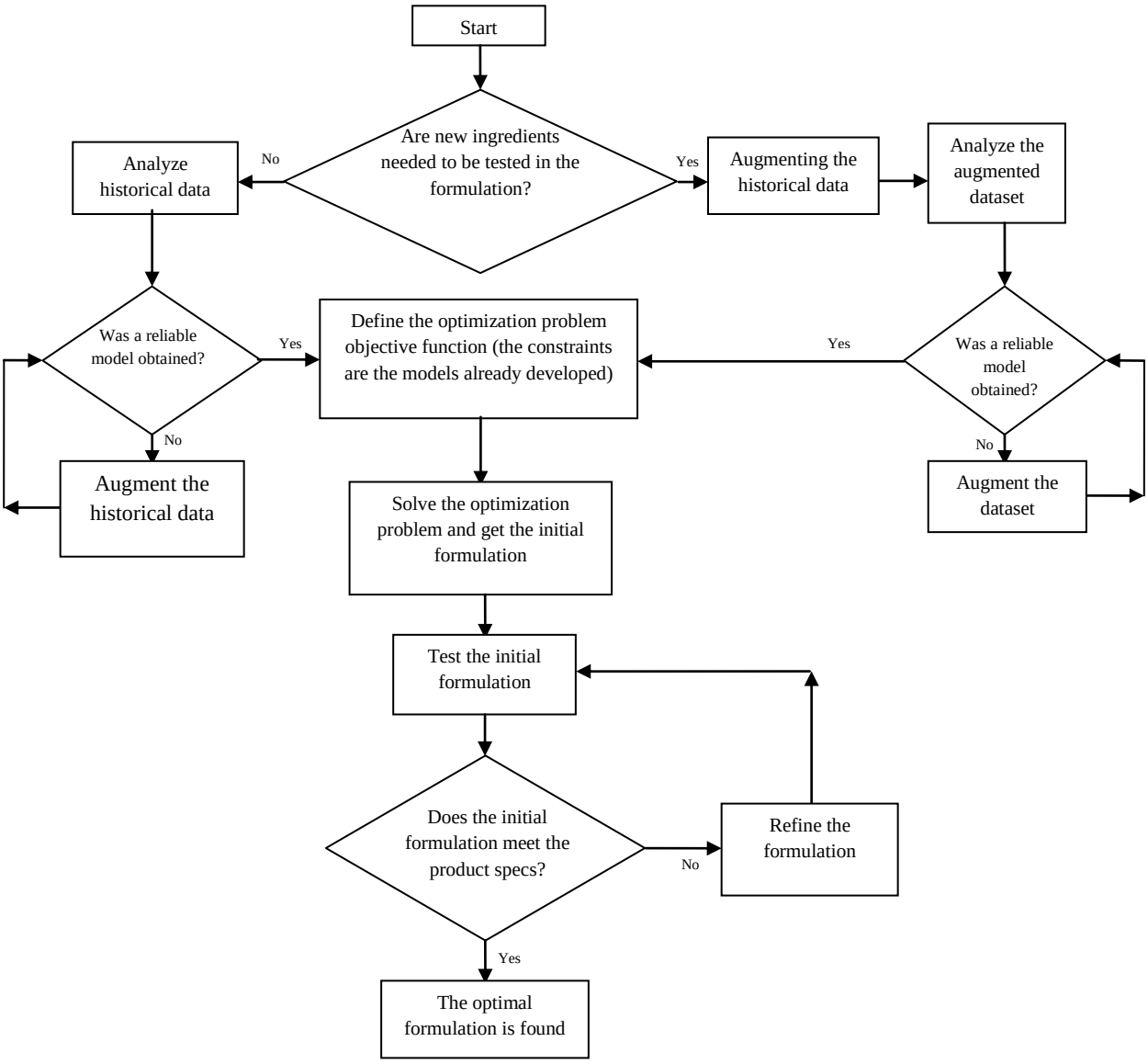

Figure 2.1- Systematic disinfecting formulation product design

2.2 Introduction to Experimental Design

Many experiments require studying the effects of several factors (Montgomery 2004). Engineers and scientists often carry out one-factor-at-a time (OFAT) experiments, which vary only one factor at a time while other factors are kept constant. Conversely, statistically designed experiments which change more

than one factor at the same time are more efficient when the objective is to study two or more factors (Czitrom 1999).

The statistical design of experiments is an efficient process for designing experiments so that the most information is obtained and the data can be analyzed to result in the best possible conclusions from minimal experimentation (Montgomery 2004).

A statistically designed experiment is more efficient to determine the effect of factors on a response than an OFAT experiment, because:

- It usually requires fewer experiments and consequently less time and cost which can be very important especially in industry.
- The estimates of the effects for each factor are more accurate because of using more observations to estimate an effect which leads to lower variability.
- The interactions between the factors can be calculated while it is not possible to estimate them by using OFAT.

DOE is proven to be a critically important tool in the science and engineering world for improving products and processes. It can be used for many applications such as:

- Improved process yield
- Formulation of new products

Of designed experiments, 2^k factorial design, response surface methodology (RSM) such as central composite or Box-Behnken designs, are widely used because they are quite general and flexible. However there are situations in which an experimenter cannot use these designs such as

1- when the design region is irregular,

2- the experimenter wants to select a nonstandard model rather than first or second order RSM models, because of some prior knowledge of the problem,

3- unusual sample size requirement, designs based on RSM require standard sample sizes,

4- where there is substantial prior knowledge available.

In such cases, a computer-aided optimal design can be implemented. In this work we make use of factorial and fractional 2^k and response surface designs so they will be briefly described in the next section.

2.2.1 Factorial Design

Factorial designs are extensively used in experiments involving numerous factors when the objective is to understand the factors' joint effect on a response. In a factorial design, all possible combinations of the factors levels are investigated. For example, if there are "a" levels of factor "A" and "b" levels of factor "B", the full experiment contains all "a × b" treatment combinations. The effect of a factor in a factorial design is defined as the amount of change in response when the factor is changed. Since this reflects the change in the primary factors of interest in the experiment; it is called a "main effect". In some experiments, the response is not simply a linear combination of the changes in the factors. When this occurs, there are said to be interactions (Montgomery and Runger 2006).

The most widely used design is the 2^k factorial, which studies k factors, each at only two levels. These levels can be either quantitative (e.g. pressure, temperature, time) or qualitative (e.g. two instruments, two different additives).

2.2.2 The 2^k Factorial Design

Since each factor is considered at high (+) and low (-) levels, a complete replicate of such a design requires 2^k observations and is called a 2^k factorial design. This design enables an experimenter to investigate "k" factors with a relatively small number of runs. Consequently, it is particularly useful in the early stages of an experimental investigation when there are usually many factors to be studied.

To design the experiments, the factor levels need to be established, and the sequence of experimental trials should be randomized (Montgomery 2004).

Since there are only two levels for each factor, the response is assumed linear over the range of the chosen factor levels. This is often adequate, particularly in the early stages of a study.

2.2.3 Fractional 2^k Factorial Design

The number of runs in a complete 2^k design increases exponentially as the number of factors increases, and therefore in many cases it is not feasible to perform a complete 2^k design. The full factorial experiment provides an experimenter with enough information to evaluate the whole set of main effects as well as all interaction effects. The main effects and lower-order interactions are usually the most significant terms (Mason, Gunst, and Hess 2003). In fact, one is usually capable of determining the main effects and the lower-order interactions by performing a fraction of the complete factorial design with little loss of information. Such a design is called a "fractional factorial" design (FFD). A 2^{k-p} fractional factorial design containing 2^{k-p} runs is called a $\frac{1}{2^p}$ fraction of the 2^k complete design, or more simply a 2^{k-p} fractional factorial design.

A potential concern regarding the fractional or full factorial design is the assumption of linearity in the factor effects. It is clear that the relationship is not necessarily perfectly linear. Some amount of non-linearity can be accommodated by adding interaction terms to a main effect or first-order model, resulting in:

$$y = \beta_0 + \sum_{j=1}^{k} \beta_j X_j \; + \sum_{i<j}^{k} \sum \beta_{ij} X_i X_j + \varepsilon \tag{2.1}$$

where ε denotes the noise or error observed in the response y and X_j is a coded variable. The relationship between the coded variable X_j and its actual variable x_j is defined by the following equation:

$$X_j = \frac{x_j - (x_{j,high} + x_{j,low})/2}{(x_{j,high} - x_{j,low})/2} \tag{2.2}$$

where $x_{j,low}$ and $x_{j,high}$ are the lowest and highest values of actual variable x_j.

The model is now capable of handling some curvature in the response function. This curvature, of course, results from the twisting of the plane induced by the interaction terms $\beta_{ij} X_i X_j$.

2.2.4 Response Surface Methodology

In many practical cases, the researcher deals with various input variables, and wants to optimize the response. Response surface methodology which is a combination of statistical and mathematical techniques is a very useful tool to model and analyze such problems (Box et al. 1978).

For example, suppose a process in which the response is a function of several input variables as shown below:

$$y = f(X_1, X_2, \ldots) + \varepsilon \tag{2.3}$$

The expected response can be given by:

$$E(y) = \hat{y} = f(X_1, X_2, \ldots) \tag{2.4}$$

where $\hat{y}$ is called a surface. The response surface can be graphically shown as a twisted plane (e.g. Equation 2.3). A quadratic model may be suitable if the response is moderately nonlinear.

$$y = \beta_0 + \sum_{i=1}^{I} \beta_k X_k + \sum_{i=1}^{I} \sum_{j>i}^{I} \beta_{ij} X_i X_j + \sum_{i=l}^{I} \beta_{ii} X_i^2 + \varepsilon \tag{2.5}$$

Almost all response surface problems use at least one of these model features. Certainly, a polynomial model is able to predict the response of a system for a fairly small region of interest with a good accuracy, but it cannot result in accurate predictions over the entire region of independent variables.

One potential approach to find the quadratic coefficients in a second-order model is to perform a 3^k factorial design; however this design is not very efficient in that the number of runs is very high and can be prohibitive.

The central composite design (CCD) is a very common and efficient design that is used to fit second-order quadratic models (Box and Wilson 1951). It is a sequential design since it consists of a 2^k factorial design (or fractional factorial design of resolution V), 2k axial runs and n_c centre runs. In CCD designs, two parameters should be specified: n_c (centre point number) and α, the distance of the axial runs from the centre point. Since CCD consist of points that are outside the studied design space, it is not feasible to perform it for chemical formulation optimization problems where at least one lower bound for the components is zero.

Another common response surface, Box-Behnken can be used in such applications (Box and Behnken 1960). Box-Behnken is a three-level design to fit second-order quadric models. It is sequential like CCD and consists of 2^k factorials with incomplete block designs. Table 2.1 shows a three-variable Box-Behnken design.

Table 2.1- A three-variable Box-Behnken design (Montgomery 2004)

Run	X_1	X_2	X_3
1	-1	-1	0
2	-1	1	0
3	1	-1	0
4	1	1	0
5	-1	0	-1
6	-1	0	1
7	1	0	-1
8	1	0	1
9	0	-1	-1
10	0	-1	1
11	0	1	-1
12	0	1	1
13	0	0	0
14	0	0	0
15	0	0	0

2.2.5 Optimal Designs

Optimal designs are experimental designs that are generated based on a particular optimality criterion and are generally 'optimal' only for a specified model. As a result, they may not satisfy desirable properties such as independence among the parameter estimates. In this work we will make use of a design methodology which selects a specified number of trials as a subset from a candidate set of trials. In this approach trials are chosen using a particular optimality criterion, normally *D*-optimality. However other design criteria could be used including *A*-, *E*- and *G*- optimality. They will be briefly described in the following sections and then their performance compared in Chapter 4.

2.2.4.1 *A*-Optimal Designs

Consider the model given by:

$$y = X\beta + \varepsilon \tag{2.6}$$

where y is an $n \times 1$, the design matrix X is $n \times [p = (k+1)]$, β is the $n \times 1$ vector of parameters to be estimated and ε is the noise vector.

The *A*-optimality criterion leads to a design matrix X which minimizes the trace of the $(X^TX)^{-1}$ matrix (dispersion matrix) (Chernoff 1953).

$$tr(X^{*T}X^{*})^{-1} = \min_{\varepsilon_n \equiv_n} tr(X^TX)^{-1} \tag{2.7}$$

where $\mathcal{E}_n \Xi_n$ represents the candidate set $\mathcal{E}_n$, chosen from $\mathcal{E}_N$.

In other words, this criterion minimizes sum of the variance of the parameter estimates (Atkinson and Donev 1992).

2.4.4.2 *D*-Optimal Designs

Many classical symmetrical designs such as 2^k factorials have desirable characteristics, one of which is called *D*-optimality. The *D*-optimality concept can also be applied to select a design when the classical symmetrical designs cannot be used, such as when the experiments chosen by a classical design are too large or when one wants to apply models that deviate from the usual first or second order ones (Aguiara et al. 1995).

D-optimality states that among all matrices that can be chosen from the matrix of candidate points ξ_N , the one that leads to a model matrix X that minimizes the determinant of the so-called dispersion matrix $(X^TX)^{-1}$ is optimal.

$$Max_{\xi_{n\equiv n}}\left\{Det(X^{*T}X^{*})\right\}=Min_{\xi_{n\equiv n}}\left\{Det(X^{*T}X^{*})^{-1}\right\} \quad (2.8)$$

Where $\xi_{n\equiv n}$ represents the group of all matrices ξ_n chosen from ξ_N.

Minimizing the determinant of the dispersion matrix in the above equation is equivalent to maximizing the determinant of the information matrix (X^TX).

2.2.4.3 Bayesian *D*-optimal Designs

In many practical cases, for example in our case study, some prior data are available. If conventional fractional factorial designs or *D*-optimal techniques are applied, one might not take advantage of the information hidden in the prior data to "optimally" design further tests. To address this issue, a Bayesian design can be contemplated, which can fully take advantage of the prior knowledge and lead to the optimal design.

It is shown, that the Bayesian D-optimal experiment is the one which minimizes $\det\left\{\left[U^{-1}+\frac{1}{\sigma^2}X^TX\right]^{-1}\right\}$, the determinant of the posterior covariance matrix, in which U is the $n\times n$ prior covariance matrix and X is a $n\times p$ regression matrix (Reilly 2006). Here n is the number of experimental trials and p is the number of parameters to be estimated. This optimization is equivalent to maximizing $G=\det\left\{U^{-1}+\frac{1}{\sigma^2}X^TX\right\}$, which may be written in the form $G=M\times\det\left(U^{-1}\right)$, where

$$M=\det\left\{I+\frac{1}{\sigma^2}XUX^T\right\} \quad (2.9)$$

and I is the $n\times n$ identity matrix (Mardia, Kent, and Bibby 1988). The quantity $\det(U^{-1})$ is a constant and the determinant in the definition of G is $p\times p$ while that in M is $n\times n$. Consequently it is advantageous to reach Bayesian D-optimality by maximizing M.

2.2.4.4 *E*-Optimal Designs

E-optimality maximizes the minimum eigenvalue of the (X^TX), information matrix (Evans 1982). In other words it minimizes the maximum variance for all potential normalized linear combination of parameter estimates (Schweitzer 1996). *E*-optimality selects experimental trials that will lead to maximum improvement in the parameter having the highest variance.

2.2.4.5 G-Optimal Designs

Another popular criterion is *G*-optimality, which seeks to minimize the maximum entry in the diagonal of the hat matrix $X^T(X^TX)^{-1}X$ (Kiefer 1961). This has the effect of minimizing the maximum variance of the predicted values corresponding to the rows of *X*.

2.3 Regression Analysis

Regression analysis is a statistical technique for investigating and modeling the relationship between variables (Montgomery, Peck, and Vining 2006). It is one of the two most widely used techniques in statistics (analysis of variance is the other) and is used in almost every field of application (Ryan 1997).

2.3.1 Multiple Linear Regression

A regression model that involves more than one manipulated variable is called a multiple regression model (Montgomery, Peck, and Vining 2006).

Suppose that the antimicrobial activity of a disinfectant (in log reduction) depends on raw material concentrations in the formulation. A multiple linear regression model that might describe the relationship is:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_m x_m + \varepsilon \tag{2.10}$$

where y denotes the response and x_1 to x_m are the input variables.

The term linear is used because Equation 2.10 is a linear function of the unknown parameters

$(\beta_0, \beta_1, \ldots, \beta_m)$.

Multiple linear regression models are often used as empirical models or approximating functions. That is, the true functional relationship between y and x_1 to x_m is unknown, but over certain ranges of the input variables, the linear regression model is an adequate approximation to the true unknown functions.

Models which are more complex in structure than Equation 2.10 might also be analyzed by multiple linear regression techniques. For example a model might have terms such as $x_j^2, x_j^3, \sqrt{x_j}, x_i x_j, \frac{1}{x_j}$, etc.

The method of least squares can be used to estimate the regression coefficients in the Equation 2.10. Suppose that $n > m$ observations are available, y_i denotes the ith observed response and x_{ij} represents the ith observation or level of input variable x_j. It is also assumed that the error term ε in the model has a

normal distribution with $E(\varepsilon)=0, Var(\varepsilon)=\sigma^2$ and that the errors are not correlated. It can be shown that the least-squares estimator of β is:

$$\hat{\beta}=(X^T X)^{-1} X^T y \tag{2.11}$$

where:

$$X=\begin{bmatrix} 1 & x_{11} & x_{12} & \dots & x_{1m} \\ 1 & x_{21} & x_{22} & & x_{2m} \\ & \vdots & & \ddots & \vdots \\ 1 & x_{n1} & x_{n2} & \dots & x_{nm} \end{bmatrix}$$

and

$$y=\begin{bmatrix} y_1 \\ y_2 \\ \vdots \\ y_n \end{bmatrix},\ \hat{\beta}=\begin{bmatrix} \hat{\beta}_0 \\ \hat{\beta}_1 \\ \vdots \\ \hat{\beta}_m \end{bmatrix} \text{ and } \varepsilon=\begin{bmatrix} \varepsilon_1 \\ \varepsilon_2 \\ \vdots \\ \varepsilon_n \end{bmatrix}$$

provided that the inverse of $(X^T X)$ exists. $(X^T X)$ matrix can always be inverted if the regressors are not linearly dependent, i.e. if any column of the *X* matrix is a linear combination of its other columns.

Since $\hat{\beta}$ is an estimator of β, therefore the estimate of the vector of y can be calculated as:

$$\hat{y}=X\hat{\beta} \tag{2.12}$$

where:

$$\hat{y}=\begin{bmatrix} \hat{y}_1 \\ \hat{y}_2 \\ \vdots \\ \hat{y}_n \end{bmatrix}$$

In order to make inferences about the parameter estimates and model predictions one also assumes the errors to be normally distributed.

2.3.2.1 Box-Cox Transformation

In some instances, the assumption that the errors are independently, identically and normally distributed are not met. An appropriate transformation of a data set can often yield a data set that does follow these assumptions. This increases the applicability and usefulness of statistical techniques based on the normality assumption.

The Box-Cox transformation is a particularly useful family of transformations. It is defined as:

$$y^{\lambda} = \begin{cases} \frac{y^{\lambda}-1}{\lambda \dot{y}^{\lambda-1}}, & \lambda \neq 0 \\ \dot{y} \ln y, & \lambda = 0 \end{cases} \tag{2.13}$$

where $\dot{y} = Ln^{-1}\left[\frac{1}{n}\sum_{i=1}^{n} \ln y_i\right]$ is the geometric mean of the observations, and fit the model:

$$y^{(\lambda)} = X\beta + \varepsilon \tag{2.14}$$

by least squares or maximum likelihood. The term $\dot{y}^{\lambda-1}$ is related to the Jacobian of the transformation which converts the response variable *y* into $y^{(\lambda)}$. In fact, it is a scale factor that makes sure that residual sums of squares for models with different values of λ are comparable (Montgomery, Peck, and Vining 2006).

The Box-Cox normality plot is a plot of the correlation coefficients for different values of the λ parameter. The value of λ corresponding to the maximum correlation on the plot is then the optimal choice for λ.

2.3.2.2 Prediction Error Sum of Square (PRESS) Statistic

The PRESS residual is defined as $e_{(i)} = y_i - \hat{y}_{(i)}$ where $\hat{y}_{(i)}$ is the predicted value of the i^{th} observed response based on a model fit to the remaining $n-1$ sample points. PRESS residuals are potentially useful in identifying observations where the model does not fit the data well or observations for which the model is likely to provide poor future predictions. It is suggested that the prediction error sum of squares (or PRESS statistic), defined as the sum of the squared PRESS residuals, can be used as a measure of model quality (Allen 1974; Allen 1971). The PRESS statistic is

$$PRESS = \sum_{i=1}^{n} \left[y_i - \hat{y}_{(i)} \right]^2 = \sum_{i=1}^{n} \left(\frac{e_i}{1 - h_{ii}} \right)^2 \tag{2.15}$$

PRESS is usually considered as a measure of how accurate a model will predict new data. One of the most important uses of the PRESS statistic is in comparing regression models. Generally, a model with a small value of PRESS is preferable to one where PRESS is large.

The PRESS statistic can be used to compute an R^2 –like statistic for prediction say

$$R^2_{\text{Prediction}} = 1 - \frac{PRESS}{SS_T} \tag{2.16}$$

where SS_T is the total corrected sum of squares. This statistic gives some indication of the regression capability of the regression model. $R^2_{\text{Prediction}}$, also known as predicted R^2, shows the percentage of the variability in the original data explained by the least-squares fit.

2.3.2 Artificial Neural Networks (ANN)

Neural networks can be used to mine patterns and distinguish trends that are too complicated to be recognized by either humans or other computer techniques. A trained neural network is like an "expert" in the domain of information in which it has been trained and can be used to give predictions for new situations of interest and answer "what if" questions.

A typical ANN is formed by an interconnection of neurons. A neuron has many inputs and a single output. Figure 2.2 shows the anatomy of a typical neuron (Baughman and Liu 1995).

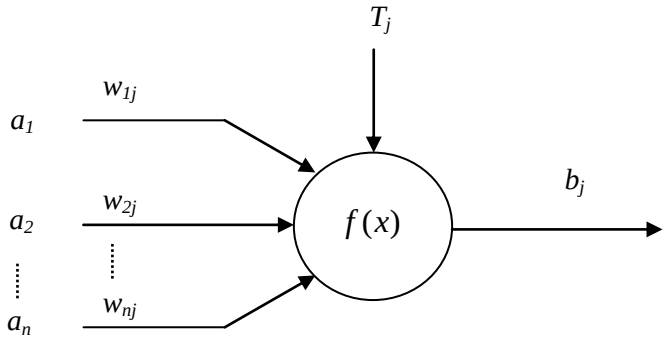

Figure 2.2- The structure of j^{th} neuron that transfers the input a_i to the j^{th} output b_j through a weight factor w_{ij} and a transfer function $f(x_j)$. T_j is the bias for the neuron j.

Each input to a neuron has a "weight" assigned to it. The weights can also be interpreted as the strength of connection between two neurons (Takayama, Fujikawa, and Nagai 1999). Inputs to each neuron are multiplied by their corresponding weights, and the product is then summed together and applied to a transfer function to create the output. T_j, bias is a factor that controls the activation of a neuron.

$$\text{Total activation} = x_j = \sum_i w_{ij} a_i - T_j \tag{2.17}$$

The output of the neuron can be shown as:

$$b_i = f(x_i) = f(\sum_i w_{ij} a_i - T_j) \tag{2.18}$$

where f is the transfer function. Sigmoid, hyperbolic tangent and the radial basis functions are the most widely used functions. Sigmoid and hyperbolic tangent are very useful for prediction purposes, and radial basis functions work well for classification networks (Baughman and Liu 1995).

The neural network can be viewed as a "black box" in which the input data is sent to each neuron in the input layer, then the input data is processed through interconnection between neurons (commonly called topology), and the output is obtained the nodes in the output layer. The neighbour neurons are interconnected with links corresponding to synapses. In summary, the input layer receives information from an outside source, and transfers the information to the hidden layer(s) to process. The hidden layer processes the information which is received from the input layer, and sends it to the output layer, and finally the output layer provides the results to an external source (Figure 2.3) (Baughman and Liu 1995).

ANN performance is controlled by normalized input and output dataset, the number of hidden layers, weights of the interconnections, transfer functions, learning rates and momentum coefficients. According to universal approximation theory, a network with a single hidden layer and a sufficiently large number of nodes can model any input versus output data (Tampe, Kulkarni, and Deshpande 1996). In most cases, input factors are not of the same order of magnitude. In order to prevent any false influence of those factors with higher magnitude, it is important to normalize them as shown by Equation 2.2.

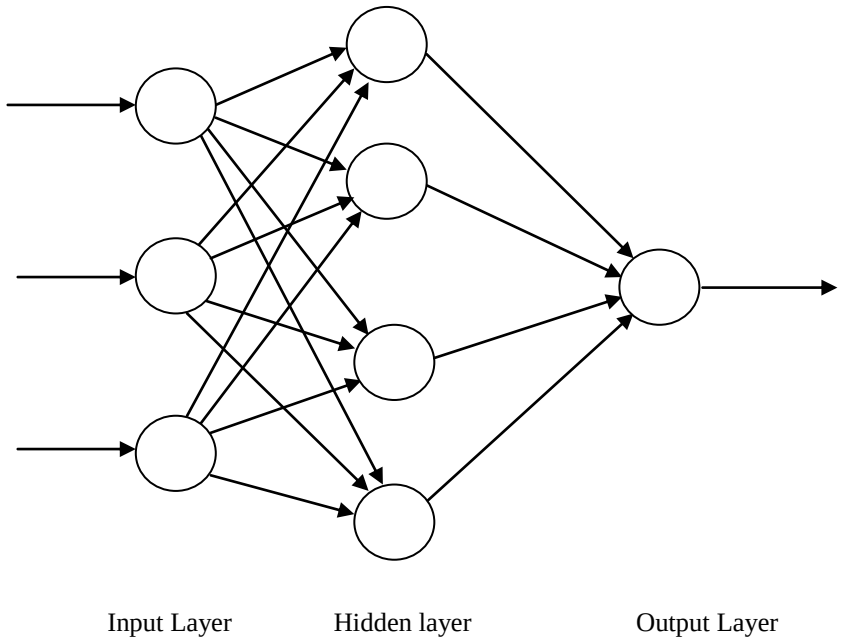

Figure 2.3- Architecture of a hidden layer feed-forward neural network

Before starting the ANN training, initial values of the interconnection weights should be randomly assigned using either a Uniform or a Gaussian distribution. These initially assigned weights are then modified using a learning rate which controls the rate of change of the weights in each sequence. The weights are modified in such a way that maps input-output correctly. Various algorithms can be used to modify the weights in order to generate the desirable outputs from the input variables. Back-propagation (BP) is one such algorithm. It is an iterative search method which modifies the weights from the output layer back to the input layer in each run until no further adjustment in MSE value is found (Alshihri, Azmy, and El-Bisy 2009).

BP requires only feed-forward interlayer connections, with each layer feeding successively into the next layer. The BP algorithm is based on the error-correction learning which is an optimization process using the steepest gradient descent principle where the change in weight is directed towards the negative of the error gradient, i.e.

$$\Delta w_n = \alpha \Delta w_{n-1} - \eta \frac{\partial E}{\partial w} \tag{2.19}$$

where w is the weight between any two neurons; Δw_n and Δw_{n-1} are the changes in a weight at n and $n-1$ iterations; α the momentum coefficient; η is the learning rate, and $\frac{\partial E}{\partial w}$ is the gradient descent correction factor.

The momentum coefficient is used to potentially skip through local minima and prevents settling into it. Outputs are calculated by transforming the input variables using a non-linear transfer function (Elkamel 1998).

The development of an ANN model usually consists of two steps. The first step is the training or learning phase, where the network is subjected to a training set of input/output patterns. The weight factors between the neurons are adjusted until the specified input factors result in the desired output set. The second step is a testing stage, where the performance of the network is tested on patterns that have not been 'seen' by the network during the learning phase.

2.4 Optimization

In optimization, one seeks to minimize or maximize a single-valued objective function f of n real variables $x_1, x_2..., x_n$, possibly subject to a finite number of constraints, which are written as inequalities or equations (Avriel 2003). In general, it can be written as:

$$\text{Min } f(x) \tag{2.20}$$

Subject to

$$g_i(x) \geq 0, \qquad i = 1,\ldots,m \tag{2.21}$$

$$h_i(x) = 0, \qquad j = 1,\ldots,p \tag{2.22}$$

where x denotes the column vector whose components are $x_1,..., x_n$. In other words, the objective is to find a vector x^* that satisfies (2.21), and (2.22) and such that $f(x)$ has a minimal, that is, optimal value.

Optimization algorithms are iterative in nature (Nocedal and Wright 2006). Initial values of the variables are guessed and sequentially improved until they reach the optimal solution. Algorithms are distinguished by different strategies which are used calculate the updates from one iteration to the next. Most optimization techniques use a function evaluation and perhaps the first and second derivatives of the function to be optimized. Some algorithms use local information from the current point while others build up information which was gathered at the previous iterations. Regardless of these specifics, all good algorithms should be robust, efficient and accurate. They should be robust so that they perform well on a

broad range of problems in their category for all logical choices or initial variables. They should also be efficient enough that they do not need too much computer storage or time, and accurate enough to identify a solution with accuracy, without being too sensitive to errors in the data or to the calculation rounding errors that occur when the algorithm is implemented on a computer. These targets may conflict. For instance, in cases where the problem is large, a fast convergent technique for nonlinear optimization may need too much computer storage. Moreover, a robust method may also be very slow (Nocedal and Wright 2006).

In this work, we used the Levenberg-Marquardt method. Levenberg-Marquardt is by its nature an improved Gauss-Newton method which incorporates steepest descent into the iterative update scheme. When the initial guess is far from the optimum point, it acts like the Steepest-Descent method and gets close to the optimum region as it gets close to the optimum it performs more like the Gauss-Newton algorithm.

The steepest-descent method is the most straightforward method in optimization, and can be shown as follows (Fang 2004):

$$x^{j+1} = x^{j} - \eta^{j} \nabla F(x^{j}) \tag{2.23}$$

The steepest-descent method converges very slowly since it only uses the first derivative of the objective function. However, it is fairly robust since it eventually converges to the optimal point even if the initial guess is far from it.

In Newton's method, the second derivative term of the objective function (from Taylor's expansion) at the current point is used to calculate the next iteration.

$$x^{j+1} = x^{j} - \left[H_f(x^{j})\right]^{-1} \nabla F(x^{j}) \tag{2.24}$$

where $\left[H_f(x^{j})\right]$ in Equation (2.24) is the second order derivative of the objective function *F(x)* at x^j (Hessian matrix). The advantage of Newton's method is that it converges faster than Steepest-Descent. In return, it is much more sensitive as to how far the initial guess from the optimal point is, and therefore it is not as robust as Steepest-Descent approach. Moreover, it has to compute the second derivative (the Hessian matrix) at each iteration. This can be problematic especially in cases where the analytical form of the objective function is not available.

If the objective function can be defined in the form of least-square optimization, i.e.:

$$\text{Min } F(x) = \|f(x)\|_2^2 = f^T(x) f(x) \tag{2.25}$$

Gauss-Newton method is a more popular technique. In this method, the second derivative matrix (the Hessian matrix) is replaced by the multiplication of the Jacobian matrix (first order derivatives) as shown in Equation 2.26:

$$x^{j+1} = x^j - \left[J_f(x^j)^T J_f(x^j)\right]^{-1} J_f(x^j)^T f(x) \tag{2.26}$$

Gauss-Newton method is advantageous over Newton's method in that it only needs to calculate the Jacobian matrix which significantly reduces the computation load. Its drawback however is that its convergence rate is slower since it uses a first order instead of a second order in Newton's method. It is also not as robust as the steepest-descent method.

Levenberg-Marquardt (Marquardt 1963; Levenberg 1944) on the other hand uses a combination of Gauss-Newton and Steepest-Descent. A new factor η is introduced here to switch between the two methods.

$$\left[J_f(x^j)^T J_f(x^j) + \eta I\right](\Delta x)^k = -J_f(x^j)^T f(x) \tag{2.27}$$

η is a factor that switches between the two methods (Gauss-Newton and Steepest-Descent). When η is close to zero, Levenberg-Marquardt method approaches to Gauss-Newton and when η has a very high value, the Levenberg-Marquardt method is reduced to the Steepest-Descent method. At the starting point, η is set to a very large value, and the method takes advantage of the robustness of the Steepest-Descent method. In each iteration, if $F(x^j + \Delta x^j) < F(x^{j-1} + \Delta x^{j-1})$ η is reduced by to speed up the convergence; and if $F(x^j + \Delta x^j) > F(x^{j-1} + \Delta x^{j-1})$ η value is increased to expand the searching area (Fang 2004).

2.5 Review of Disinfectant Chemicals

The word "disinfect" was first used in the 17th century, when some "effluvia" or mystifying emanations were believed to cause sickness and could be inactivated by some chemicals such as burning sulfur (Dychdala 2001). Today, a disinfectant is known as a chemical agent that kills, not just inhibits microorganisms in the vegetative stage.

2.5.1 Disinfecting Active Agents

2.5.1.1 Chlorine and Chlorine Compounds

It was not until the first half of the 19th century that the disinfecting and deodorizing properties of chloride of lime were first recognized. Then, purifying and disinfecting properties of hypochlorites in water treatment was established. Extensive use of chlorine as an antimicrobial began during World War I, when a 0.45% sodium hypochlorite solution was introduced for disinfection (Dychdala 2001; Dakin 1915).

Hypochlorites are the oldest and most extensively used of the active chlorine-based compounds used for disinfection. They are proven and powerful germicides, non-poisonous to human at use dilutions, free of poisonous residuals, easy to handle, and most economical to handle (Lesser 1949). Sodium hypochlorite, NaClO, is commonly known as bleach and has many applications such as drinking water treatment, swimming pool water treatment, sewage and waste water treatment, spas and hot tubs, surface disinfection in health care facilities, sanitizing of food processing plants, etc (Fukuzaki 2006). It is mass produced by the chlorination of soda ash (sodium carbonate).

Other chlorine compounds used as disinfectants are chlorine dioxide, inorganic chloramines, organic chloramines, chloramine-T, etc.

2.5.1.2 Iodine and Iodine Compounds

Iodine was first used as a wound disinfectant about 140 years ago and was found to be effective in inactivating bacteria, viruses, fungi, and spores (Gershenfield 1956; Anon 1965; Sykes 1965; Kelsey and Maurer 1972). Iodine can be dissolved in water or alcohol for usage. Since it is not easily soluble in cold water, potassium iodide can be used instead to deliver the iodine for disinfection purposes. Iodine solutions are mostly used as teat-dip sanitizers to decontaminate cow udders. Iodine disadvantages such as its unpleasant smell and sparingly water solubility can be overcome by mixing it with a carrier to form iodophors. The carriers can be neutral polymers such as polyvinylpyrrolidone, polyether glycols, polyvinyl alcohols, etc. These carriers improve iodine properties by increasing its solubility, providing a controlled-release reservoir of iodine, and decrease the equilibrium concentration of free molecular iodine (Gottardi 2001).

2.5.1.3 Hydrogen Peroxide

Hydrogen peroxide can be considered as nature's own antimicrobial agent. It is naturally present in milk and honey, which it helps to preserve from spoilage and is a normal resident of tissues as a result of cellular metabolism. Further, it protects us from infection (Block 2001), and in the mouth works as a strong oxidizing agent either alone or in combination with thiocyanate and peroxidase in the saliva

(Thomas and Aune 1978). Hydrogen peroxide has been in use as a disinfectant since the 19th century. Hydrogen peroxide solutions at 0.5-3% are used as hard surface disinfectants and at 6-7% as medical instrument sterilizers.

2.5.1.4 Peracetic Acid

Peracetic acid (PAA) is a more effective disinfectant than hydrogen peroxide (Eggensberger 1979; Baldry 1983) and has no toxic residuals (Schroder 1984; Block 1986). It decomposes to water, oxygen and acetic acid and therefore has no environmental disadvantage. Like hydrogen peroxide, peracetic acid is effective in inactivating bacteria, viruses, fungi, and spores. Unlike chlorine, it is not easily inactivated in the presence of organic matter. Peracetic is an effective sporicide even at low temperatures. It is more potent in acidic than alkaline media; however higher concentrations in alkaline solution still have germicidal properties. Peracetic acid finds extensive use in food processing plants as a sanitizer. It is also used as a medical instrument sterilizer.

2.5.1.5 Alcohols

Many alcohols have disinfecting properties against bacteria, viruses, and fungi, but they are not effective against spores (Ali et al. 2001). In general, those alcohols with antimicrobial activity are very fast acting. Ethanol and isopropyl alcohol are amongst the most popular ones in disinfection application. Isopropyl alcohol is more favourable than ethanol because it has a slightly higher flash point. Alcohols have disadvantages such as flammability, strong alcoholic odor, toxicity, being recognized as volatile organic compounds (VOC) which are not environmentally favourable. Alcohols have an extensive use in hand disinfection due to their fast disinfection activity.

2.5.1.6 Quaternary Ammonium Compounds (QAC)

These compounds are a class of cationic surfactants with strong bactericidal, but weak detergent prosperities. QACs are effective against bacteria, viruses (mostly enveloped viruses), and fungi (Lawrence 1950; Klein and Deforest 1963). They are not effective against non-enveloped viruses, mycobacteria, and spores even at high concentrations (Merianos 2001). QACs are widely used in many disinfection applications including hard surface disinfection, and food hygiene in hospitals. Benzalkonium chloride, is a typical QAC used in many disinfectant formulations.

2.5.1.7 Aldehydes

Formaldehyde, glutaraldehyde (glut) and ortho-phthaldehyde (OPA) are of the most importance of this class. Formaldehyde is used as liquid or vapour as a disinfectant, however it is known to pose a carcinogenic risk to humans (Russell, Hugo, and Ayliffe 1999). Glutaraldehyde is widely used as a medical instrument disinfectant especially for heat sensitive medical devices. Since glut has high toxicity

(Ballantyne and Berman 1984; Maibach and Prystowsky 1977; Gannon et al. 1995; Corrado, Osman, and Davies 1986), its application as a disinfectant has been decreasing in recent years. OPA has favourable materials compatibility like glut, but has less toxicity concerns, and has therefore replaced glut at least partially in many disinfection applications.

2.5.1.8 Other Disinfecting Active Agents

There are many other antimicrobial active ingredients that have some applications in disinfection industry including but not limited to acetic acid, propionic acid, lactic acid, benzoic acid, salicylic acid, propamidine, dibromopropamidine, chlorohexidine, derivatives of imidazoles and hexamine, essential oils, ethylene oxide, ozone, etc.

2.5.2 Factors Affecting Disinfectant Activity

The effective use of disinfectants is an important factor in the prevention of hospital-associated infections (Rutala and Weber 2001). Disinfectants are assessed for their antimicrobial activity using standard test methods. These methods require a specified disinfectant concentration, ratio of disinfectant to the microbial culture, test temperature, soil load, contact time, etc. Each of these factors can play an important role in the activity of a disinfectant. Here is a brief description of some of these effects:

2.5.2.1 Temperature

It is known that the activity of a disinfectant is often enhanced if the temperature is increased. To measure the effect of the temperature change on antimicrobial activity, the following equation can be used:

$$\theta^{T_2-T_1} = \frac{t_1}{t_2} \tag{2.28}$$

where t_1 and t_2 are the respective times to completely inactivate organisms at T_1 and T_2 (Russell, Hugo and Ayliffe 1999), θ is the temperature coefficient, which is dimensionless, and refers to the effect of temperature per each 1˚C increase and is usually between 1 and 1.5 (Bean 1967). It is more useful to compare the disinfection activity in every 10˚C (θ^{10}) which can be shown as (Denyer, Hodges, and Gorman 2004):

$$\theta^{10} = \frac{Time\ to\ kill\ at\ T}{Time\ to\ kill\ at\ (T+10)} \tag{2.29}$$

While θ^{10} values of chemical and enzyme-catalyzed reactions vary between 2 and 3, values for disinfection vary more widely, for example 45 for ethanol, 4 for phenol, and 1.5 for formaldehyde (Russell, Hugo, and Ayliffe 1999; Denyer, Hodges, and Gorman 2004). This means that a 2˚C decrease in

temperature will result in 2.14, 1.32 and 1.08 times slowdown in disinfection activities of ethanol, phenol and formaldehyde, respectively. Therefore, while applying the disinfectants, the operating temperature must be taken into account. Furthermore, chemistries with higher θ^{10} must be tested in the lower bound of the temperature range due to their significant activity reduction by temperature drop.

2.5.2.2 Environmental pH

pH can influence disinfection activity in different ways including:

a) Changes may happen in the molecule. Active ingredients such as sorbic acid, phenol, and benzoic acid are mainly effective in non-ionized form. pH increases results in their dissociation and therefore inactivity. Chlorine is more lethal at acidic pH, while glutaraldehyde is more active at the alkaline pH.
b) Changes may happen in the cell surface. The number of negatively charged groups on the bacterial cell surface increases with pH, which results in a higher activity of positively charged molecules such as QACs at alkaline pH (Hugo 1991).

2.5.2.3 Organic Mater

Organic matter exists in different forms: blood, earth, food residues, serum, faecal material, and milkstone (dried residue of milk). Organic matter is believed to react with the disinfectant active ingredient, thus leaving a decreased concentration of the biocidal active ingredient to attach microorganisms. This reduced activity is particularly observed with highly reactive antimicrobial active ingredients such as chlorine-based disinfectants (Grossgebauer 1970; Russell 1991). Active ingredients with lower reactivity such as iodine and iodophors are interfered by the organic matter lesser than chlorine (Sykes 1965).

2.5.2.4 Type of Organism

Different microorganisms show varying resistance to disinfectants. The reasons are not completely known, but research continues on finding these reasons (Russell 1995).

2.5.2.4.1 Bacteria

Gram positive bacteria are more sensitive to disinfectants than gram negatives (Baird-Parker and Holbrook 1971). The main reason for this increased sensitivity is due to the relative composition of the cell wall (Russell and Chopra 1996). Gram positive bacterial cells do not have an outer membrane and the wall is made of multiple layers of peptidoglycan which builds a thick and rigid layer (Shuler and Kargi 2007). In gram negative bacteria, an outer membrane is supported only by a thin layer of peptidoglycan.

2.5.2.4.2 Mycobacteria

Resistance to mycobateria is associated with the composition of the cell wall, which is composed of unusually high lipid content. This results in a hydrophobic wall which makes them less susceptible to disinfectants. The sensitivity of mycobateria to disinfectants is between that of vegetative bacteria and bacterial spores (Spaulding, Cundy, and Turner 1977; Favero and Bond 1991).

2.5.2.4.2 Bacterial Spores

Bacterial spores are significantly more resistant to disinfectants than vegetative bacteria. In some cases, they are up to 100,000 times more resistant than vegetative bacteria (Philips 1952). They are the most resistant microorganisms. Many disinfectants such as QACS, alcohols, phenolics, bisbiguanides have little or no sporicidal activity. Bacterial spores contain an outer protective spore coat, an intermediate cortex and an inner protoplast. Experimental data suggests that the spore coat is associated with resistance to many disinfectants by preventing penetration of the disinfectant inside the spore (Russell, Hugo, and Ayliffe 1999).

2.5.2.4.3 Viruses

Non-enveloped viruses (e.g., polio, parvo, coxsackie) are less susceptible to disinfectants than enveloped viruses (e.g., Influenza, HIV, Herpes simplex) (Klein and Deforest 1983). Enveloped viruses are quite sensitive to most disinfectants with a lipophilic character such as phenol derivatives and QACS. Non-enveloped viruses however are not effectively inactivated by QACS or alcohols and stronger antimicrobial agents such as oxidizing agents (e.g., chlorine or hydrogen peroxide).

2.5.3 Disinfection Kinetics

Microbial inactivation depends on many factors such as disinfectant concentration, contact time, pH, temperature, etc. Different models have been proposed to describe the kill-rate behaviour. The models are either based on assumed inactivation mechanisms or empirical models (Finch et al. 2001).

The major assumptions which are used in such models include uniform dispersion of organisms and disinfectant molecules, sufficient mixing to prevent rate limiting of liquid diffusion, constant temperature and pH during the disinfection time (Gyürèk and Finch 1998).

2.5.3.1 Chick's Model

The first disinfection kinetic model was used by Chick (Chick 1908) as a first order kinetic model:

$$r_d = \frac{dN}{dt} = -kN \tag{2.30}$$

where r_d is the inactivation rate, N is the concentration of organisms, and k is the reaction rate constant. In batch systems, N decreases exponentially, assuming that k is constant. If the disinfectant concentration is

assumed constant (which happens when the ratio of disinfectant to microorganisms is very high), Equation 2.30 can be integrated and resulted in:

$$\ln\frac{N}{N_0} = -kt \tag{2.31}$$

2.5.3.2 Chick-Watson Model

In this hypothetical model (Watson 1908), the reaction rate is assumed to be related to the disinfectant concentration,:

$$k = k'C^n \tag{2.32}$$

where *n* is the coefficient of dilution and *k'* is assumed to be independent of disinfectant and microorganism concentration.

$$r_d = \frac{dN}{dt} = -k'C^nN \tag{2.33}$$

If *C*, *n*, and *k* are constant, Equation 2.33 may be integrated to:

$$\ln\frac{N}{N_0} = -k'C^nt \tag{2.34}$$

2.5.3.3 Hom Model

The Hom model is used to describe complex inactivation kinetics (Hom 1972; Haas and Joffe 1994) as shown below:

$$r_d = \frac{dN}{dt} = -mNC^nt^{m-1} \tag{2.35}$$

If *m*=1, Equation 2.35 becomes identical to the Chick-Watson model (Equation 2.33).

2.5.3.4 Other Models

There are other models such as Rational Model (Power Law), which is the generalized power law formulation (Majumdar, Ceckler, and Sproul 1973), Hom Power Law, which includes subsets of both the Hom and Rational models (Anotai 1996), series Event Model, which describes disinfection as series of events happening in a discrete stepwise fashion (Gyürèk and Finch 1998; Severin, Suidan, and Engelbrecht 1983).

2.5.4 Disinfectant Mechanism of Action

Antimicrobial agents in general intereact with cytoplasmic membrane, cytoplasm and cell wall (Denyer and Stewart 1998). Chemical properties of antimicrobial agents, chemical composition and morphology of the cell, and extra-cellular materials are the main factors that influence the entry of the disinfectant inside the cell. The major mechanisms of interactions at the target sites are sorption and partitioning.

These actions can be affected by factors such as disinfectant concentration, type of microorganism, external conditions (e.g., humidity, temperature, pH). The target sites are usually positioned at the cell wall or inside the cytoplasmic area of the cell (Kaymak 2003).

Nonetheless, the cytoplasmic membrane contains a richer environment, where there are balanced interactions between enzymatic/structural proteins and phospholipids.

The maintenance of intracellular homeostasis and vectorial transport/metabolism and the control of impermeability are ensured by the cytoplasmic membrane. These sensitive functions and the great expanse for interaction result in more susceptibility of the cytoplasmic membrane to disinfectant attack (Denyer and Stewart 1998). Besides cell membrane, the integrity of DNA and maintenance of the folding proteins are essential for cell survival (Booth 2002). Disinfectant chemistry also influences the extent or rate of diffusion of disinfectant through the cell wall and injure to the cell (Denyer and Stewart 1998; Stewart and Olson 1996).

2.5.5 Disinfectant Toxicity

The toxicity of a chemical in general is its ability to cause damage once inside the body. Chemicals' major entry methods into the body are inhalation, absorption through the skin and ingestion. Vapours, gases, mists, and aerosols can be inhaled and consequently affect the skin, eyes and mucous membranes. Ingestion is uncommon even though probable due to poor personal hygiene, subconscious hand-to-mouth contact, or accidents. Chemicals can directly affect skin, even when undamaged. Permeability of the skin also results in the entry of the chemicals into the body (Carson and Mumford 2002).

2.5.5.1 Types of Toxic Chemicals

2.5.5.1.1 Irritant Chemicals

Irritant chemicals result in a local inflammatory effect which is one of the body's defence mechanisms (Carson and Mumford 2002). Inflammation is the reaction of a tissue to damage which is not enough to destroy the tissue and is characterized by compression of the tiny vessels in the affected area, expansion of the blood vessels, amplified permeability of the vessel walls, and passage of the white blood cells to the attacking toxic substance. The goal is to increase water and protein level in the area to reduce the effect of the harmful substance. Therefore, the production of new cells will be accelerated and damaged surface cells discarded.

The respiratory system is the major organ that is targeted by mist, gas, or vapour. Skin and eyes are also susceptible to contact or exposure. Irritation is considered a reversible response. In extreme conditions irritant chemicals become corrosive. Corrosive chemicals attack living tissues and in severe conditions

can cause burns with degradation of biochemicals, destroy cells and perhaps predispose to secondary bacterial attack. Corrosion and burn are considered as irreversible due to the destruction of cells at the contact site (Carson and Mumford 2002).

2.5.5.1.2 Sensitizers

Sensitizers normally do not induce any symptoms, even though cellular changes can be caused and the person's immune system affected, however some substances may act as both primary irritants and sensitizers. Repeated exposures to the same or similar substances may result in allergic reactions, which indicate that the person is sensitized. Normally, the degree of exposure and extent of the reaction cannot be correlated by a mathematical model. Sensitization to a substance is typically very specific and usually occurs within ten days and remains for life in most cases.

2.5.5.1.3 Carcinogens

Cancer is a disorder in control of the growth of cells in body. The disease may be genetic or influenced by life style or exposure to certain chemicals, termed carcinogens.

2.5.5.1.4 Other Types of Toxic Chemicals

Asphyxiants, anaesthetics and narcotics, systematic poisons, and respiratory fibrogens are considered as other types of toxic chemicals (Carson and Mumford 2002).

2.5.5.1.4 Hazard Assessment

Indicators of toxicity hazards include LD_{50}, LC_{50}, risk phrases, plus an extensive range of in vitro and in vivo techniques for assessment of skin and eye irritation, skin sensitization, mutagenicity, acute and chronic dermal and inhalation toxicity, reproductive toxicology, carcinogenicity, etc.

LD_{50} is the dose that produces lethality in 50 percent of animals (Williams, James and Roberts 2000) and therefore is an indicator of acute toxicity, which is usually measured by ingestion using rats or mice. LD_{50} can also be determined by other routes such as skin absorption in rabbits. The values are influenced by sex, age, species, etc.

The LC_{50} is the lethal concentration of a substance in air or water which causes the death of 50% of the sample population. This is most suitable as an indicator of the acute toxicity of chemicals in air breathed or in water, for aquatic organisms.

R-phrases (short for Risk Phrases) are defined in Annex III of European Union Directive 67/548/EEC: Nature of special risks attributed to dangerous substances and preparations. The list was consolidated and

republished in Directive 2001/59/EEC. These risk phrases are used internationally not just in Europe as another method for hazard assessment.

2.5.5.2 Assessing Toxicity of Disinfectants

Disinfectants ideally should be non-irritating to skin, eyes and the respiratory tract and also not cause burns. In vivo test methods are available to evaluate these properties. However these methods are costly, slow to yield results and, most importantly, require the use of animals. There are alternative methods available to quantify the toxicity level of different formulations using individual ingredients. One such method is used by the Dangerous Substances Directive (Directive 67/548/EEC; Bender and Philipp Eisenbarth 2007). Directive 67/548/EEC describes in details the classification system which covers almost any chemical or mixtures of chemicals ranging from highly toxic to benign. In our disinfectant case study, none of the ingredients falls into the classifications of very toxic and toxic, based on this method. Therefore, the concern for formulations based on our mixtures of ingredients would be to fall into the category of 'harmful, severe eye damage, eye irritant, skin irritant, and respiratory irritant'. This method explains how to predict if a formulation falls into any of these categories. In Chapter 8, this method has been used to put some constraints on the optimization of the problem and ensure that the product will not fall into any of these categories. This method has been briefly described in the following section for those categories that have been used in this work.

2.5.5.2.1 Harmful (Xn)

The formulation is categorized as harmful if it contains one or more substances categorized as very toxic, toxic or harmful and that cause such effects in individual concentrations greater than or equal to:

(a) either the concentration indicated in Annex I to Directive 67/548 for the concerned substances, or

(b) the concentration indicated at point 1 (Part B of this Annex) where the substances are not given in Annex I of Directive 67/548/EEC or mentioned in it with no maximum limit.

Formulations which contain more than one substance categorized as very toxic, toxic or harmful in lower individual concentrations than the limits indicated above if:

$$\sum_{j=1}^{k}\left(\frac{P_{T+}}{L_{x_n/T^+}}+\frac{P_T}{L_{Xn/T}}+\frac{P_{Xn}}{L_{Xn}}\right)\geq 1 \tag{2.36}$$

where P_{T+} is the weight or volume percentage of each very toxic substance in the formulation, P_T is the weight or volume percentage of each toxic substance in the formulation, P_{Xn} is the weight or volume percentage of each harmful substance in the formulation, L_{Xn} is the respective harmful limit accredited for each harmful, very toxic, toxic substance, stated as weight or volume percentage.

2.5.5.2.2 Severe Eye Damage, X_i (R41)

The formulation will be categorized as irritant if expected to cause serious eye damage and given the symbol X_i. Formulations which contain one or more substances categorized as irritant for which phrase R41 applies (risk of severe damage to eyes) individual concentrations equal to or greater than:

(a) either the concentration indicated in Annex I for the concerned substances, or

(b) the concentration indicated at point 4 (Part B of this Annex) where the substances are not given in Annex I of Directive 67/548/EEC or mentioned in it without a maximum limit;

Formulations which contain more than one of the substances categorized as irritant to which phrase R41 applies, or categorized as corrosive to which phrase R35 or R34 apply, in lower individual concentrations than the limits indicated under above sections if:

$$\sum_{j=1}^{k}\left(\frac{P_{C,R35}}{L_{Xi,R41/R35}}+\frac{P_{C,R34}}{L_{Xi,R41/R34}}+\frac{P_{Xi,R41}}{L_{Xi,R41}}\right)\geq 1 \qquad (2.37)$$

where $P_{C,R35}$ is the weight or volume percentage of each corrosive substance to which phrase R35 applies in the mixture, $P_{C,R34}$ is the weight or volume percentage of each corrosive substance to which phrase R34 applies in the mixture, $P_{Xi,R41}$ is the weight or volume percentage of each irritant substance to which phrase R41 applies in the mixture, $L_{Xi,R41}$ is the respective irritant limit R41 indicated for each corrosive substance to which phrase R35 or R34 apply or irritant substance to which phrase R41 applies, expressed as weight or volume percentage.

2.5.5.2.3 Eye Irritant, X_i (R36)

The formulation is considered irritant to eyes and given the symbol X_i, the warning of danger "irritant" and the risk phrase is R36;

Formulations which contain substances categorized as corrosive to which phrase R35 or R34 apply or as irritant to which phrase R41 or R36 apply in concentrations greater than or equal to:

(a) either the concentration mentioned in Annex I of Directive 67/548/EEC for the concerned substances , or

(b) the concentration indicated at point 4 in Part B of this Annex where the substances are not given in Annex I of Directive 67/548/EEC or mentioned in it without concentration limit;

Formulations which contain more than one substance categorized as irritant to which phrase R41 or R36 apply or as corrosive to which phrase R35 or R34 apply, in lower individual concentrations than the limits indicated above if:

$$\sum_{j=1}^{k}\left(\frac{P_{C,R35}}{L_{Xi,R36/R35}}+\frac{P_{C,R34}}{L_{Xi,R36/R34}}+\frac{P_{Xi,R41}}{L_{Xi,R36/R41}}+\frac{P_{Xi,R36}}{L_{Xi,R36}}\right)\geq 1 \qquad (2.38)$$

where $P_{C,R35}$ is the weight or volume percentage of each corrosive substance to which phrase R35 applies in the formulation, $P_{C,R34}$ is the weight or volume percentage of each corrosive substance to which phrase R34 applies in the formulation, $P_{Xi,R41}$ is the weight or volume percentage of each irritant substance to which phrase R41 applies in the formulation, $P_{Xi,R36}$ is the weight or volume percentage of each irritant substance to which phrase R36 applies in the formulation, and $L_{Xi,R36}$ is the respective irritant limit R36 indicated for each corrosive substance to which phrase R35 or R34 apply or irritant substance to which phrase R41, or R36 apply, stated as weight or volume percentage.

2.5.5.2.4 Skin Irritant, X_i (R38)

Formulations which contain one or more substances categorized as irritant to skin and to which phrase R38 applies or as corrosive to which phrase R35 or R34 apply, in individual concentrations greater than or equal to:

(a) either the concentration indicated in Annex I of the Directive for the concerned substances, or

(b) the concentration indicated at point 4 (Part B of this Annex) where the substances are not given in Annex I of the Directive or mentioned in it without maximum limits;

Formulations which contain more than one of the substances categorized as irritant to which phrase R38 applies, or as corrosive to which phrase R35 or R34 apply, in lower individual concentrations than the limits mentioned above if:

$$\sum_{j=1}^{k}\left(\frac{P_{C,R35}}{L_{Xi,R38/R35}}+\frac{P_{C,R34}}{L_{Xi,R38/R34}}+\frac{P_{Xi,R38}}{L_{Xi,R3}}\right)\geq 1 \qquad (2.39)$$

where $P_{C,R35}$ is the weight or volume percentage of each corrosive substance to which phrase R35 applies in the formulation, $P_{C,R34}$ is the weight or volume percentage of each corrosive substance to which phrase R34 applies in the formulation, $P_{Xi,R38}$ is the weight or volume percentage of each irritant substance to which phrase R38 applies in the formulation, and $L_{Xi,R38}$ is the respective irritant limit R38 for each corrosive substance to which phrase R35 or R34 apply or irritant substance to which phrase R38 applies, expressed as weight or volume percentage.

2.5.5.2.5 Respiratory Irritant, X_i (R37)

Formulations which contain one or more substances with phrase R37 in individual concentrations greater than or equal to:

(a) either the concentration indicated in Annex I of the Directive for the concerned substances, or

(b) the concentration indicated at point 4 (Part B of this Annex) where the substances are not given in Annex 1 of the Directive or mentioned in it without maximum limits.

Formulations which contain more than one substance categorized as irritant to which phrase R37 applies in lower individual concentrations than the limits indicated under above sections (a) or (b) if:

$$\sum_{j=1}^{k}\left(\frac{P_{C,R37}}{L_{Xi,R37}}\right)\geq 1 \qquad (2.40)$$

where $P_{Xi,R37}$ is the weight or volume percentage of each irritant substance to which phrase R37 applies in the formulation, $L_{Xi,R37}$ is the irritant limit R37 given for each irritant substance which phrase R37 applies, expressed as weight or volume percentage.

2.6 Microbiology of the Organism Studied

Staphylococcus aureus, abbreviated to *S. aureus* or *Staph aureus* in the medical literature, is the most common cause of staphylococcal or staph infections. However, when abbreviated to *S. aureus,* it should not be confused with the similarly named (and also medically relevant) species of the genus *Streptococcus*.

S. aureus is a spherical bacterium, frequently a part of the human body's microbiota often residing on the skin and/or in the nose. Almost 20% of humans are long-term carriers of *S. aureus* (Kluytmans, Van Belkum, and Verbrugh 1997). *S. aureus* can cause various diseases from insignificant skin infections, such as pimples and boils to more serious conditions such as pneumonia and meningitis. Parts of the body affected include the skin, soft tissues, the respiratory system, bones, joints, and endovascular organs. It is still one of the most frequent causes of nosocomial infections, which often causes post-operative wound infections.

S. aureus was discovered by the surgeon Sir Alexander Ogston in 1880 in Scotland in purulence from surgical infections (Ogston 1984). Each year some 500,000 patients in American hospitals suffer from staphylococcal infection (Bowersox 1999).

S. aureus is a Gram-positive coccus, facultatively anaerobic, which is similar to grape-like clusters under a microscope and has large, golden-yellow round colonies, frequently with hemolysis, when it is grown on blood agar plates (Ryan and Ray 2004). The golden look is the historical Latin source of its name "*aureus:* which means "golden". Figure 2.4 shows the colorized scanning electron micrograph (SEM) of *Methicillin-Resistant Staphylococcus aureus* usually referred to MRSA.

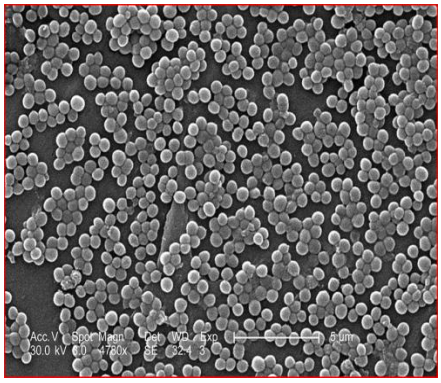

Figure 2.4- Colorized clumps of MRSA, Magnified 4780 (http://en.wikipedia.org/wiki/File:MRSA7820.jpg)

2.6.1 Role in Disease

S. aureus may live on human skin without causing any harm; it can also be found in the nose regularly (approximately a third of the population) (Abigail and Dixie 2002) and less commonly in the throat. The presence of *S. aureus* in such cases does not necessarily mean infection and consequently does not always need treatment (in fact, treatment may be useless and lead to re-colonisation). *S. aureus* can also be found in domestic animals including horses, chicken, dogs, and cats. In chicken, it can cause bumble foot. *S. aureus* can also host phages, which results in an increase in its virulence.

When normal barriers are broken through, *S. aureus* can infect other tissues such as skin or mucosal lining which causes boils. In infants *S. aureus* infection can lead to a severe disease called Staphylococcal scalded skin syndrome (Curran and Al-Salihi 1980).

Some of *S. aureus* strains are able to produce a carotenoid pigment (staphyloxanthin) which acts as a virulence factor. Staphyloxanthin has an antioxidant action which protects the organism against reactive oxygen used by the host immune system. It is believed that staphyloxantin is responsible for *S. aureus*' typical golden colour (Clauditz et al. 2006) and therefore a normal strain of *S. aureus* is more likely to survive exposure to an oxidizing chemical such as hydrogen peroxide than a mutant strain which is modified to lack the yellow coloration.

2.6.2 Diagnosis

Biochemical or enzyme-based tests can be used to identify *Staph*. At first, a Gram stain test is applied to show Gram-positive cocci in colonies. Then, the organism is cultured in mannitol salt agar (a selective medium with 7–9% NaCl). *S. aureus* grows in this medium and produces yellow colonies. In addition, catalase, coagulase, DNAse, lipase, and phosphatase tests can be performed to differentiate the species.

2.6.3 Infection Control

S. aureus can be spread through contact with purulence or drainage from an infected wound, contact with objects such as clothing, towels, or other personal items used by an infected person. Lately, numerous cases of the pathogen in hospitals across North America have been reported. *S. aureus* easy transportation in medical facilities is mostly due to inadequate healthcare worker hygiene. *S. aureus* is an extremely resilient bacterium, and can survive on a piece of polyester for about three months (Neely and Maley 2000), polyester being the main material used in hospital privacy curtains. *S. aureus* can transfer itself on the hands of healthcare workers who, for example, get it from an apparently healthy patient who carries a "benign" or commensal strain and then give it to the next person. When entered into the bloodstream, it can result in a variety of problems such as endocarditis, meningitis, and, if it is extensive, sepsis - toxins infecting the whole body. Hand-washing and surface disinfection are therefore very effective in preventing the spread of *S. aureus.*

2.7 Hydrogen Peroxide Decomposition

For product development purposes, it is not feasible to age the solution at room temperature since it may take years to develop a product using this approach. Instead, an accelerated stability method is implemented so that the stability test is carried out at a higher temperature for a shorter period of time. The relationship between the temperature and stability test duration can be calculated as follows:

The decomposition rate for H_2O_2 can be written by an expression of the form:

$$\frac{-d(H_2O_2)}{dt} = K.g(C_{H_2O_2}, C_{H_2O}, C_{O_2}, \ldots) \tag{2.41}$$

where K = Kinetic constant, g= a function of the concentration of the relevant species affecting the decomposition rate, t = time, K is given by the *Arrhenius* expression (Levenspiel 1999):

$$K = K_0 e^{-E/RT} \tag{2.42}$$

where K_0 = constant, E = Activation Energy of H_2O_2, R = Gas Law Constant, T = Temperature (K)

Integrating equation (2.41) yields:

$$-\int_{[H_2O_2]_i}^{[H_2O_2]_f} \frac{dC_{H_2O_2}}{g(C_{H_2O_2}, C_{H_2O}, C_{O_2}, \ldots)} = K\left[t_f - t_i\right] \tag{2.43}$$

where $[H_2O_2]_f$ = Concentration of H_2O_2 at time $t = t_f$, $[H_2O_2]_i$= Concentration of H_2O_2 at time, $t = t_i$

Therefore, the change in concentration of H_2O_2 at a fixed temperature T, and over a time period of t_f is given by rewriting equation (2.43) as:

$$\int_{[H_2O_2]_i}^{[H_2O_2]_f} \frac{dC_{H_2O_2}}{g(C_{H_2O_2}, C_{H_2O}, C_{O_2}, \ldots)} = K_0 e^{-E/RT} t_f \tag{2.44}$$

$$\Delta C_{H_2O_2} = [H_2O_2]_f - [H_2O_2]_i \tag{2.45}$$

In fact, we need to measure the equivalent H_2O_2 loss for a system at temperature T_1 over the length of time t_1 in reference to a system at temperature T_2 over the length of time t_2. Thus, from equation (2.45):

$$\Delta C_{H_2O_2\ system1} = \Delta C_{H_2O_2\ system2} \tag{2.46}$$

or

$$K_0 e^{-E/RT_1}.t_1 = K_0 e^{-E/RT_2}.t_2 \tag{2.47}$$

$$e^{\frac{-E(1/T_2 - 1/T_2)}{R}} = \frac{t_2}{t_1} \tag{2.48}$$

$$\frac{E}{RT_1T_2}.[T_1 - T_2] = \ln\left(\frac{t_2}{t_1}\right) \tag{2.49}$$

$$\frac{0.4342E[T_1 - T_2]}{RT_1T_2} = \log_{10}\left(\frac{t_2}{t_1}\right) \tag{2.50}$$

Equation 2.50 can be approximated to (Schumb, Satterfield, and Wentworth 1955):

$$A[T_1 - T_2] = \log_{10}\left(\frac{t_2}{t_1}\right) \tag{2.51}$$

For our temperature range: A = 0.0342, T_1 = test temperature (°C), T_2 = 20°C, t_1 = test duration (days)

t_2 = shelf-life at 20°C.

In our stability tests, we age the samples at 70˚C for a one week period in a water bath. A volume of about 230 mL of each sample is placed in a glass precleaned 250 mL Erlenmeyer flask and is placed in the water bath. For pre-cleaning, Erlenmeyer flasks are washed with detergent and then filled with a 5%

Etidronic acid solution for 5 minutes to eliminate any possible metal contamination, rinsed with deionized water and dried in the oven at 50˚C for an hour. During the stability test, Erlenmeyer flasks are closed with caps to avoid evaporation of the test solution. The hydrogen peroxide in the solution is measured before and after the stability test using an iodometric titration method as described by Blanco (Blanco, Coello, and Sanchez 2006), and the peroxide loss is calculated as:

$$Peroxide\,loss\,\% = \frac{\left[H_2O_2\ \%\right]_i - \left[H_2O_2\ \%\right]_f}{\left[H_2O_2\ \%\right]_i} \times 100 \tag{2.52}$$

2.8 Corrosion

Corrosion is the chemical or electrochemical reaction between the environment and a material, typically a metal, which degrades the properties or mass of the material over time. It is the normal behaviour of the compositional elements of a material to go back to their most thermodynamically stable condition (Schweitzer 1996). Metal corrosion can be defined as the undesirable deterioration of a metal or alloy (Uhlig 1948). In the metal corrosion process, the metal turns into a combined condition from its elementary state (Evans 1982), which means that it forms basic metallic compounds such as oxides or sulphides which are typically considered as ores. Corrosive environment can consist of liquids which may or may not be electrolytic conductors. Only inert atmospheres and vacuums can be considered free of corrosion for most metallic materials. Metal corrosion can take place in many forms as follows:

2.8.1 Uniform Corrosion

Uniform or general corrosion, which is considered to be the simplest form of corrosion, is defined as a uniform rate of metal degradation over the contacted surface. Uniform corrosion is one of the most predictable and easily measured types of corrosion.

Since the material degradation is even in uniform corrosion, corrosion rates are usually shown in terms of material depth loss per unit of time such as “mils per year”.

2.8.2 Galvanic Corrosion

This type of corrosion is a result of an electrochemical reaction where a metal is corroded preferentially when it is in electrical contact with another metal, and both metals are exposed to an electrolyte.

2.8.3 Erosion Corrosion

Erosion corrosion is the increasing degradation caused by an electrochemical corrosion process and mechanical actions from the relative movement between the degrading surface and the electrolyte (Roberge 1999).

2.8.4 Pitting Corrosion

Pitting corrosion is a localized form of degradation by which holes or cavities are made in the material. Pitting corrosion is more dangerous than uniform corrosion since it is not easy to identify, predict or even prevent. It usually happens when a small area in the material comes under rapid attach while the adjacent areas remain almost untouched (Roberge 1999).

2.8.5 Corrosion Effect of Disinfectant Formulations

The corrosivity of cleaning or disinfecting products can be described as a uniform corrosion. In the case of hydrogen peroxide solutions, the corrosion is mainly due to dissolved oxygen which is the natural decomposition product of hydrogen peroxide. Oxygen has known corrosive properties toward ferrous metals, with well documented pH, temperature, pressure and salinity effects. For dilute solutions of H_2O_2 (< 1%), corrosion rates of < 0.5 mpy have been reported (Perry and Green 1997). In complex systems, where there is a combination of hydrogen peroxide and other ingredients such as acids, the corrosion will depend on the concentration of the present ingredients, pH, temperature, and possibly the interaction of the different ingredients.

Since disinfectants are used to decontaminate environmental surfaces and medical devices, they have to be compatible with such items or at least cause only minor corrosion. In general, materials that come into contact with disinfectants are either plastics or metals. Plastics are typically compatible with disinfectants. However, some types of plastics are not compatible with alcohols and deteriorate gradually. Metals are either coated or uncoated. Coated metals are mostly compatible since the coatings are mainly chemical resistant. Among uncoated metals, stainless steel is compatible with most chemicals, but some reactive substances such as chlorine corrode it. Copper and brass are compatible with most disinfectants including but not limited to alcohols and QACs, but are not often compatible with products based on hydrogen peroxide and acids.

CHAPTER 3

MATERIALS AND METHODS

3.1 Microorganism

Staphylococcus aureus (ATCC 6538) was used as the surrogate for the bactericidal activity tests in the following efficacy tests.

3.1.1 Stock Culture Preparation

Stock suspension of *Staphylococcus aureus was* prepared by culturing it in tryptic soy broth (TSB) for 24 hours at 37˚C.

3.2 Evaluation of Antimicrobial Activity of Disinfectants

Antimicrobial activity of disinfectants is tested using in-vitro or in-vivo methods. In-vitro tests consist of suspension, capacity, and carrier tests. In suspension tests, the microorganisms are suspended in a pool of disinfectant. In carrier tests, the microorganisms are dried on the carriers and then exposed to the disinfectant. Capacity tests are performed by incremental addition of a bacterial suspension to the disinfectant pool, and sub-culturing of the pool for survivors to see if the capacity of the disinfectant solution has been exhausted by consecutive additions of bacteria. In-vivo testing involves controlled laboratory tests simulating practical conditions.

Suspension or carrier tests can be either quantitative or qualitative. The qualitative method only concerns the presence or absence of growth in the subculture, while the quantitative method involves counting the number of surviving microorganisms and compare them to the initial number and calculate the reduction percentage or log reduction. In this work, a quantitative carrier test method (ASTM E2111) was used for all bactericidal tests.

3.2.1 Quantitative Carrier Test Methods, Tier 1

The quantitative carrier test (QCT) is a method of evaluating the antimicrobial activity of disinfectants against different microorganisms. It has been designed to: 1) permit the determination of the exact number of colony forming units (CFU) placed on each carrier and the CFU remaining after the drying of the inoculum, 2) avoid wash-off of any of the test organism, 3) allow complete recovery of the inoculum from the carrier surface, 4) arrest the test product's activity by dilution immediately at the end of the contact time, 5) in the case of bactericidal tests, capture of all the bacterial cells of the test organism on a membrane filter before and after exposure to the test product, 6) removal of any residual germicidal activity by a thorough rinsing of the membrane filter, 7) allow a ratio of 1:100 between the volume of the

test microbial inoculum and the volume of the product being evaluated, 8) incorporation of glass inserts to eliminate any false-positive results due to the generation of micro-aerosols in the carriers and 9) give a precise determination of log10 reduction in CFU of the test organism after exposure to the product under test (Springthorpe and Sattar 2003). Therefore this test method eliminates the deficiencies associated with the AOAC Use-Dilution Test (AOAC 1998) while meeting the Canadian General Standards Board's requirements for germicide test methodology (CGSB 1997).

3.2.1.1 Carriers

The inside bottom surface of glass vials (Galaxy Co., Newfield, New Jersey) was used as the carrier.

3.2.1.2 Soil Load

For inoculation of the carriers, the test organism was first suspended in bovine serum (Gibco BRL Life Technologies Cat. No. 16000-044, NY, USA), at a final concentration of 5% to simulate the dirty condition in the environment.

3.2.1.3 Neutralizer, Microbial Diluent and Filter Rinse

Letheen Broth (with 0.1% sodium thiosulfate pentahydrate) was used as the neutralizer and to rinse the membrane filters and the filter holder unit. Normal saline was used to make dilutions of the bacterial suspensions and as the final rinse of the carrier vials and the filter holder unit to aid in rinsing off the froth created by the Letheen broth.

3.2.1.4 Controls

Control carriers were used in the same manner as test carriers except that normal saline was applied to the dried inoculum instead of the test product.

3.2.1.5 Neutralization Verification

One part of the use-dilution of the product was mixed with 14 parts of the neutralizer. The test organism was added to the neutralized solution to give an estimated 20-100 CFU. The neutralizer alone was used as the control solution. At the end of a contact time of 3 minutes at 20°C, the mixture was passed through a membrane filter to capture the bacteria. The filters were placed on the appropriate recovery medium. The plates were incubated and the colonies counted.

3.2.1.6 The Method for Testing Bactericidal Activity

The inside bottom surface of glass vials was used as the carrier. For inoculation of the carriers, all test organisms were first suspended in bovine serum at a final concentration of 5%. Letheen Broth (with 0.1% sodium thiosulfate pentahydrate) was used as the neutralizer and to rinse the membrane filters and the filter holder unit. Normal saline was used to make dilutions of the bacterial suspensions and as the final

rinse of the carrier vials and the filter holder unit to aid in rinsing off the froth created by the Letheen broth.

The test involved drying the bacterial suspension (*staphylococcus aureus*) on a hard surface carrier and covering the dried inoculum with the disinfectant for the specified contact time (3 min) at room temperature. At the end of the contact time, an eluent/rinse was used to recover the reaction mixture from the carrier and the eluate was passed through a membrane filter (0.22μm pore diameter) to capture the test organism. The filters were then placed on plates of suitable recovery agar medium (Tryptic soy agar) and incubated to allow viable organisms to form visible colonies. The numbers of colony forming units (CFU) were recorded and the level of inactivation of the test organism was calculated.

The general equipment and procedure for testing are given in Figure 3.1 and 3.2, respectively.

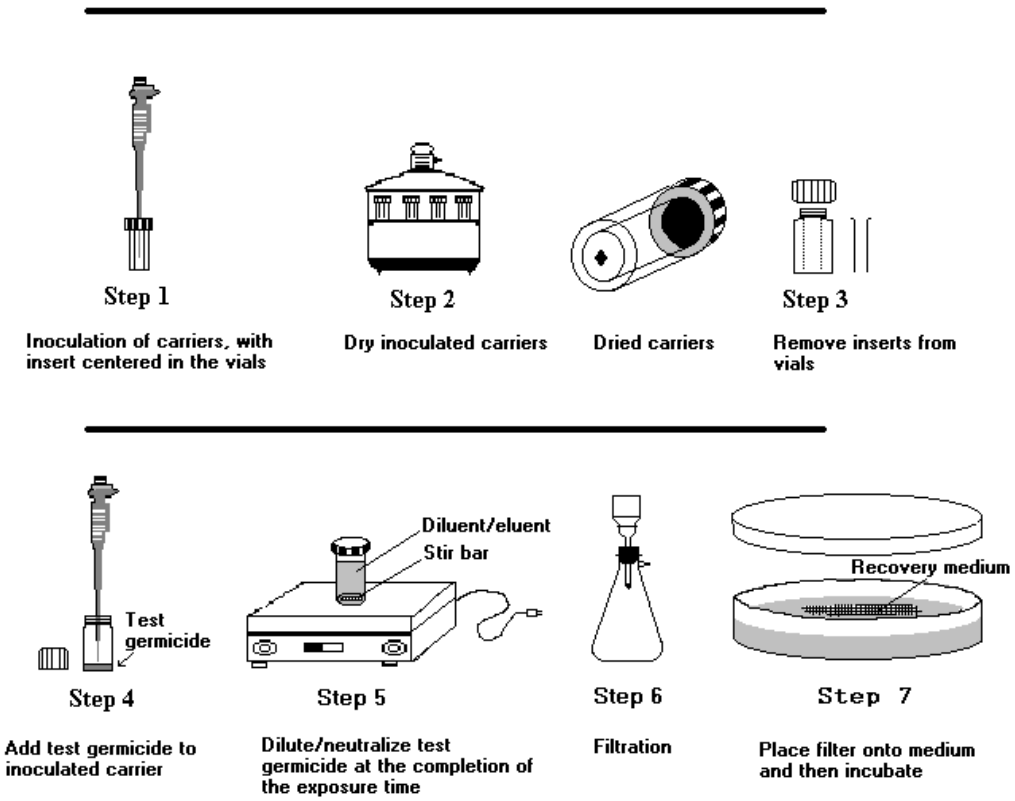

Figure 3.1- General Steps for the Quantitative Carrier Test (QCT) (Springthorpe and Sattar 2003)

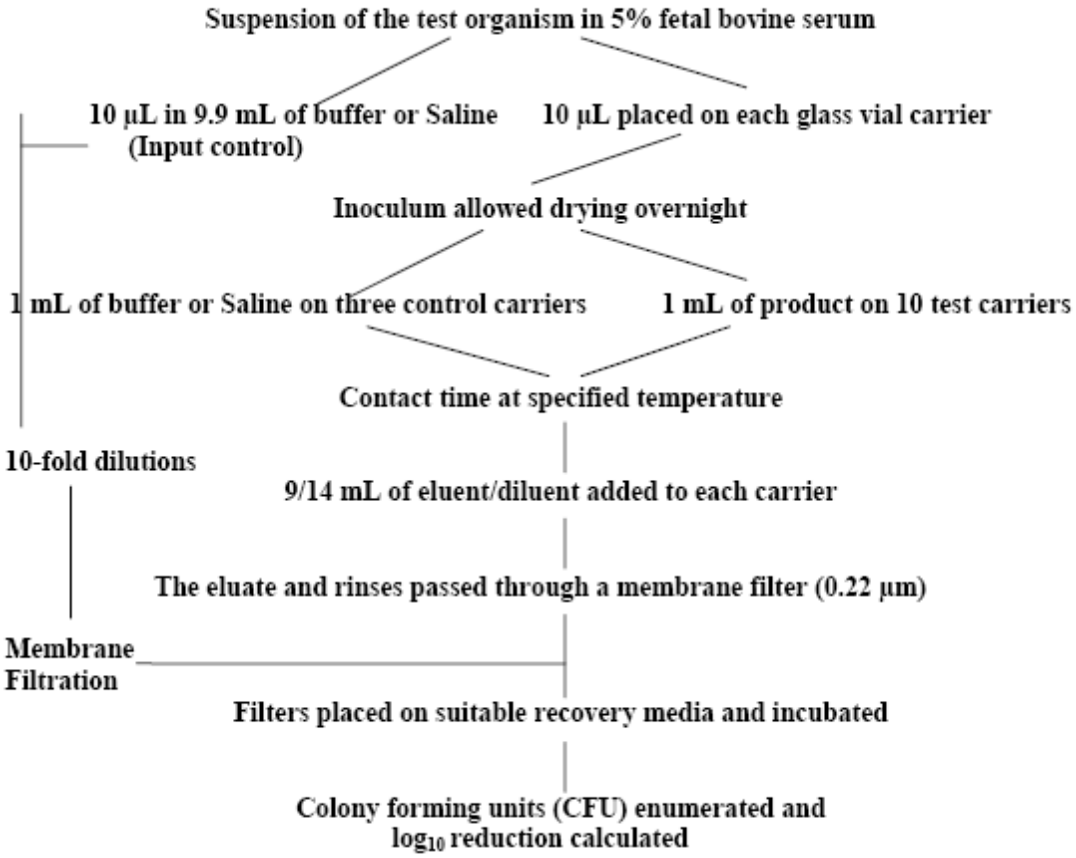

Figure 3.2- The basic quantitative carrier method for testing the bactericidal activity of liquid chemical germicides (Springthorpe and Sattar 2003)

3.2.1.7 Experimental error sources in antimicrobial testing

The experimental error in antimicrobial testing may come from different sources as follows:

1) Experimenter error: since such methods are very laborious, technician error in different parts of the test procedure may take place.

2) Variability in the resistance of microorganisms to test solution: this may be due to using different batches of complex media (due to the variability in the media composition). Also, the microorganism itself may go through some mutation over several sub-culturing steps.

3) Temperature variability: temperature is a very important factor in antimicrobial activity, since the activity of a solution is exponentially increased as temperature increase over time. That said, even small temperature variations (even 2 to 3˚C) from one test to another can result in some variation in the results. Depending on the chemistry, this variation can be moderate to significant.

4) Initial and final titre readings variability: in QCT 1 test, 3 control carriers are used to average the initial titre for the test microorganism. Also 3-5 replicates are used for the test solution, and therefore the results shown would show some error.

3.3 Hydrogen Peroxide Measurement Method

Hydrogen peroxide concentration was determined in this study by an iodometric titration (Degussa; Solvey). The principle of the method was that H_2O_2 oxidizes iodide to iodine in acidic media and in the presence of molybdate catalyst. The resulting iodine was then titrated with thiosulfate solution combined with a starch indicator.

$$H_2O_2 + 2\ KI + H_2SO_4 \longrightarrow I_2 + K_2SO_4 + 2\ H_2O \qquad (3.1)$$

$$I_2 + 2\ Na_2S_2O_3 \longrightarrow Na_2S_4O_6 + 2\ NaI \qquad (3.2)$$

3.3.1 Interferences

The method is widely applicable because organics do not interfere. The pH must be sufficiently acidic: if not, the proper color will not develop.

3.3.2 Equipment

1. Digital burette; readability 0.01 mL (Note: digital burette is more accurate and is the preferred instrument, but a glass burette with readability of 0.1mL is acceptable.
2. Analytical balance; readability 0.0001g
3. Class A volumetric pipettes
4. 500 and 250 mL erlenmeyer flasks
5. Magnetic stir bar
6. Magnetic stirrer
7. Graduated cylinder

All glassware used should be thoroughly cleaned and rinsed with deionized water.

3.3.3 Water

Low conductivity water <12 Micromhos/cm or deionized water

3.3.4 Reagents

1 Potassium Iodide(KI), Crystals

2. 10% (w/w) Potassium Iodide solution

2.1 Weigh 10.0 ± 0.1g Potassium Iodide and add deionized water to a final weight of 100.0 ± 0.1g.

2.2 Mix thoroughly.

2.3 Prepare this reagent fresh on each test day.

3. Ammonium Molybdate, Tetrahydrate

4. 5% (w/w) Ammonium Molybdate solution

4.1.1 Weigh 5.0 ± 0.1g Ammonium Molybdate and add deionized water to a final weight of 100.0 ± 0.1g.

4.1.2 Mix thoroughly.

5. Potassium Iodate (KIO_3), 0.1000N

6. Sodium Thiosulfate, 0.1000N

7. Starch Indicator Solution, 1% W/V

8. Hydrochloric Acid, 1.000N

9. Sulfuric Acid, (1+4) V/V

10. Hydrogen Peroxide, 3% W/V, Stabilized

3.3.5 Titration Method

1. Sodium Thiosulfate Standardization

1.1 Fill the burette with 0.1000 N Sodium Thiosulfate solution ($Na_2S_2O_3$).

1.2 Volumetrically pipet a 25-mL aliquot of 0.1000 N potassium iodate (KIO_3) primary standard solution into a clean 250-mL Erlenmeyer flask and dilute to 50 mL with deionized water.

1.3 Add 0.5 ± 0.05g of Potassium Iodide (KI) crystals and swirl to dissolve.

1.4 Add 7.5 ± 0.1mL of 1.000 N Hydrochloric Acid (HCl).

1.5 Titrate immediately with 0.1000 N Sodium Thiosulfate solution ($Na_2S_2O_3$) until the solution becomes pale yellow.

1.6 Add 1 ± 0.1mL of starch indicator and complete the titration until the solution becomes colourless.

1.7 Calculate the exact normality of the Sodium Thiosulfate ($Na_2S_2O_3$) solution as follows:

Normality = $(25 \times 0.1) \div$ (mL of sodium thiosulfate)

2. Sample Analysis: Test all samples and controls according to the following steps.

2.1 Weigh about 5 g of the sample solution into a clean 500-mL Erlenmeyer flask. Record sample weight to the nearest 0.0001g.

2.2 Add 400 ± 0.1g of deionized water.

2.3 While stirring with a magnetic stir bar add 10 ± 0.1mL of 1:4 Sulfuric Acid solution.

2.4 Volumetrically pipet 25 mL of 10% (w/w) Potassium Iodide solution into the flask.

2.5 Add 6 drops of 5% (w/w) ammonium molybdate solution.

2.6 Titrate immediately with Sodium Thiosulfate until the solution reaches a pale yellow colour.

2.7 Add 1 mL of Starch Indicator and titrate until solution is colourless.

2.8 Calculate the peroxide content as follows:

$$\%H_2O_2 = \frac{\text{ml thiosulfate} \times \text{thiosulfate normality} \times 0.017 \times 100}{\text{weight of sample}} \quad (3.3)$$

3.4 Hydrogen Peroxide Stability Test Method

Based on the *Arrhenius* equation, it was shown in Section 2.7 that hydrogen peroxide stability is dependent on temperature. To accelerate the stability test of peroxide solutions, instead of monitoring the peroxide stability at room temperature for a period of months or a year, it is possible to perform the stability test at raised temperatures in much shorter times. Figure 3.3 shows the hydrogen peroxide loss at different time and temperatures. This graph has been obtained by using Equation 2.51.

As can be seen in this Figure, a one week test at 70˚C is equivalent to one year at room temperature. This rationale has been previously used and the actual room temperature results were found to be close to those of accelerated tests (Omidbakhsh 2006). Therefore, the accelerated test method at one week, 70˚C was used in this study.

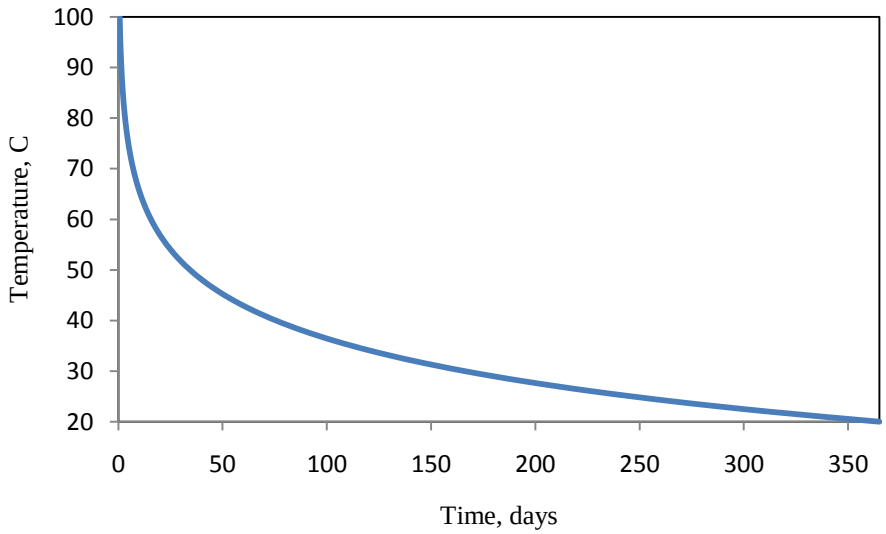

Figure 3.3- The accelerated stability test duration versus test temperature

3.5 Corrosion Coupon Test Method

The metal panels were sanded, using a #50 sand paper and a #120 for final touch. The panels were then washed with bleach-free detergent and a soft brush. The panels were rinsed with isopropanol (IPA). A

galvanized wire was attached through the hole in the panels. The wire was then suspended on a horizontal hanger for the panels to be air-dried for about two hours. The dried panels were then weighed on an analytical balance to the nearest 0.1 mg. Caution was taken while handling the panels so as to eliminate contact with any soiling substance, such as the technician's hands, etc. This can be accomplished by holding the panels at the sides with just two fingers.

The panels were then suspended on a plastic hanger, using a plastic string, inside a 1 L canister. The panels were suspended in such a way that they do not touch each other. This can be done by suspending up to three panels parallel to each other.

The panels were then completely immersed in 500 ml of the solution and covered using a plastic lid (see Figure 3.4). Since panels were not marked with any identification, the 1-L containers should be marked with numbers to identify the panels accurately (ASTM G3-72; ASTM G1-03).

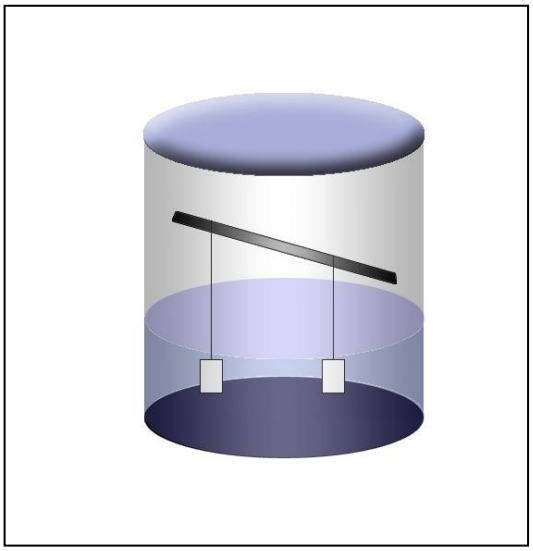

Figure 3.4- Metal coupons hanging in plastic container

The containers were then placed in the water bath, which is set at 20°C. A small map of the setup of the containers in the water bath can be useful in order to identify the panels once they are taken out of the water bath. The water bath was then covered to prevent water evaporation. After the specified period of time which can vary from a few hours to a few days, the containers are taken out of the water bath. The panels were scrubbed with tap water and a soft brush, until the surface is clean, rinsed with IPA solution dried in a 50°C for 2 hours, cooled and then weighed to the nearest 0.1mg. Assuming that localized or internal corrosion is not present, the corrosion rate expressed as mils penetration per year (mpy) can be calculated by the equation:

$$Mpy = \frac{Weight\ loss \times 5.45 \times 10^{6}}{area \times time \times metal\ density} \tag{3.5}$$

where weight loss is in grams, area was in cm^2 of metal surface exposed, time was hours exposed and metal density was in g/cm^3.

CHAPTER 4

COMPARING PREDICTION POWER AND MODEL DEPENDENCIES OF *A*-, *D*-, *E*-, AND *G*-OPTIMAL DESIGNS

4.1 Introduction

As already explained in Chapter 2, statistically designed experiments are superior to OFAT. To use a DOE, one should choose the most relevant approach for the problem among the variety of available methods including 2^k full factorial design, 2^k fractional factorial design, central composite design, Box Behnken and designs based on optimality criterion such as *A*-, *D*-, *E*-, and *G*-optimality. Designs based on *D*-optimality for example are utilized in Chapter 6. In simple cases, where a single objective is considered, such as achieving minimum parameter variance, such a selection can be simple. In the latter *D*-optimality can be employed. Another example would be the case where there are no constraints on the design space and on the number of trials in which case a fractional factorial design can be a good start, and later response surface methodology (RSM) might be implemented. However in cases where more than one objective is considered, for example simultaneous minimization of parameter variances and model prediction errors, the solution is not that simple. Also the issue of model mismatch can lead to complications. By this we mean that the experiment is designed using one model but in the end the model selected after regression analysis is different.

In this chapter, such issues will be addressed. Two objectives were considered here; the first objective was to find a design which gives the best prediction capability as well as the lowest parameter variances given a known model. In the second objective the robustness of each optimality criterion was compared for different optimality criteria given a wrong initial model. All the numerical comparisons here were performed for linear-in-parameters models and for only four factors.

4.2 Numerical Approach and Examples

4.2.1 Algorithm for Numerical Comparison of the Predictive Capability and Parameters Variance of *A*-,*D*-,*E*-,and *G*- Optimality

In order to compare the predictive capability and parameter variances of *A*-, *D*-, *E*- and *G*-- optimality, the following approach was taken:

1- Randomly select some prior data for 4 factors.

2- Assume a linear-in-parameters model, model coefficients and a normally distributed model error to estimate the response.
3- Augment the prior data with different numbers of designed experiments using the *A*-, *D*-, *E*-and *G*-optimality criteria, and find the determinant of X^TX for the augmented design for each case.
4- Simulate the responses for the augmented dataset, then regress the responses versus the input variables, and find Predicted R^2 for each case.
5- Repeat steps 1 to 4, 100 times, and average the Predicted R^2 and $det(X^TX)$ for each case.

Example 1

Assume the following model:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_{14} x_1 x_4 + \beta_{34} x_3 x_4 \quad (4.1)$$

where β is [-0.2 -0.424 -0.116 -0.207 0.991 0.2 0.3]' . It is also assumed throughout this chapter that the response in data simulation has a normal distribution of N(0,0.15). The values of β have been chosen in the range of -1 to 1 at random.

In this comparison 3, 4, 5 and 6 experiments have been added to the 7 randomly selected historical data at each iteration using different optimality criteria. For each optimality criterion, first 3 experiments are designed for each criterion and the response is estimated using the above model. Then the determinant of X^TX matrix and predicted R^2 are calculated. This is repeated for all four optimal design criteria. The responses are estimated again and the same procedure is repeated for 100 times, and the average estimates of $det(X^TX)$ and Predicted R^2 are calculated. Again, the historical data is randomly selected and this time, the above procedure is repeated for 4, 5, and 6 optimal experiments. The reason for using historical data here is to avoid singularity of the X^TX matrix. The results are illustrated in Figures 4.1 and 4.2.

This methodology has been used in examples 1-3.

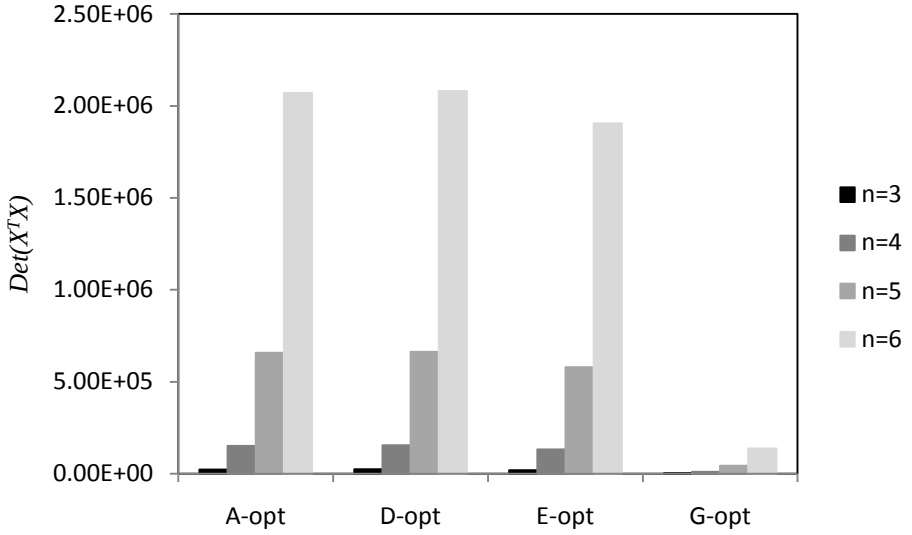

Figure 4.1- Model (4.1), 7 historical experiments. The legend shows the number of augmented trials.

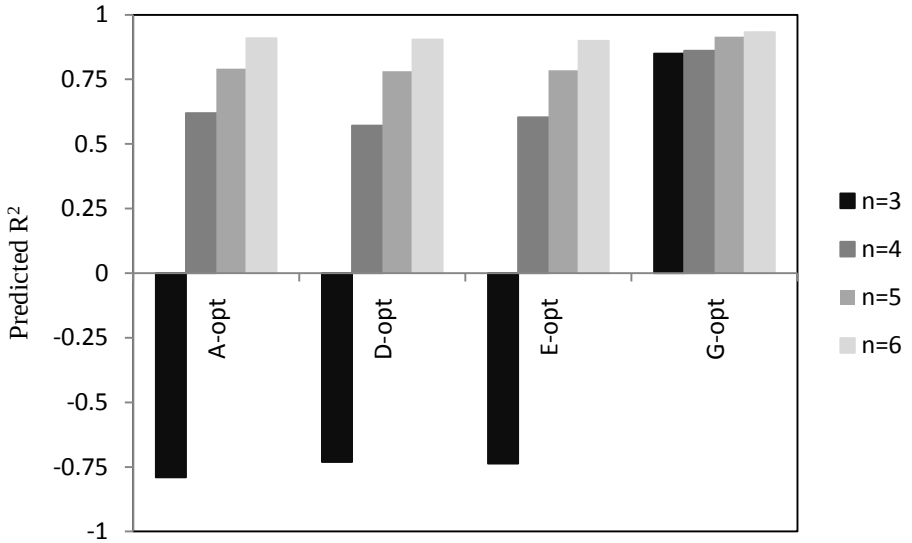

Figure 4.2- Predicted R^2, Model (4.1), 7 historical experiments

As can be seen the predicted R^2 for the *G*- optimality criterion compared to the other criteria is much higher when the number of optimal designs is small (3 in this case), but it rises rapidly for *A*-, *D*-, and *E*- as the number of optimally designed trials increase to 5 or 6. At n = 6, the predicted R^2 for all optimal designs are almost the same, but the determinant of X^TX for the *G*-optimality criteria is significantly smaller, indicating that even by increasing the number of optimal experiments, the variability of the

coefficients does not decrease significantly. It is possible for the predicted R^2 to take on a negative value. This is simply an indication of a model that has no predictive capability.

Example 2:

Assume the model:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 \tag{4.2}$$

where β is [-0.2 -0.424 -0.116 -0.207 0.991]'

Figures 4.3 and 4.4 show the average $det(X^TX)$ and predicted R^2 for model 4.2.

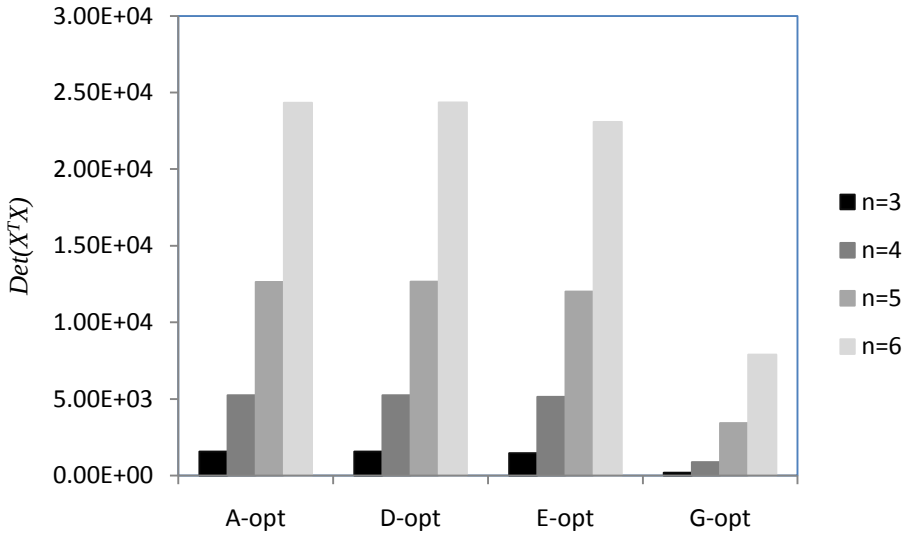

Figure 4.3- Model (4.2), 4 historical data

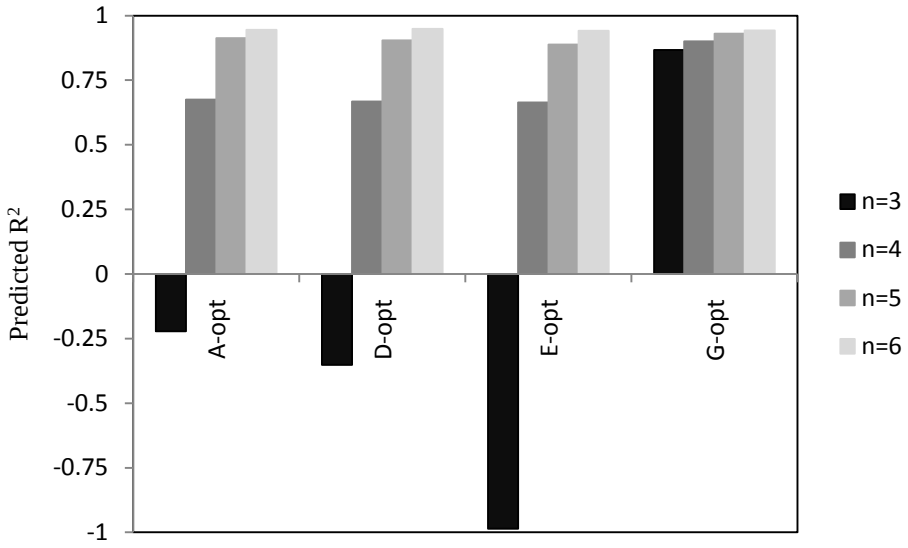

Figure 4.4- Predicted R^2 Model (4.2), 4 historical data

Again, $det(X^TX)$ does not increase significantly for *G*-optimality compared to the other 3 optimality criteria. On the other hand, the Predicted R^2 for *A*-, *D*-, and *E*- optimality is very low when the number of trials is low (3 in this case), but significantly jumps when the number of experiments increase and becomes almost the same as that of *G*-optimality when the number of optimal trials reaches 6.

Example 3:

Assume the model:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_{11} x_1^2 + \beta_{33} x_3^2 \tag{4.3}$$

where β is [-0.2 -0.424 -0.116 -0.207 0.991 0.2 0.3]'.

The average $det(X^TX)$ and predicted R^2 are shown in Figures 4.5 and 4.6.

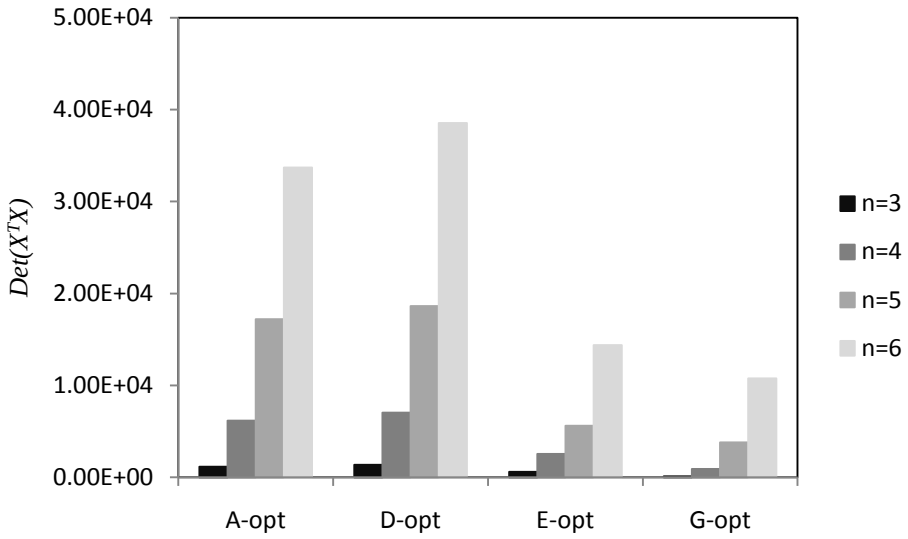

Figure 4.5- Model (4.3), 6 historical trials

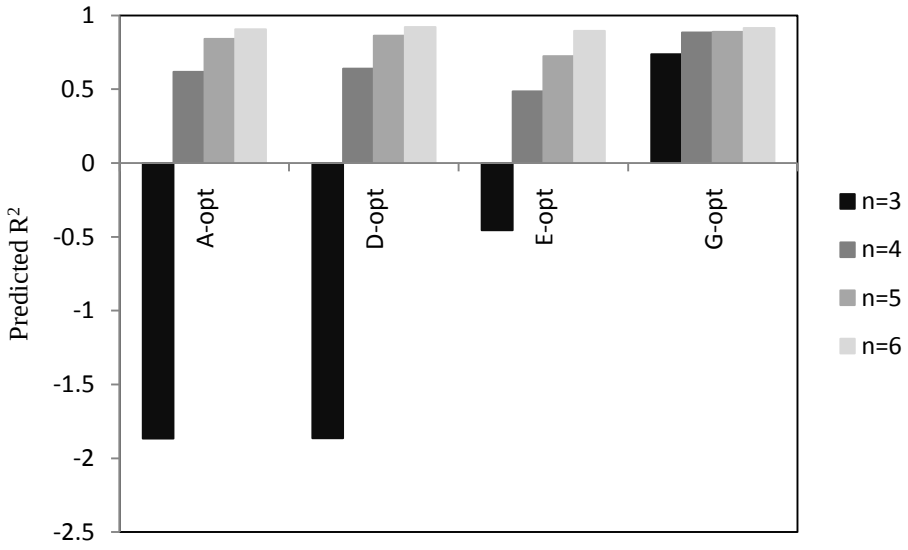

Figure 4.6- Predicted R^2 , Model (4.3), 6 historical trials

For the model 4.3 with some quadratic terms, the *det* (X^TX) for *G*- and *E*-optimality do not increase much compared to *A*- and *D*-optimality, while the predicted R^2 values for *A*-, *D*- and *E*-optimality criteria reach almost to that of *G*-optimality when the number of optimal trials increase from 3 to 6.

4.2.2 Algorithm for Numerical Comparison of the Design Robustness of *A*-, *D*-, *E*- and *G*-Optimality

In this section we investigate the design robustness of the experiments suggested by the various design criteria. By "design robustness" we mean the effect seen when experiments are designed on the basis on one model and in fact another model is found to be correct upon analysis of the designed dataset. In order to compare the design robustness of *A*-, *D*-, *E*-, and *G*- optimality, the following approach was taken:

1- Randomly select some prior data for four factors.
2- Select a model ($y = f_1(x)$) and use it to augment the prior data using *A*-,*D*-,*E*-, and *G*-optimality criteria for different numbers of further experiments, and calculate the determinant of (X^TX) matrix.
3- Assume a second model ($y = f_2(x)$) and simulate the responses for each augmented dataset, regress the dataset, and calculate the Predicted R^2.
4- Repeat steps 1 to 3 for 100 times, and average the Predicted R^2 and $det(X^TX)$ for each case.

Example 4:

The initial model assumed to obtain optimal designs based on the optimality criteria is:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_{14} x_1 x_4 + \beta_{34} x_3 x_4 \quad (4.4)$$

It is also assumed that the following model is the actual model:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 \quad (4.5)$$

where β is [-0.2 -0.424 -0.116 -0.207 0.991]'.

6 historical data are generated randomly with factors from -1 to 1. Using each optimality criterion, and the assumed model (Equation 4.4), the historical data are augmented with 2 further experiments and the $det(X^TX)$ is calculated. Next, the responses are estimated using the above actual model (Equation 4.5) and the predicted R^2 is then calculated. This procedure is repeated 100 times and the average predicted R^2 is calculated. Next, the augmented data are eliminated from the dataset, and 3 experiments are added to the historical data based on each optimality criterion, and the procedure is repeated. Finally the $det(X^TX)$ and predicted R^2 are averaged for each optimality criterion at each number of optimal trials. Figures 4.7 and 4.8 show the $det(X^TX)$ and Predicted R^2 respectively.

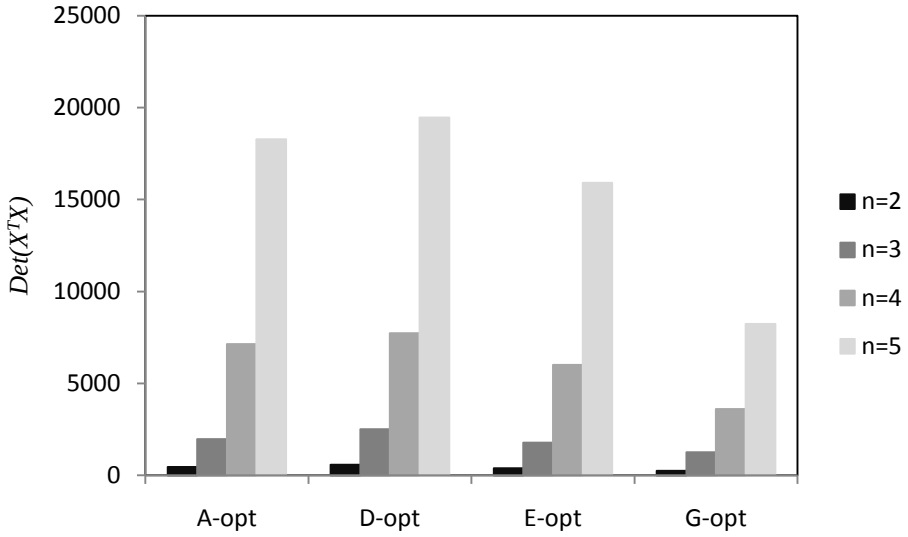

Figure 4.7- Determinant of X^TX matrix for 6 historical data

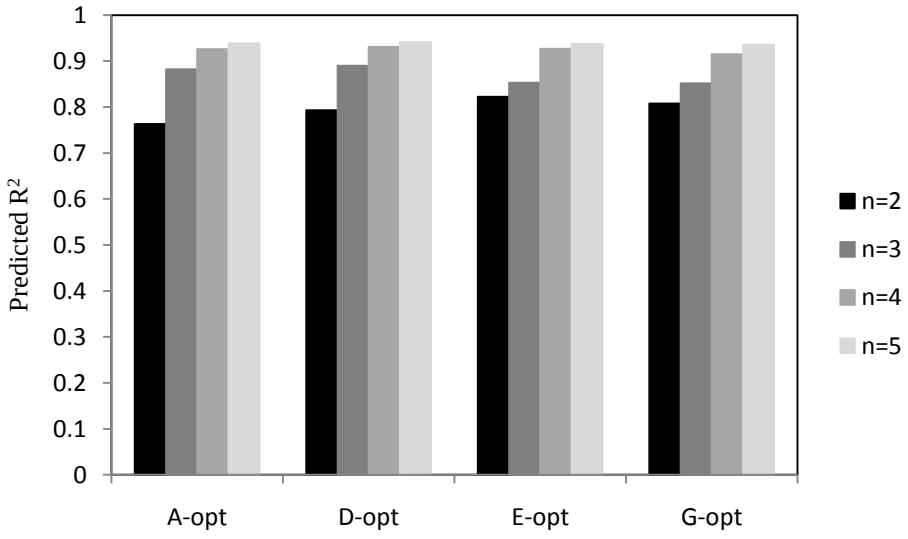

Figure 4.8- Predicted R^2 for 6 historical data

It can be seen from Figure 4.7 that the det(X^TX) for *G*-optimality criterion does not increase as much as that for the other three. Furthermore Figure 4.8 shows that the predicted R^2 is almost the same for all optimality criteria even starting with 2 optimal trials. In fact in this case the predicted R^2 is relatively

high for all criteria for all numbers of trials. This is likely attributable to the fact that the "actual" model (Equation 4.5) is nested within the model assumed for design purposes (Equation 4.4).

Example 5:

In this case we study the opposite situation where the assumed model (Equation 4.6) is nested within the actual model (Equation 4.7). In other words a more complex model is being fitted to data that come from a design based on a simpler model. Assume that the initial model used for optimal designs is:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 \tag{4.6}$$

and the actual model is:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_{14} x_1 x_4 + \beta_{34} x_3 x_4 \tag{4.7}$$

where β is [-0.2 -0.424 -0.116 -0.207 0.991 0.2 0.3]'.

Figure 4.9 and 4.10 at the following show the average of $det(X^TX)$ and predicted R^2 respectively for different optimality criteria at various trial numbers. It is observed in Figure 4.9 that the average for $det(X^TX)$ for *G*-optimality does not increase as much the other three criteria. Also Figure 4.10 shows that the predicted R^2 is also better for *A*-, *D*-, and *E*- than *G*-optimality. We also note that the predicted R^2 is considerably lower here than in the previous case implying that when data are used based on designs from a simpler model, the resulting predictive capability of a more complex model is poorer and more trials are required to augment the historical data.

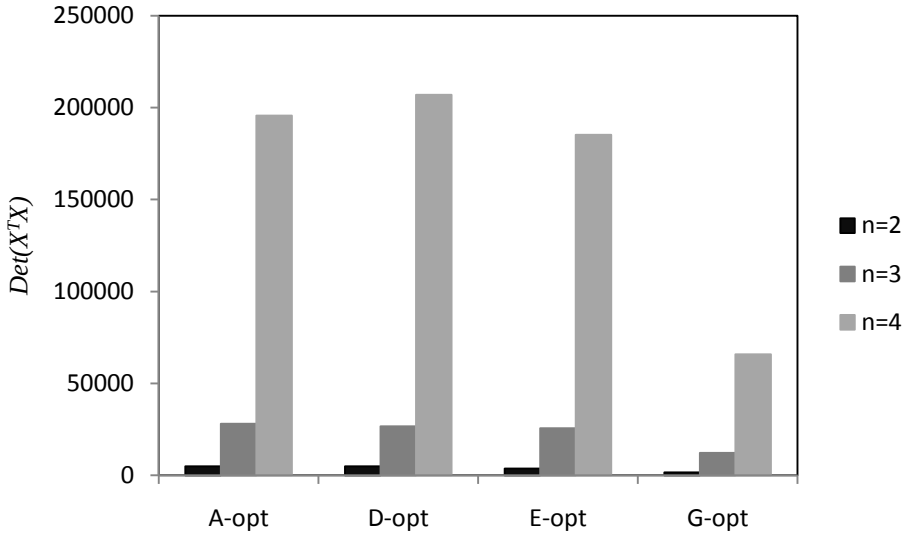

Figure 4.9- Determinant of X^TX matrix for 6 historical data, number of augmentations: 2 to 4

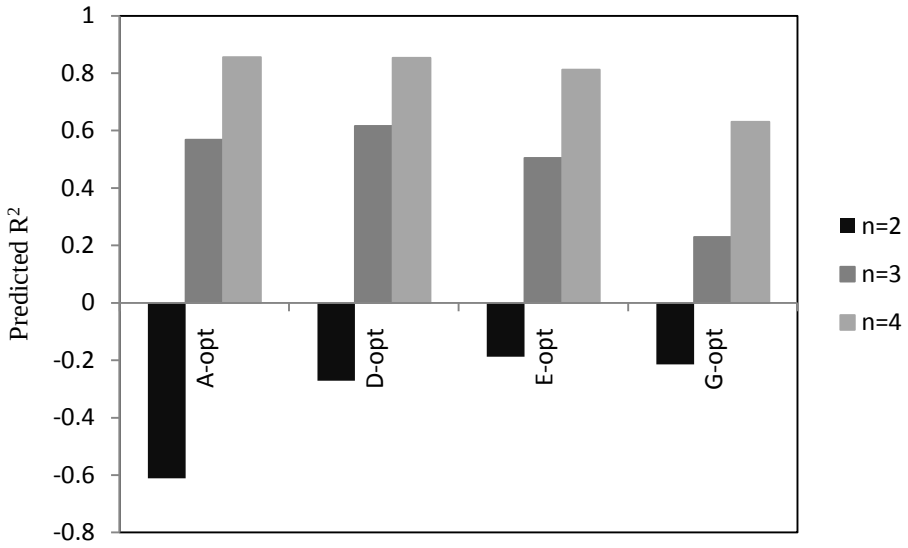

Figure 4.10- Predicted R^2 for 6 historical data, number of augmentations: 2 to 4

Example 6:

This example is similar to example 5, the difference being in the structure of the "actual" model which contains quadratic terms. The initial model is:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 \tag{4.8}$$

The following model is the actual model:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_{33} x_3^2 + \beta_{44} x_4^2 \tag{4.9}$$

where β is [-0.2 -0.424 -0.116 -0.207 0.991 0.2 0.3]'.

Figure 4.11 shows that the $det(X^TX)$ is significantly smaller than the other three criteria and it does not increase as much as the others by augmenting the number of trials. Figure 4.12 shows the predicted R^2 for *A*-, and *D*-optimality is as good as *G*-optimality at least especially when the number of trials is increased. The results are comparable to those found in example 5.

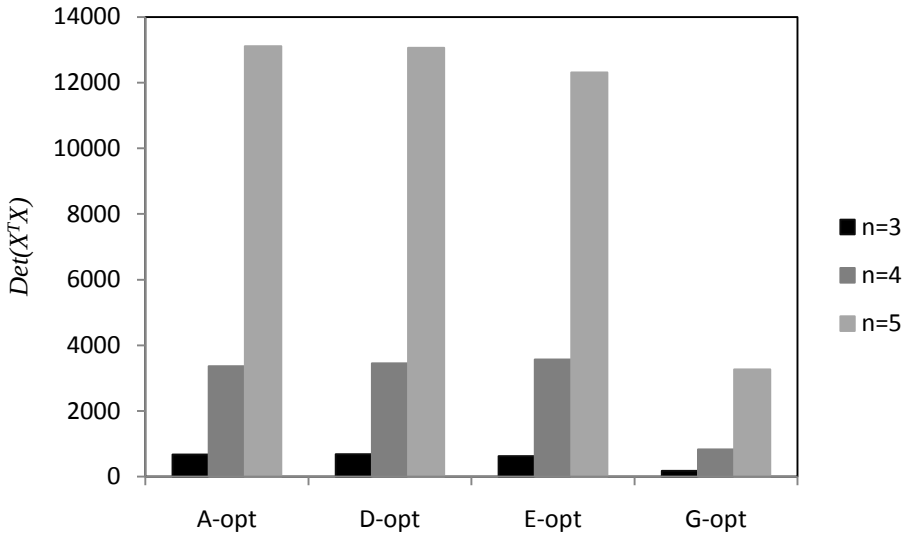

Figure 4.11- Determinant of X^TX matrix for 6 historical data, number of augmentations: 3 to 5

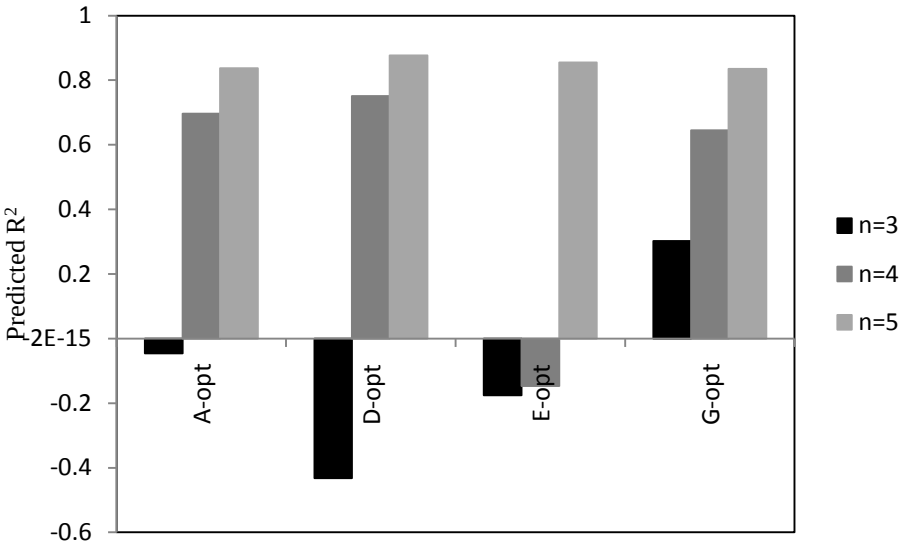

Figure 4.12- Predicted R^2 for 6 historical data, number of augmentations: 3 to 5

4.3 Conclusions

Examples 1 to 3 illustrated that the *D*-optimality criterion gave simultaneously good parameter estimates and good prediction capability providing the number of trials was sufficient. *G*-optimality on the other hand also gave models with good predictive power; however the quality of the parameter estimates as measured by the determinant of the X^TX matrix is poor by comparison. *A*- and *E*- optimality behaved close to *D*-optimality, since their criteria focus on some aspects of parameter estimation similar to *D*-optimality. In examples 4 to 6, we found that when the model used to fit the data in the end is a subset of the model used to design the experiments, models with good predictive capability were obtained with very few additional trials. Again *D*-optimality gave superior performance in terms of parameter uncertainty. When the actual model found was more complex than the model used to design experiments, the predictive power as measured by the predicted R^2 was poor initially and required more augmented trials to reach a reasonable level. While these results are limited to the types of models used in the examples, they are representative of the models used in this work.

CHAPTER 5

EXPERIMENTAL DESIGN FOR ANTIMICROBIAL ACTIVITY

5.1 Introduction

Antimicrobial activity is one of the most important criteria of a disinfectant formulation and consequently any disinfectant formulation must be tested for its antimicrobial activity using relevant in-vivo or in-vitro test methods. In this work, disinfectant formulations based on an oxidizing agent, carboxylic acid, and surfactants have been studied. The prior knowledge from the previous tests was augmented using a fractional factorial design, the results were analyzed and modeled, and then a product formulation was optimized to deliver the minimum required antimicrobial activity. The two different approaches taken in this work have been published in the journal of Industrial and Engineering Chemistry Research (Omidbakhsh et al. 2010a) and Chemical Product and Process Modeling (Omidbakhsh et al. 2010b).

5.2 Prior Knowledge

Table 5.1 shows the historical data from previous experiments performed at Virox Technologies, Ontario, Canada. For confidentiality purposes, the ingredients in the formulations are illustrated by variables x_1, x_2, x_3,..., x_6 where x_1 is an oxidizing agent, x_2 and x_5 are surfactants, x_3 is a chelating agent. x_4 is pH of the solution, x_6 is a buffering agent, and y is the microbial log reduction (LR) at 3 min contact time against *Staphylococcus aureus* using the QCT test method as described in Chapter 3. In the following, where the factors have been shown in capital letters (X_1 to X_6), they represent the coded factors and the small letters (x_1 to x_6) represent the actual values of the weight percentages except for x_4 which presents the actual value of the pH.

Table 5.1- Historical data for antimicrobial tests against *Staphylococcus aureus*

Trial#	X_1	X_2	X_3	X_4	X_5	X_6	y
1	0.88	0.73	0.87	-1	0.73	0.67	6.13
2	1.2	1	0.92	1.63	1	1	4.3
3	0.88	0.73	0.87	-1	1	0.67	6.24
4	0.88	0.73	0.87	-1	1	-1	6.24
5	1.2	1	-1	-0.94	1	-1	6.85
6	1.2	1	0.92	1	1	1	3.5
7	2.2	0.67	-1	-1	-1	-1	6.76
8	1.4	0.67	-1	-1	-1	-1	6.03
9	0.6	0.67	-1	-1	-1	-1	4.96
10	2.2	0.67	0.92	-1	-1	-1	6.95

It can be easily seen from Table 5.1 that the change in X_1, and X_2 are not large enough thus leading to a poor estimate of the effects of these factors. Since it is already known from the literature that these two factors are effective antimicrobials, analyzing the above historical data cannot be conclusive. These have been generated during previous formulation development projects in the past and are not based on statistically designed experiments. It should be also noted that the standardization here was performed based on Equation 2.2 where the upper and lower values for each ingredient was set based on those values used in the fractional factorial design in the next section for consistency purposes. Values of 0.88 to 2.2 for X_1 means that the actual values of this factor were in the upper range for this variable far beyond the upper value used in the designed experiment reported in the next section.

5.3 Historical Data Augmentation

The historical data were augmented with additional experiments so that the new dataset would yield a more conclusive model. In this case study, two new ingredients were tested in addition to the existing ones. These were selected on the basis of their known low toxicity, and potential antimicrobial activity. x_7 is a carboxylic acid and x_8 is a surfactant. Furthermore, it was decided to eliminate x_6 from the experiments since it is known from the literature that this ingredient has no antimicrobial activity. Ordinarily, the general approach to designing experiments for determining an optimal disinfectant recipe is to use mixture designs (Cornell 1990). Recipe formulation experiments differ from other experiments involving process variables such as pressure and temperature in that we are interested in the effects of the proportions rather than the absolute level of a factor. Of course the proportion over all the ingredients must equal 100%. However in the case where the majorities of the ingredients are present in small quantities and can be considered to be additives to the major component like water in the disinfectant formulation, the strategy which can be used is to study the effects of the different amounts of the additives using a factorial design. The amount of water used is therefore equal to 100% minus the sum of the additives. This approach was used here.

Two-level or fractional two-level factorial designs are particular efficient designs to use for this purpose (Montgomery 2004). They are designed to yield uncorrelated estimates of the effects of the input factors and their interactions and hence maximize the information obtained. It is anticipated that the addition of information obtained from such an experiment to the historical data set will significantly improve the predictive capabilities of the resulting models.

In this case study, a resolution IV fractional factorial design was performed as shown in Table 5.2. A resolution R design confounds main effects with interactions of no more than (R-1) factors. So if a design is of resolution IV, main effects are confounded with effects involving at least three factors, two factor interactions with effects involving at least two factors, etc.

Table 5.2- Fractional factorial design to augment the historical data

Trial#	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	y
1	-1	1	1	1	-1	-1	-1	-1	1.5
2	1	-1	-1	-1	1	-1	1	1	7
3	-1	1	1	-1	1	-1	-1	1	3.24
4	0	0	0	0	0	-1	0	0	4.0
5	1	-1	-1	1	-1	-1	1	-1	3.11
6	-1	-1	1	-1	-1	-1	1	1	3.9
7	0	0	0	0	0	-1	0	0	3.89
8	-1	-1	1	1	1	-1	1	-1	1
9	1	1	-1	-1	-1	-1	-1	1	6.28
10	0	0	0	0	0	-1	0	0	3
11	1	1	-1	1	1	-1	-1	-1	2.34
12	-1	1	-1	1	-1	-1	1	1	1.33
13	-1	1	-1	-1	1	-1	1	-1	6.46
14	1	-1	1	-1	1	-1	-1	-1	4.5
15	1	-1	1	1	-1	-1	-1	1	1.12
16	0	0	0	0	0	-1	0	0	4.06
17	-1	-1	-1	-1	-1	-1	-1	-1	2.04
18	1	1	1	1	1	-1	1	1	4.1
19	1	1	1	-1	-1	-1	1	-1	6.68
20	-1	-1	-1	1	1	-1	-1	1	1.8

5.4 Regression Analysis

5.4.1 Linear Least Squares Regression Analysis

The fractional factorial design experiments data were added to the historical data and the full dataset was then analyzed using a multiple regression analysis. This analysis was performed to develop a model for the microbial log reduction versus ingredient concentrations and pH.

Although the model being developed is empirical in nature, some prior knowledge about the nature of the relationship between variables was injected. For example, it is known from experience that log reduction (LR) varies with the reciprocal of pH (x_4). Therefore, new predictive variables were generated by dividing each variable by x_4. The new variables will be z_1, z_2, z_3, z_5, z_6, z_7 and z_8 where $z_i = \frac{x_i}{x_4}$. In many cases the original metric of the response variable (y) may not necessarily be the correct one to use. To ensure that the standard assumptions of linear regression are met, we allowed for a possible transformation of y. Here we employed the Box-Cox transformation methodology to search for an optimal metric. The model therefore had the general form:

$$LR^{\lambda} = \beta_0 + \beta_1 z_1 + \beta_2 z_2 + \beta_3 z_3 + \ldots + \varepsilon \tag{5.1}$$

Figure 5.1 shows the results of the Box-Cox analysis. This plot shows the log-likelihood function for the parameter λ. The optimum value was 1.7; however the confidence interval for λ includes 2 which was the value used here.

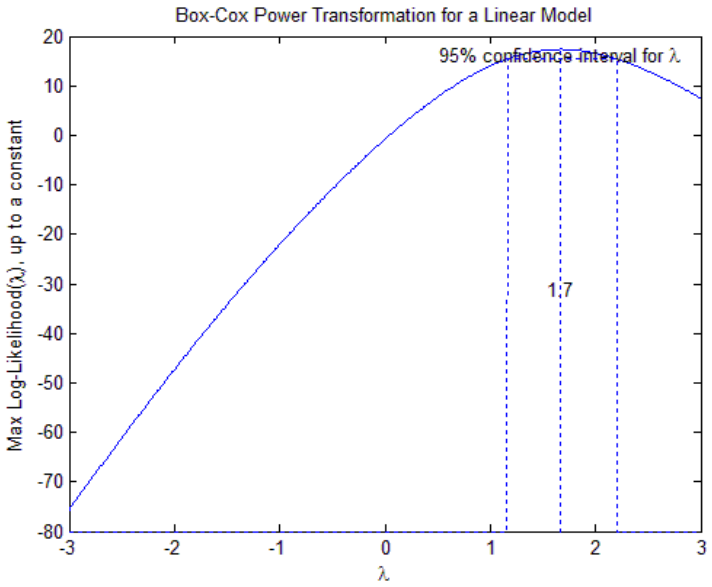

Figure 5.1- Box-Cox transformation for the microbial data

All possible models containing quadratic terms, and interactions were tried and the best fitted model was found to be:

$$LR^{2} = -3.24 + \frac{87.4x_1 + 145x_2 - 15.8x_3 + 89.53x_5 + 647.7x_7}{x_4} \tag{5.2}$$

R^2 = 93.7%

Adjusted R^2= 92.4%

Predicted R^2 = 88.92%

Both the R^2 and adjusted R^2 values are reasonably large, given the relative large error which is present in the log reduction measurements. Furthermore the predicted R^2 which is defined as $1 - \frac{PRESS}{SST}$, where PRESS represents the prediction error sum of squares and SST is the total sum of squares, is also high, indicating that the model has good predictive capability.

The normal probability plot as shown in Figure 5.2 shows that the residuals are reasonably close to normally distributed which satisfies the normality assumption of the least square regression analysis. The residual plot in Figure 5.3 does not show a trend.

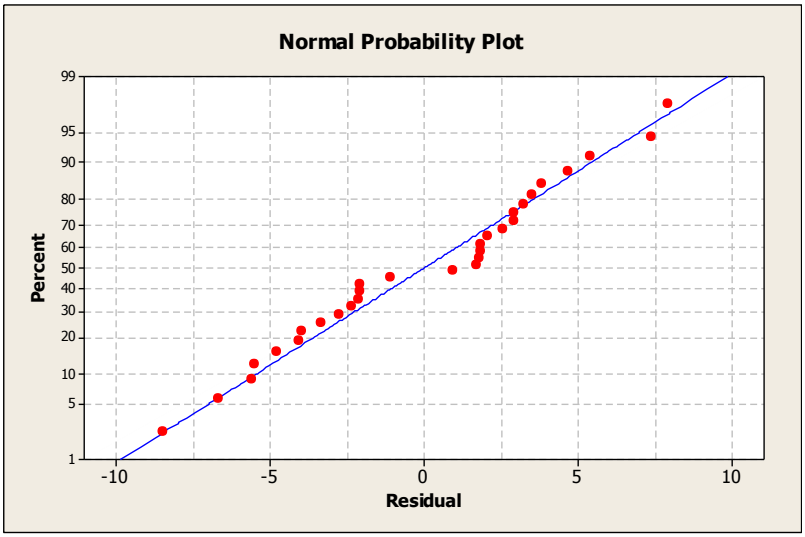

Figure 5.2- Normal probability plot of the residuals

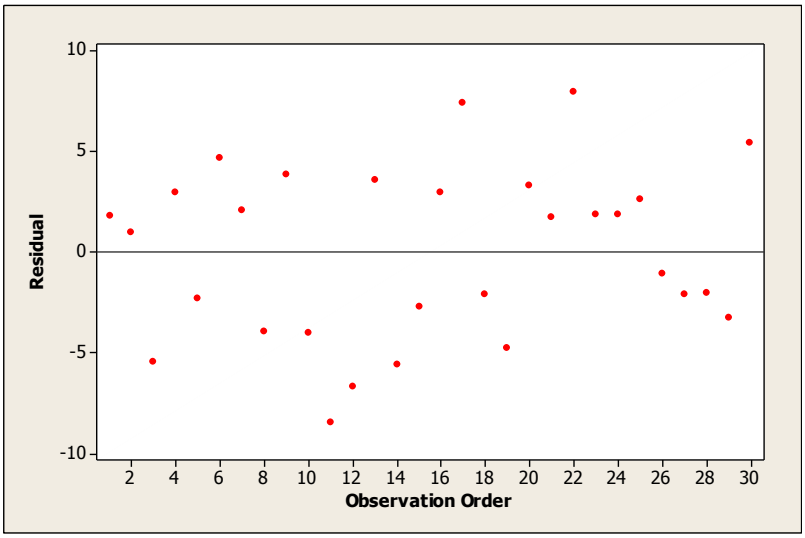

Figure 5.3- Plot of Residuals versus observation order

Also the measured log reductions versus the predicted values in Figure 5.4 are quite acceptable. Table 5.3 shows the analysis of variance. The p-value corresponds to hypothesis test on the regression model. A p-value of less than 0.1 indicates that the parameter is significant. The regression p-value of less than 10^{-3} shows that the model is significant. The pure error calculated in Table 5.3 comes from both the replicates

in the historical data and the centre points of the fractional factorial design. p-value of 0.161 shows that the model does not have a lack of fit. The above mentioned diagnostics indicate that the model gives a reasonably good fit to the data and provides good predictive power.

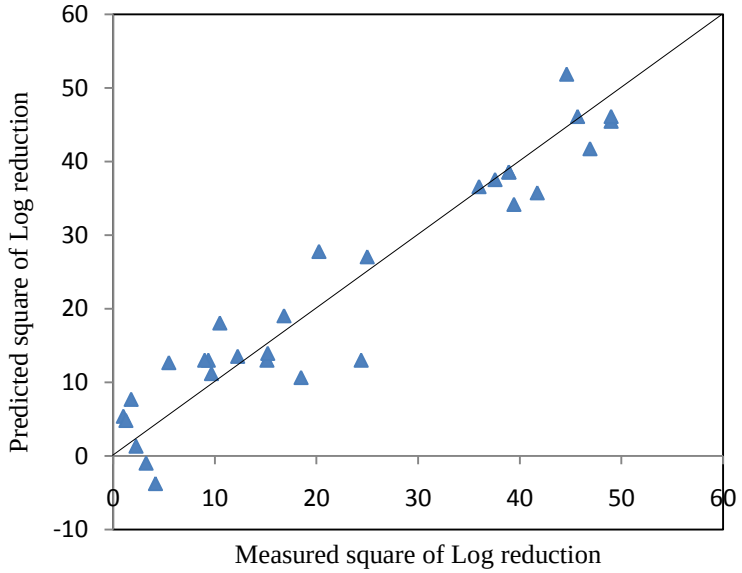

Figure 5.4- Measured log reduction versus calculated

Table 5.3- Analysis of Variance

Source	**DF**	**SS**	**MS**	**F**	***P*-value**
Regression	5	7712.8	1512.8	71.18	0.000
Residual	24	520.1	21.7		
Lack of Fit	20	485	24.3	2.83	0.161
Pure Error	4	34.3	8.6		
Total	29	8232.9			

5.4.2 Artificial Neural Networks

Matlab software version 7.6 from Mathworks, Natick, Massachusetts was used for ANN calculations in this research.

Trainlm, a built-in Matlab function, which is a back propagation technique, was used in the analysis. It is a network training function that updates weight and bias values according to Levenberg-Marquardt algorithm. Trainlm is frequently the fastest back propagation algorithm in Matlab toolbox, and is strongly suggested as a first-choice supervised algorithm, even though it does need more memory than other algorithms.

In the ANN program, one hidden layer was used. Furthermore, the hyperbolic tangent transfer function, tansig, was used for the hidden layer and the linear transfer function, purelin, was used for the output. Also 1000 epochs were used in the test.

The ANN program used here starts with random initial weights in the neural network and adjusts them based on back-propagation technique. To find the best possible weights, the program was run many times until it produced a test R^2 not less than 0.95. This was done by writing an algorithm in Matlab where the program starts with given hidden layers, neuron numbers in hidden layers, epoch numbers. It then trains a network and uses it to calculate the estimates of the test dataset. If R^2 of the test dataset (R^2 here is calculated by regressing estimated versus actual results) is less than 0.95, the training starts over until R^2 becomes greater than 0.95. Table 5.2 was used as the training set and Table 5.4 was used as the validation set.

Table 5.4- Validation dataset for antimicrobial tests against staph in coded format

Trial#	X_1	X_2	X_3	X_4	X_5	X_6	y
1	0.88	0.73	0.87	-1	0.73	0.67	6.13
2	1.2	1	0.92	1.63	1	1	4.3
3	0.88	0.73	0.87	-1	1	0.67	6.24
4	0.88	0.73	0.87	-1	1	-1	6.24
5	1.2	1	-1	-0.94	1	-1	6.85
6	1.2	1	0.92	1	1	1	3.5
7	0.6	0.67	-1	-1	-1	-1	4.96
8	-1	1	-1	1	-1	-1	1.14
9	-1	1	-1	-0.25	-1	-1	2.17
10	-0.2	-1	-1	-0.875	-1	-1	2.2
11	-1	-1	-1	1	-1	-1	0

Different numbers of neurons were also tried in the program; however, the lower number of neurons was preferred since higher numbers tend to over train the dataset and result in unstable models which perform inaccurately for new observations.

Figure 5.5 shows the total data (training+validation) actual data versus predicted data. Comparing training and validation data shows that the network has not been over trained, and there is not much difference between the R^2 for training and testing data.

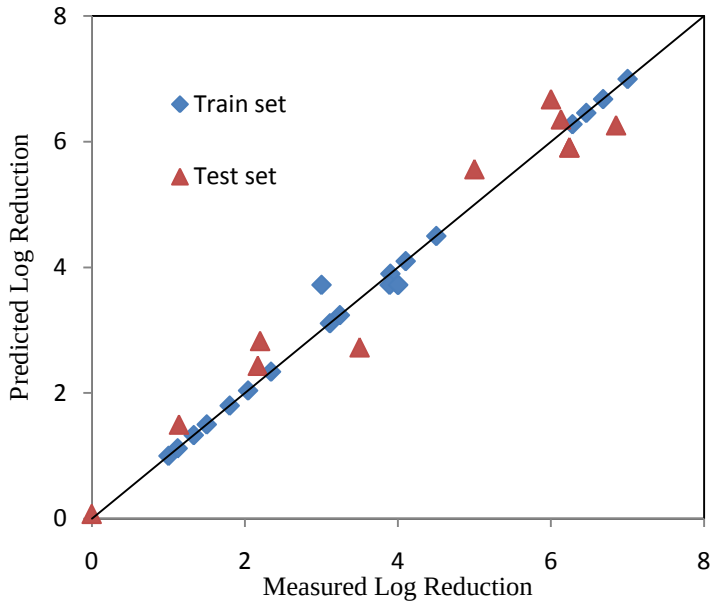

Figure 5.5- The predicted log reduction versus the actual log reduction for the whole dataset (train+test).

5.5 Formulation Optimization

In the previous section, two models were developed to predict the microbial kill. Here the question of how to design a product that will possess certain specified properties will be considered. In order to accomplish this inversion, and predict the formulation based on desired product specifications, optimization techniques are employed.

The objective of the case study is to minimize the toxicity of the formulation. The toxicity of a disinfectant can be measured in several ways, but usually human toxicity is estimated based on test results on rats and other animals. One such measure is the acute oral toxicity (LD_{50}) and is defined in Chapter 2. Based on its definition, the higher the LD_{50} of a substance, the less its toxicity. Therefore, the objective function will be set as:

$$f = \sum_{i=1}^{n} \frac{x_i}{LD_{50}(x_i)} + \frac{1}{x_4} \tag{5.3}$$

This objective function encourages the optimization program to use the least amount of ingredients while the pH is as close to neutral as possible. In our case study, the solutions are acidic, and therefore the highest possible pH is the closest to the neutral value of 7.

In addition the following two constraints are applied:

(1) Microbial log reduction $\geq$ specified value

(2) Ingredients concentration and pH upper and lower bounds: to be in the defined range

The microbial log reduction is a nonlinear term and therefore a nonlinear optimization technique should be implemented. Since two different LR models have been developed using least squares linear regression analysis and artificial neural networks, these models will be used separately as one of the optimization constraints. Therefore, there will be two different optimized solutions. Matlab software version 7.0 is used to solve the optimization program.

1- Using microbial LR model obtained by least squares linear regression analysis:

Objective function:

$$\textit{Product toxicity} = \sum_{i=1}^{n} \frac{x_i}{LD_{50}(x_i)} + \frac{1}{x_4} \tag{5.4}$$

Constraints:

$$\textit{Microbial log reduction} \geq 6 + \sqrt{t_{\alpha/2,n-p} \sqrt{\hat{\sigma}^2 (x_0^T (X^T X)^{-1} x_0)}} \tag{5.5}$$

$$LR = \sqrt{-3.24 + \frac{87.6x_1 + 145x_2 - 15.8x_3 + 647.7x_5 + 89.5x_6}{x_4}} \geq 6 + \sqrt{t_{\alpha/2,n-p} \sqrt{\hat{\sigma}^2 (x_0^T (X^T X)^{-1} x_0)}} \tag{5.6}$$

The formulation of the constraint on LR takes into account the model prediction error. In other words, the LR is predicted in this work using a regression model, which leads to a prediction which is subject to uncertainty. Therefore the constraint is specified as the upper limit of the prediction interval. To simplify the optimization program, the average prediction error is calculated and used here. Simulation results for 1000 random x_0 show an average of 1.55 for the prediction error.

The second constraint, namely the upper and lower bounds, is set from -1 to +1 for each ingredient in coded format. In actual format, the lower bounds for all ingredients were set to zero since the ingredients concentration cannot be less than zero percent. If an ingredient must be present in the formulation for some reason, then the lower bound for this ingredient will be set accordingly. The upper bounds have been defined based on the maximum allowable ingredient concentration. These allowable concentrations can be defined based on regulatory requirements, marketing demands, etc. It should be noted that these ranges should conform to those used in developing the microbial log reduction model due to the fact that the constraint model is empirical and any extrapolation can result in high model prediction error.

The optimized formulation turned out to be:

X_1= 1, X_2=1, X_3=-1, X_4= -0.25, X_5= 1, X_6= -1, X_7=1, X_8= -1

This formulation is prepared in the lab and tested for its antimicrobial activity, which is 7.62. It therefore meets the required antimicrobial activity criteria. The estimated LD_{50} for this mixture is 279.2 g/Kg.

2- Using microbial log reduction obtained by Artificial Neural Networks

It is also possible to use the antimicrobial efficacy model obtained by Artificial Neural Networks instead of using the model developed by linear regression analysis. The result of this optimization turns out to be:

X_1= 1, X_2=1, X_3=-1, X_4= -0.17, X_5= 0.77, X_6= -1, X_7=0.2, X_8= -1

This formulation is prepared in the lab and tested for its antimicrobial activity, which turns out to be 6.66, and it therefore meets the minimum required antimicrobial activity. The estimated LD_{50} for this mixture is calculated as 301.5 g/kg.

In both cases, the formulation is optimized in one step, without the need for further fine-tuning experiments. Also comparing the toxicity of these optimized formulations to an existing product (LD_{50} of 202 g/kg) shows a significant improvement for the optimized formulations.

CHAPTER 6

EXPERIMENTAL DESIGN FOR STABILITY OF HYDROGEN PEROXIDE

6.1 Historical Data Analysis

This work has been published in the Canadian Journal of Chemical Engineering (Omidbakhsh et al. 2010c). Formulation chemists have usually some knowledge about the different ingredients that they intend to use. Often, they have test results from previous projects which also relate to the ingredients of the current project. These models can be used to save time and cost in product development as will be illustrated here. In this study, a few stability test results were available from the past (Table 6.1).

Table 6.1- Historical stability data

Trial#	X_3	X_4	X_5	X_7	y
1	1	0.09	0	0	0.5
2	1	0.14	0	0	0.7
3	1	0.09	0	0	0.3
4	0	0.14	0	0	0.6
5	0	0.94	1	-1	4.84
6	-1	-1	-1	-1	0.8

The variables X_3 to X_7 are the same as those in Chapter 5. As mentioned in the previous chapter, capital letters for X denote the coded factors, and lower case letters of x refer to the actual w/w% concentrations.

Similar tests had been performed at Virox Technologies in the past using similar but not identical raw materials, and the following model had been obtained:

$$Log_{10}\, loss = \beta_0 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_7 X_7 + \beta_{34} X_3 X_4 + \beta_{47} X_4 X_7 + \varepsilon \quad (6.1)$$

where loss is the hydrogen peroxide loss in the accelerated stability test.

6.2 Historical Data Augmentation Using Bayesian D-optimality Approach

The traditional approach is to use OFAT testing such that in each experiment only one factor is changed. OFAT is very inefficient and in particular, fails to find the interactions. A better alternative is to use

experimental design methods such as a full or fractional 2^k factorial design. A full 2^k factorial design for 4 factors would require 16 trials, but there is a resolution IV fractional factorial design with only 8 trials. However, since we already have some existing knowledge, it is more efficient if we design our further experiments in the light of this knowledge. One such method is Bayesian D-optimality. In Figure 6.1, the determinant of the $(I+\frac{1}{\sigma^2}XUX^T)$ matrix is shown for different numbers of trials based on the Bayesian D-optimality criterion explained in Chapter 2.

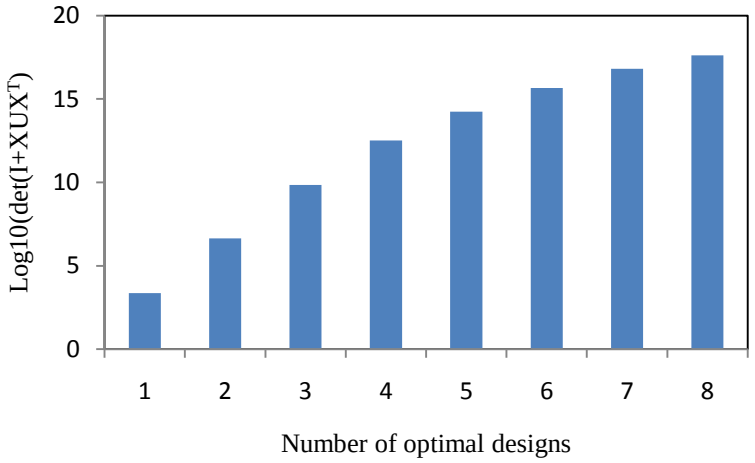

Figure 6.1- $\log_{10}\det\left(I+\frac{1}{\sigma^2}XUX^T\right)$ versus the number of optimal trials

In each case, the *X* matrix is that of the Bayesian *D*-optimal design for the number of trials shown. In the calculations below, the prior covariance matrix is calculated as:

$$U=\left[A^{-1}+X_p^T X_p\right]^{-1} \tag{6.2}$$

where $A=400I$ and I is a $p\times p$ identity matrix. A is also known as the vague initial covariance matrix where the knowledge of each parameter is independently judged as having variance 400, which is a reasonable assumption in our case study. X_p is an $n\times p$ matrix of the prior available trials (Table 6.1).

It can be seen from Figure 6.1 that the $\det\left(I+\frac{1}{\sigma^2}XUX^T\right)$ increases substantially for 1 to 6 optimal trials and the rate of the increase decreases afterwards. In the following, we demonstrate different

scenarios to design respectively 4, 5, and 6 trials based on the Bayesian *D*-optimality approach as well as a resolution IV fractional factorial design which contains 8 trials.

In the Bayesian *D*-optimality calculations shown below, σ^2 is assumed to be 0.05. This is based on the results for similar analysis performed before.

Case#1, augmenting the prior data with 4 trials using Bayesian *D*-optimality.

Table 6.2 shows the optimal four-trial experiment designed to augment the previous data.

Table 6.2- Bayesian *D*-optimality design to augment the prior data with 4 trials

X_3	X_4	X_5	X_7
-1	1	-1	1
-1	-1	1	1
1	-1	1	-1
1	1	1	1

In practice, it was not possible to set X_4 (pH) as shown in Table 6.2 since it is difficult to adjust it precisely. The experiment actually performed is shown in Table 6.3.

Table 6.3- Actual experiment for Bayesian *D*-optimality along with the test results for 4 trials

Trial#	X_3	X_4	X_5	X_7	y
1	-1	0.89	-1	1	7.94
2	-1	-0.94	1	1	0.1
3	1	-0.94	1	-1	0.02
4	1	1	1	1	1.95

The observations of Table 6.3 were added to the prior data (Table 6.1) and the whole dataset was analysed and the following model was obtained:

$$\log_{10} y = -0.174 - 0.306X_3 + 0.975X_4 - 0.192X_7 - 0.272X_5 + 0.255X_3X_4 + 0.0126X_4X_7 \quad (6.3)$$

Even though R^2 and adjusted R^2 are high (respectively 98.3% and 95%), the predicted R^2 which is an indicator of the model predictive capability was very low (0.00%) and therefore this model is not desirable for prediction purposes.

Case#2, augmenting the prior data with 5 trials using Bayesian *D*-optimality.

Table 6.4 shows the resulting optimal 5-trial design, however as mentioned above, it is not possible to set X_4 exactly to -1 and 1 and Table 6.5 shows the actual experiment that was carried out.

Table 6.4- Bayesian *D*-optimality design to augment the prior data with 5 trials

X_3	X_4	X_5	X_7
-1	-1	-1	-1
1	-1	1	-1
-1	1	-1	1
-1	-1	1	1
1	1	1	1

Table 6.5- Actual experiment for Bayesian *D*-optimality along with the test results for 5 trials

Trial#	X_3	X_4	X_5	X_7	y
1	-1	-1	-1	-1	1
2	1	-0.94	1	-1	0.02
3	-1	0.89	-1	1	7.94
4	-1	-0.94	1	1	0.1
5	1	1	1	1	1.95

The results of Table 6.5 were added to the prior dataset (Table 6.1) and the whole dataset was analysed and the following model was obtained:

$$\log_{10} y = -0.17 - 0.313X_3 + 0.973X_4 - 0.204X_7 - 0.279X_5 + 0.269X_3X_4 + 0.0214X_4X_7 \quad (6.4)$$

It is observed here again than even though the number of optimal designs has been increased from 4 to 5, the predictive capability of the model is not increased (0.00%) although R^2 and the adjusted R^2 were high (98.2% and 95.5% respectively).

Case#3, augmenting the prior data with 6 trials using Bayesian *D*-optimality.

Table 6.6 shows the resulting 6-trial optimal design and Table 6.7 shows the actual experiment that was carried out.

Table 6.6- Bayesian *D*-optimality design to augment the prior data with 6 trials

X_3	X_4	X_5	X_7
-1	1	-1	-1
-1	-1	1	1
-1	1	1	1
1	-1	-1	1
1	1	-1	1
1	-1	1	-1

Table 6.7- Actual experiment for Bayesian *D*-optimality along with the test results for 6 trials

Trial#	X_3	X_4	X_5	X_7	y
1	-1	0.83	-1	-1	13.33
2	-1	-0.94	1	1	0.1
3	-1	1	1	1	10.2
4	1	-0.94	-1	1	0.01
5	1	1	-1	1	3.3
6	1	-0.94	1	-1	0.02

The results of Table 6.7 were added to the prior data and the whole dataset were analysed and the following model was obtained:

$$\log_{10} y = -0.181 - 0.425X_3 + 1.04X_4 - 0.111X_5 - 0.187X_7 + 0.196X_3X_4 + 0.131X_4X_7 \quad (6.5)$$

A significant increase in the model predictive capability is observed now by adding 6 optimal trials to the prior data. (Predicted R^2 =75.7%).

Since the prediction error for the new observations is acceptable, the model worth testing for its significance and lack of fit, as shown in Table 6.8:

Table 6.8- Analysis of variance

Source	**DF**	**SS**	**MS**	**F**	***P*-value**
Regression	6	10.5496	1.7583	41.00	0.000
Residual Error	5	0.2144	0.0429		
Lack of Fit	4	0.1898	0.0474	1.93	0.489
Pure Error	1	0.0246	0.0246		
Total	11	10.764			

Table 6.8 shows that the model is significant and the lack of fit is not significant.

Case#4, augmenting the prior data using a resolution IV fractional factorial design.

Table 6.9 shows 8 trials designed based on the resolution IV fractional factorial design, while Table 6.10 shows the actual experiment conducted.

Table 6.9- Prior data augmentation using 8 trials based on Resolution IV

X_3	X_4	X_5	X_7
-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

Table 6.10- Actual design based on the resolution IV fractional factorial design.

Trial#	X_3	X_4	X_5	X_7	y
1	-1	1.17	1	-1	15.6
2	1	1	1	1	1.95
3	1	1	-1	-1	3.9
4	-1	-1	-1	-1	1
5	1	-0.94	-1	1	0.01
6	1	-0.94	1	-1	0.02
7	-1	0.89	-1	1	7.94
8	-1	-0.94	1	1	0.1

Results of Table 6.10 were added to the prior data (Table 6.1) and the whole dataset was analysed and the following model was obtained:

$$\log_{10} y = -0.198 - 0.431X_3 + 0.986X_4 - 0.122X_5 - 0.215X_7 + 0.201X_3X_4 + 0.125X_4X_7 \quad (6.6)$$

The predictive R^2 is about 88% for this model. The analysis of variance is shown in Table 6.11:

Table 6.11- Analysis of variance

Source	DF	SS	MS	F	*P*-value
Regression	6	10.8226	1.8038	49.35	0.000
Residual Error	7	0.2559	0.0366		
Lack of Fit	5	0.2266	0.0453	3.09	0.262
Pure Error	2	0.0293	0.0147		
Total	13				

Table 6.11 shows that the regression model is significant and the lack of fit is not significant.

To compare the predictive capability of both models, i.e. the model obtained when augmenting with 6 Bayesian *D*-optimal points and the one obtained using the 8-trial fractional factorial, the following two plots were prepared. In the first, Figure 6.2, the predicted versus observed data are shown for both models. Subjectively, the quality of the fit is similar.

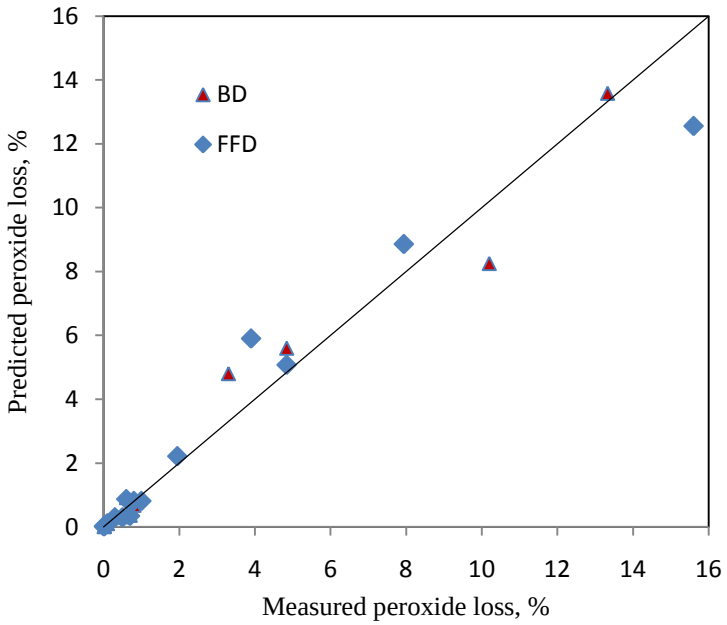

Figure 6.2- Comparison of measured peroxide loss versus calculated peroxide loss for models obtained by augmenting historical data with 6 Bayesian *D*-optimality and 8 FFD designs.

Comparing the Bayesian D-optimal and FFD designs shows that there are only three common trials in these two designs. That said, the FFD design, out of 8 trials, 5 are different from the Bayesian *D*-optimal design. To compare the predictions based on equal numbers of data points, we add the three extra data points that were not included in the FFD design, and estimate their peroxide loss using the model developed for FFD. We take the same approach for the BD model, and add the 5 data points that were not included in it (they are from FFD design), and predict their peroxide loss using the BD model. Now we have two equal sets for BD and FFD and can fairly compare them as shown in Figure 6.3. Again, the quality of the fits are comparable.

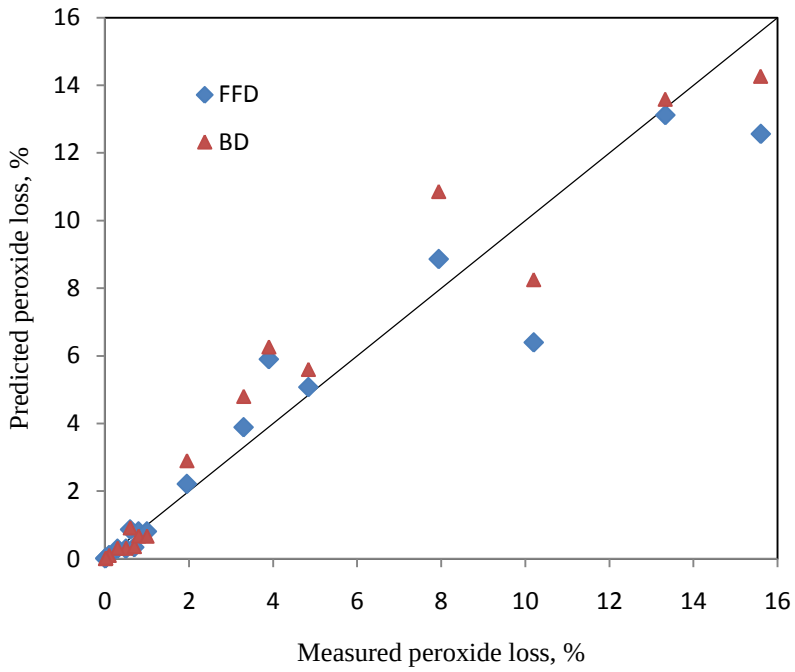

Figure 6.3- Comparison of measured peroxide loss versus calculated peroxide loss for models obtained by BD and FFD designs using equal data points.

6.3 Conclusions

It is demonstrated in this study that using a Bayesian *D*-optimality method, one can develop a model that shows a comparable prediction capability to a model based on a fractional factorial design which requires more trials. In this way, it is possible to reduce the number of trials without sacrificing the precision of the results. This method can be very useful especially where the experiments are very expensive to perform.

It should be pointed out that in all this work the model is assumed known. However the concept here is to take advantage of the prior information and in practice there are many situations where this can be done.

In summary the presented methodology is superior to factorial designs in that the number of experiments is flexible and can be less than that required for them. Moreover, in the case of expensive experiments, Bayesian *D*-optimality can be performed sequentially where the prior data is updated each time. Furthermore, it is advantageous over conventional *D*-optimality in that it easily deals with the singularity problem, and more importantly fully takes advantage of the prior information. In other words, if you have sufficient prior information then you can estimate the parameters no matter how few trials are performed.

CHAPTER 7

EXPERIMENTAL DESIGN FOR CORROSION

7.1 Introduction

A disinfectant formulation must have acceptable materials compatibility. Most plastics and most grades of stainless steel have high resistance against low concentrations of acidic peroxide solutions. The most susceptible materials against such solutions are soft metals such as brass and copper, which are widely used in many areas especially in health care facilities. Previous brass and copper corrosion tests from Virox Technologies have shown that the corrosion results for brass/copper are similar, and it is not necessary to test both; therefore in this study, only brass was tested. In this chapter, an empirical model for corrosion versus ingredients concentration will be developed, and used later as a constraint for optimization of disinfectant product formulation. The corrosion method used here has been described in Chapter 3.

7.2 Preliminary Experimental Design

For the development of these models no useful prior information was available. Therefore a designed experiment was used to perform experiments in an attempt to obtain an empirical model for the corrosion rate of brass coupons in terms of mils per year (mpy). Seven factors (X_1 to X_7) were studied here. X_8 was not included in the tests, since it was known to the experimenter by experience that it does not affect metals.

As mentioned in Chapter 5, X_1 is an oxidizing agent, X_2 ,and X_5 are surfactants, X_3 is a chelating agent, X_4 is pH, X_6 is a buffering agent and X_7 is a carboxylic acid. A fractional factorial design is performed as shown in Table 7.1.

Table 7.1- Fractional Factorial Design for brass corrosion test

X_1	X_2	X_3	X_4	X_5	X_6	X_7
1	-1	-1	-1	1	-1	1
-1	1	1	1	-1	1	-1
-1	1	1	-1	1	-1	-1
0	0	0	0	0	0	0
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
0	0	0	0	0	0	0
-1	-1	1	1	1	-1	1
1	1	-1	1	1	-1	-1
0	0	0	0	0	0	0
-1	-1	1	-1	-1	1	1
1	1	-1	-1	-1	1	-1
1	1	1	-1	-1	-1	1
0	0	0	0	0	0	0
1	1	1	1	1	1	1
-1	-1	-1	1	1	1	-1
-1	-1	-1	-1	-1	-1	-1

In practice, it was not possible to set X_4 (pH) to the values shown in Table 7.1 since it is difficult to adjust pH precisely. Therefore the experiment was actually performed according to the values shown in Table 7.2. It should be noted that all the trials are performed in duplicates in all corrosion tests and their average is used as the result of each trial. Furthermore the last trial was not practical to perform since it is a solution that has no solutes in it, and it is therefore ignored in the testing.

Table 7.2- Actual Performed Fractional Factorial Design

Trial#	X_1	X_2	X_3	X_4	X_5	X_6	X_7	y
1	1	-1	-1	-0.3	1	-1	1	18.1
2	-1	1	1	1.1	-1	1	-1	1.3
3	-1	1	1	-1.0	1	-1	-1	9.8
4	0	0	0	0.5	0	0	0	101.2
5	1	-1	-1	1.0	-1	1.1	1	2.7
6	-1	1	-1	-0.9	1	1	1	7.1
7	1	-1	1	-1.0	1	1	-1	427.8
8	-1	1	-1	-0.7	-1	-1	1	3.9
9	1	-1	1	1.3	-1	-1	-1	165.0
10	0	0	0	0.5	0	0	0	90.2
11	-1	-1	1	1.0	1	-1	1	0.0
12	1	1	-1	1.1	1	-1	-1	0.0
13	0	0	0	0.5	0	0	0	95.7
14	-1	-1	1	-1.0	-1	1	1	6.8
15	1	1	-1	-0.9	-1	1	-1	211.0
16	1	1	1	-1.0	-1	-1	1	325.9
17	0	0	0	0.5	0	0	0	100.8
18	1	1	1	1.2	1	1	1	203.4
19	-1	-1	-1	1.1	1	1	-1	0.3

The data in Table 7.2 is analyzed; however due to nonlinearity in the data, a reliable model could not be derived. This fractional factorial design along with practical experience only indicate that X_2, X_5, and X_7 are not important. The data has to be augmented at this time in order to find a proper model. Response surface methodology is used here to design experiments that can capture the nonlinearity of the system.

7.3 Response Surface Methodology (RSM)

RSM is typically based on the Central Composite Design (CCD). However CCD is not applicable here since it would design formulations which have percentages of ingredients less than zero which is not practical. Therefore, a Box-Behnken (BB) design is used. Four factors are selected from FFD regression analysis. A BB design will result in 24 trials plus a few centre points. Table 7.3 shows the Box-Behnken design for the corrosion dataset.

Table 7.3- Box-Behnken Design for corrosion tests

X_1	X_3	X_4	X_6
-1	-1	0	0
1	-1	0	0
0	0	1	1
-1	1	0	0
1	1	0	0
0	0	-1	-1
0	0	-1	1
0	0	1	-1
0	-1	0	1
-1	0	-1	0
-1	0	1	0
1	0	-1	0
1	0	1	0
0	1	0	1
0	1	0	-1
-1	0	0	1
0	-1	1	0
1	0	0	1
1	0	0	-1
0	1	1	0
-1	0	0	-1
0	1	-1	0
0	-1	0	-1
0	-1	-1	0

The formulations according to Table 7.4 were prepared and tested for their brass corrosion. Table 7.4 shows the formulations that were tested.

Table 7.4- Actual experiments for the Box Behnken design

Trial#	X_1	X_3	X_4	X_6	y
1	-1	-1	0	0	0
2	1	-1	0	0	7.1
3	0	0	1.2	1	77
4	-1	1	0	0	1.8
5	1	1	0.5	0	195.6
6	0	0	-0.73	-1	134.1
7	0	0	-0.9	1	284.6
8	0	0	1.5	-1	65.6
9	0	-1	0	1	9.6
10	-1	0	-0.8	0	5.3
11	-1	0	1.2	0	0
12	1	0	-0.8	0	304.9
13	1	0	1.1	0	70.7
14	0	1	0.5	1	192.5
15	0	1	0.6	-1	196.2
16	-1	0	0	1	2.1
17	0	-1	1.2	0	0
18	1	0	0.35	1	73.9
19	1	0	0.4	-1	82.2
20	0	1	1.3	0	187.0
21	-1	0	0	-1	0.7
22	-0.1	1	-0.9	0	337.4
23	0	-1	0	-1	7.3
24	0	-1	-0.6	0	72.0

Several empirical models were examined to find a relationship between input variables and the corrosion response; however the attempts were unsuccessful due to the substantial nonlinearity of the data. Therefore a nonlinear method, namely ANN, was used to model the data.

7.4 Artificial Neural Networks

Artificial Neural Networks used the back propagation technique of Matlab version 7.6 (2008). Fractional factorial design plus ten of the Box-Behnken trials were used as the training set, and the rest of the Box-Behnken design was used as the validation set. Table 7.5 shows the Box-Behnken trials that were used for training in addition to FFD trials.

Table 7.5- 10 selected trials from Box-Behnken design

X_1	X_3	X_4	X_6	y
-1	-1	0	0	0
1	-1	0	0	7.1
1	1	0.5	0	195.6
0	0	-0.9	1	284.6
0	-1	0	-1	7.3
0	-1	0	1	9.6
-1	0	0	1	2.1
0	-1	1.2	0	0
-1	0	0	-1	0.7
0	1	-0.9	0	337.4

One hidden layer, and 1000 epoch were used. Linear transformations were used for the input and output layers and tangent hyperbolic function was used for the hidden layer. Different numbers of neurons were tried and an R^2 of greater than 0.95 was used as the program termination criterion. To ensure that the network is not over trained, another criterion was also incorporated by generating an arbitrary set of 100 formulations. When the ANN model gave an R^2 of greater than 0.95, the network was then used to predict the corrosion of this dataset. If the corrosion results were higher than 500 mpy or less than -30 mpy, then the model was assumed to be unstable and the training was started over. Among several tested number of neurons, the best result was achieved at 4 neurons. Figure 7.1 shows the actual versus predicted corrosion for both training and validation data based on ANN. The data indicates that the predictions for the validation data are good enough and the model can be reliably used. This model will be used in the next chapter as one of the constraints to optimize a disinfectant formulation.

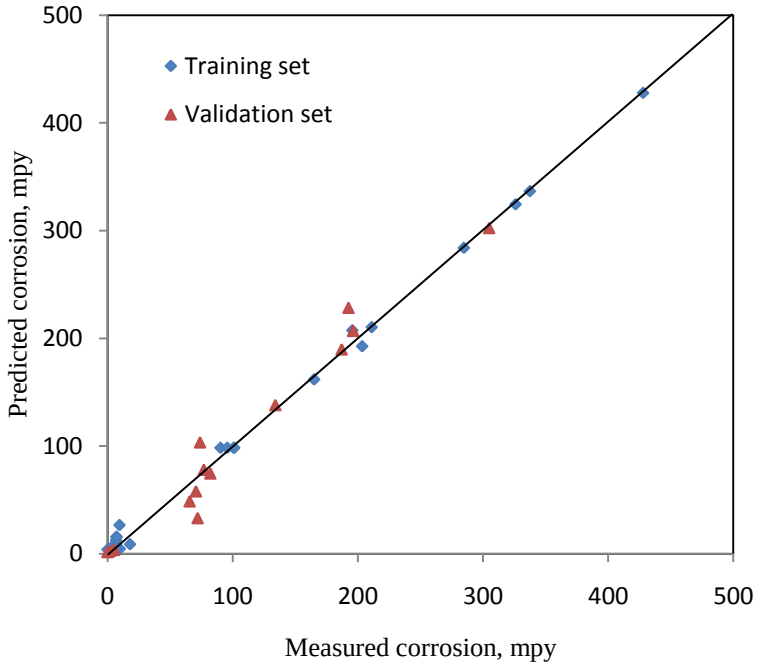

Figure 7.1- Training and Test datasets for ANN

CHAPTER 8

FINAL FORMULATION OPTIMIZATION

8.1 Introduction

The objectives in previous chapters were to build models for the product properties as a function of the input variables. These models will then be used as constraints for product optimization. In this chapter, all those constraints will be implemented, and the optimized formulation will be designed and tested.

An ideal product should be optimal in the sense of its desired characteristics. An ideal disinfectant formulation should have the following properties:

- Minimum cost
- Minimum toxicity
- Meets the minimum antimicrobial criteria
- Remains stable for a reasonable period of time
- Should show as little corrosivity as possible

The antimicrobial activity, product stability, and corrosion have been modeled in the previous chapters. The toxicity models are also available from the literature as described in Chapter 2. An optimization program is performed to minimize the cost of the formulation while meeting the specified criteria as elaborated below.

8.2 Optimization

In the previous chapters, empirical models were developed to predict the microbial kill, peroxide stability and brass corrosion. In this chapter, the question of how to design a product that will possess certain specified properties will be considered. In order to accomplish this inversion, and predict the formulation based on desired product specifications, an optimization technique is employed.

The objective of the case study is to minimize the cost of the formulation. Therefore the objective function can be written as:

$$\text{Minimize } \left(\sum c_i x_i\right) \tag{8.1}$$

where c_i is the cost of ingredient i per weight unit, and x_i is the percentage of the ingredient in the formulation.

In addition, the following constraints are applied:

Microbial log reduction $\geq$ 5

Desired log reduction is based on marketing demand. Here, 99.999% reduction in the bacterial count is aimed. To compensate for the model error, safety factors of 1.55 and 1.5 were added to the minimum log reduction for linear regression and artificial neural networks models respectively as explained in Chapter 5.

Peroxide loss $\leq$ 10%

Peroxide loss of less than 10% is required for one year shelf life. However to compensate for the model error, a safety factor was added to the peroxide loss limit. The safety factor was calculated as the average prediction error for 1000 random trials (x_0) and resulted in the value of 2.2%.

$$\text{Peroxide loss} \leq 10 - \sum_{x_0} \left(10^{t_{\alpha/2,n-p}\sqrt{\hat{\sigma}^2 (x_0^T (X^T X)^{-1} x_0)}} \right) / 1000 \tag{8.2}$$

Therefore, the peroxide loss maximum was 7.8%, and was rounded-off to 7%.

Brass corrosion $\leq$ 100 mpy

Based on experience, corrosion rates of higher than 100-150 mpy result in moderate cosmetic damage to yellow metals upon extended exposure. In the corrosion model, the highest discrepancy between a measured and predicted value was 38. This value was increased by 50% to simulate the worst case scenario, and therefore it is expected that setting the corrosion limit to 43, does not result in higher values than 100 mpy in practice. Here we decided on a round-off value of 50 mpy as the specification for the corrosion. It is unlikely that the true corrosion of the new formulation will exceed 100 mpy based on this specification.

The product should not be harmful if ingested, should not cause severe eye damage, and should not be skin and respiratory irritant. Constraints 8.3 to 8.6 satisfy these requirements.

$$\sum_{j=1}^{k} \left[\frac{P_{T^+}}{L_{Xn/T^+}} + \frac{P_T}{L_{Xn/T}} + \frac{P_{Xn}}{L_{Xn}} \right] < 1 \tag{8.3}$$

$$\sum_{j=1}^{k} \left[\frac{P_{C,R35}}{L_{Xi,R36/R35}} + \frac{P_{C,R34}}{L_{Xi,R36/R34}} + \frac{P_{Xi,R41}}{L_{Xi,R36/R41}} + \frac{P_{Xi,R36}}{L_{Xi,R36}} \right] < 1 \tag{8.4}$$

$$\sum_{j=1}^{k} \left[\frac{P_{C,R35}}{L_{Xi,R38/R35}} + \frac{P_{C,R34}}{L_{Xi,R38/R34}} + \frac{P_{Xi,R38}}{L_{Xi,R38}} \right] < 1 \tag{8.5}$$

$$\sum_{j=1}^{k}\left[\frac{P_{C,R37}}{L_{Xi,R37}}\right]<1 \tag{8.6}$$

Ingredients X_1 to X_8 are either neutral or acidic. X_1, X_5, X_8 are almost neutral and have no effect on pH. X_2 and X_3 are strong acids, and X_6 and X_7 are weak acids. Therefore the pH will be:

$$pH=-\log_{10}[H^+] \tag{8.7}$$

$$[H^+]=[H^+]_{from\ strong\ acids}+[H^+]_{from\ weak\ acids} \tag{8.8}$$

$K_{a,\,x_7} \ll K_{a,\,x_6}$ therefore the $[H^+]$ from the weak acids comes mostly from x_6.

The dissociation for x_6 will be:

HA	$\rightleftharpoons$	H^+	+	A^-
$C-x$		$[H^+]_{from\ strong\ acids}+x$		x

$$K_a=\frac{[H^+][A^-]}{[HA]}=\frac{([H^+]_s+x)x}{C-x} \tag{8.9}$$

where $[H^+]_s$ is the hydrogen cation from strong acids. x can be now calculated from a second order equation as follows:

$$x=\frac{-(K_a+[H^+]_s)+\sqrt{([H^+]_s+K_a)^2+4CK_a}}{2} \tag{8.10}$$

where C is the molarity of the weak acid, x_6 , in the mixture, x_6 is the weight/weight concentration of the weak acid, and K_a is the acid dissociation constant. All ingredients in the formulation are in low concentrations, and even in the case where all ingredients exist in the formulation at their highest level, the density is 1.002 g/cm^3, therefore with a reasonable approximation, the density of the mixture is estimated as 1.

$$C=\frac{10x_6}{MW_{x_6}} \tag{8.11}$$

Therefore:

$$x_4=-\log_{10}([H^+]_s+\frac{-(K_a+[H^+]_s)+\sqrt{([H^+]_s+K_a)^2+\frac{40x_6K_a}{MW_{x_6}}}}{2}) \tag{8.12}$$

where $[H^+]_s = \frac{10x_2}{MW_{x_2}} + \frac{10x_3}{MW_{x_3}}$

Since two different models for log reduction are developed, one based on linear least square and the second one based on artificial neural networks, we have used them separately and therefore obtained two different optimal formulations.

In the first case, the linear least square model for log reduction is used. The optimization problem results in the formulation O1:

X_1=1, X_2=1, X_3=-1, X_4=-0.72, X_5=-0.86, X_6=-0.96, X_7=1, X_8=-1

The same optimization procedure is repeated using the log reduction model developed by the Artificial Neural Networks and the following formulation (O2) is achieved:

X_1=0.74, X_2=0.92, X_3=-1, X_4=-0.71, X_5=-1, X_6=-1, X_7=0.01, X_8=-1

8.3 Optimized Formulation Verification

Formulations O1 and O2 are prepared and tested for their antimicrobial activity, brass corrosion, and peroxide stability and the test results are compared to the estimated results. Table 8.1 shows the results for formulation O1.

Table 8.1- O1 Optimized Formulation (using Least Square Regression for LR) Actual Test Results Versus Predicted Results

Optimized Formulation Test	**Acceptable criteria**	**Predicted Results**	**Actual Test Results**
Microbial Efficacy, LR	>5	6.55	6.39
Peroxide Stability, %	<10	0.28	0.81
Corrosion, mpy	<100	50.0	24.5

It is observed that the actual test results meet the criteria. The microbial log reduction criterion was 5, and the result is 6.39 which is more than 5 LR. The peroxide loss limit was 10% and the formulation had 0.81% loss. The corrosion limit was 100, and the measured value was 24.5 mpy.

The optimization results for formulation O2 are summarized in Table 8.2.

Table 8.2- O2 Optimized Formulation (using ANN for LR) Actual Test Results Versus Predicted Results

Optimized Formulation Test	Acceptable criteria	Predicted Results	Actual Test Results
Microbial Efficacy, LR	>5	6.5	5.71
Peroxide Stability, %	<10	0.57	0.94
Corrosion, mpy	<100	9.68	15.97

The required microbial log reduction for this formulation was 5, and the measured result is 5.71 which is greater than 5. The peroxide loss is 0.94% which is less than the 10% limit. The corrosion rate is greater than the predicted value, but it is still less than the acceptable limit, 100 mpy.

8.4 Comparing the Optimized Formulations to an Available Product

An available disinfectant product (product A) which is considered to be a successful product and has been developed based on trial and error methodology has the following formulation:

X_1=1, X_2=1, X_3=1, X_4=-1, X_5=1, X_6=1, X_7=-1, X_8=-1

Table 8.3 shows the comparison of this formulation with the two optimized formulations.

Table 8.3- Comparison of the optimized formulations with a conventional product

Formulation	Cost kg	Antimicrobial activity, (LR)	Peroxide loss, (%)	Corrosion (mpy)	LD_{50} (g/kg)
Product A	4.042	>6	Less than 1	420	201.8
O1	1.2288	>6	0.81	24.5	305.0
O2	0.9893	>5	0.94	15.97	367.1

As shown in this table, optimizing the formulation significantly reduces product cost, toxicity, and corrosion. The peroxide loss is in the acceptable range. As long as it is less than 10%, the product can have one year shelf life. Therefore both formulations O1 and O2 will have 2 years of shelf life.

Comparing O1, and O2 reveals that O2 is cheaper and less toxic, however it has been obtained based on the LR model developed by ANN, while O1 formulation is based on the LR model using simple linear least square regression analysis.

In general we did not see large differences between the LR models obtained by ANN compared to those obtained using ordinary linear least squares. Since the latter are simpler to develop on might give some

preference to those. However in the example shown above the formulation obtained using the ANN model is somewhat cheaper, less corrosive and less toxic. An assessment would have to be made if the differences are significant or not in order to prescribe which model is preferred.

Using this methodology, it is very easy to develop various products with different specifications. For example, if one is interested in a very green product, then the toxicity of the mixture should be maximized. If a very strong formulation for outbreaks is required, then objective can be to maximize the log reduction.

Other constraints can be added to the optimization to broaden the product design criteria. For example, cleaning tests can be performed based on designed experiments, and a cleaning model can also be added to the constraints. Furthermore, disinfectant products have often virucidal, fungicidal, and mycobactericidal claims. Further models can be developed in the same way for those criteria and added to the constraints.

While formulations O1 and O2 are superior to product A in many respects such as lower toxicity, lower corrosion, and lower costs, they are also more sustainable in that they release smaller amounts of chemicals to the environment. Taking into account 10 million liters consumption for product A, O1 and O2 release almost 75 and 86 tons less chemicals to the environment respectively which is a significant contribution for a greener environment. Moreover, there is about $300,000 savings in raw materials.

CHAPTER 9

CONCLUSIONS AND RECOMMENDATIONS

9.1 Conclusions

This thesis presented a new methodology which is the application of statistical methods in an algorithmic approach to efficiently develop new product formulations. This section summarizes the major and novel findings of this thesis.

- Design of experiments techniques, and in particular fractional factorial designs were successfully implemented and resulted in recognizing the significant factors involved in antimicrobial activity, product stability, metal corrosion toxicity, and cost.
- Multiple linear regression analysis was able to obtain acceptable empirical models to predict the product characteristics. Box-Cox transformation was successfully used to linearize/normalize the model and fit an acceptable model. The prediction power calculations revealed that the empirical models are able to predict the characteristics of a new formulation with adequate precision.
- Artificial Neural Networks technique was used to model the antimicrobial activity versus ingredients' concentration and resulted in a model with a prediction power similar to the one developed by least square regression analysis. Since the model based on linear least square is simpler, it should be preferred for this case study providing the difference in cost, corrosion and toxicity are not significant.
- In the corrosion data analysis, it was not possible to obtain a model with acceptable prediction accuracy based on linear least square technique, and the model based on Artificial Neural Networks was successfully used. This indicates the presence of nonlinearity in the corrosion data which was not detected in the log-reduction and stability data.
- Bayesian *D*-optimality was used to augment the prior dataset and was found to be more efficient than fractional factorial design since it takes advantage of the historical data in order to develop further trials.
- A nonlinear optimization was used to develop the optimal product formulation. The optimization resulted in developing a product which has the lowest possible toxicity and cost, and meets all the required criteria.
- The methodology developed here saves time and money significantly since it does not require laborious trial and error testing. Further experiments can also be added to the dataset, and the empirical models can then be updated.

9.2 Recommendations

- Disinfectant formulations are tested against different classes of microorganisms including bacteria, viruses, fungi, and sometimes mycobacteria and bacterial spores. In this study, for antimicrobial activity we focused on bacteria and its surrogate, *staph*, however it is recommended to model other classes of microorganisms such as viruses, fungi and mycobacteria, and use them in the optimization program to better optimize the formulation.
- The statistical design technique used here is based on *D*-optimality, which tries to minimize a function of the variances of parameter estimates. It would be interesting to develop a criterion which minimizes prediction error.
- The Bayesian *D*-optimality concept tested here was based on a linear model. Since in many practical cases, nonlinear models may be dealt with, a nonlinear Bayesian D-optimality concept could and should be implemented.
- The microbial log reduction model is based on a multiple linear regression analysis, and can be further improved by using a multiple nonlinear regression analysis.
- Disinfectant efficacy test methods are very labour intensive and time consuming and it would be great to develop new methods which are easier, faster, and more accurate.
- Software can and should be developed to help the product formulators who are not familiar with the statistical methods to augment the historical data, design further experiments, and optimize the product formulations based on the chemist's inputs.

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ABBREVIATIONS

ACS	American Chemical Society
ANN	Artificial neural networks
Adjusted R^2	Adjusted coefficient of determination
BP	Back propagation
CFU	Colony forming unit
DF	Degrees of freedom
Directive 67/548/EEC	The Dangerous Substances Directive is one of the main European Union laws concerning chemical safety.
DOE	design of experiments
F	F-value
Glut	glutaraldehyde
GN	Gauss-Newton search method in optimization
$[H_2O_2]_f$	Concentration of hydrogen peroxide at time t = tf
$[H_2O_2]_i$	Concentration of hydrogen peroxide at time zero
IPA	Isopropanol
LD	Lethal dose
LM	Levenberg–Marquardt search algorithm in optimization
LR	Log reduction
LXn	the respective harmful limit specified for each very toxic, toxic or harmful substance
LXi,R36	the respective irritant limit R36 specified for each corrosive substance
LXi,R38	the respective irritant limit R38 specified for each corrosive substance
LXi,R37	the irritant limit R37 specified for each irritant substance
MSE	Mean sum of squares of residuals (errors)

MSR	Mean sum of squares of the model
MPY	Mils per year
OPA	orthophetaldehyde
OFAT	One-factor-at-a-time
PRESS	Prediction Error Sum of Squares
PAA	Peracetic acid
Predicted R^2	Predicted coefficient of determination
QAC	Quaternary Ammonium compounds
QCT	Quantitative carrier test
SST	Total sum of squares
SSE	Error sum of squares
SSR	Regression (or model) sum of squares

NOMENCLATURE

e_i	Model error at point i
$e_{(i)}$	The error of model at point i where the point i was not included to evaluate the model
ε	Noise in the response
$E(x)$	Expected value of random variable X
E	Activation Energy
ξ_N	Matrix of candidate points
$\xi_{n \equiv n}$	Group of all matrices ξ_n chosen from ξ_N
$\frac{\partial E}{\partial w}$	the gradient descent correction factor
$H_f(x^k)$	Hessian matrix of function
$J_f(x^k)$	Jacobian matrix
k	k is the reaction constant
n_c	Replicates run at the point $x_j=0$
R	Gas Law Constant
r_d	inactivation rate
R36	Eye irritant risk phrase
R38	Skin Irritant risk phrase
R37	Respiratory irritant risk phrase
R^2	Coefficient of determination
$\hat{y}_i$	Predicted value of y at point i where the point i were not included to obtain the model
$\left[H^+\right]$	Hydrogen cation
$\left[OH^-\right]$	Hydroxide cation

GREEK LETTERS

α	the momentum coefficient
η	the learning rate
θ	the temperature coefficient