Empirical and Kinetic Models for the Determination of Pharmaceutical Product Stability

by

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AUTHOR'S DECLARATION

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

I understand that my thesis may be made electronically available to the public.

ABSTRACT

Drug stability is one of the vital subjects in the pharmaceutical industry. All drug products should be kept stable and protected against any chemical, physical, and microbiological degradation to ensure their efficacy and safety until released for public use. Hence, stability is very important to be estimated or predicted.

This work involved studying the stability of three different drug agents using three different mathematical models. These models included both empirical models (linear regression and artificial neural network), and mechanistic (kinetic) models. The stability of each drug in the three cases studied was expressed in terms of concentration, hardness, temperature and humidity. The predicted values obtained from the models were compared to the observed values of drug concentrations obtained experimentally and then evaluated by calculating the mean of squared.

Among the models used in this work, the mechanistic model was found to be the most accurate and reliable method of stability testing given the fact that it had the smallest calculated errors.

Overall, the accuracy of these mathematical models as indicated by the proximity of their stability measurements to the observed values, led to the assumption that such models can be reliable and time-saving alternatives to the analytical techniques used in practice.

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Especially, I would like to give my sincere thanks to my husband, Mohamed Elmeddah whose patient love and sacrifice enabled me to complete this work.

DEDICATION

To my beloved son, Yaseen, my husband, Mohamed Elmeddah, and my parents, Dr. Masaud

Khalifa and Jamia Ali who gave me all encouragement and most importantly the love I need

to reach my goals.

Table of Contents

CHAPTER 3. STABILITY MODELLING AND TECHNIQUES

CHAPTER 4. STABILITY OF ASPIRIN TABLETS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

API	Active Pharmaceutical Ingredient
ANN	Artificial Neural Network
ASA	Acetyl Salicylic Acid
DF	Degree of Freedom
FDA	Food and Drug Administration
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
HPTLC	High Performance Thin Layer Chromatography
ICH	International Conference on Harmonization
K	Kelvin degree
kp	kilo-pound
MSE	Mean Sum of Squares
NMR	Nuclear Magnetic Resonance
ODE	Ordinary Differential Equation
PDE	Partial Differential Equation
RH	Relative Humidity
SIAMs	Stability Indicating Assay Methods Chromatography
SSE	Sum of Squares Error
SSR	Sum of Squares regression
SST	Total Sum of Squares
TLC	Thin Layer Chromatography
USP-NF	United States Pharmacopeia –National Formulary)

NUMENCLATURE

A	frequency factor ($days^{-1}$)
a_j	inputs of data set into the neuron
b_j	output of data set from the neuron
C_{A0}	concentration of reactant A at time zero (mole/liter)
C_A	concentration of reactant A at time t
C_B	concentration of reactant B at time t
dc/dt	rate of chemical reaction decomposition
E_a	activation energy (cal/mole)
e_i	the residual (error)
$f(x_j)$	transfer function in neural network
k_A	reaction rate constant
$k_{\alpha+\beta}$	overall rate constant
ln k	natural logarithm of reaction rate constant
R	molar gas constant (mole/cal. K)
R^2	coefficient of determination
$-r_a$	rate of chemical decomposition
T	Temperature
T_j	internal thresholds
T	Time
w_j	weight factor

X	independent variable (regressor)
Y	dependent variable

Greek Letters

$\beta_0, \beta_1, \beta_n,$	estimated parameters
α and β	partial orders of the reaction
σ^2	Variance
ε	Error

CHAPTER 1

INTRODUCTION AND RESEARCH OBJECTIVES

1. Introduction

The drug as a chemical agent can easily be altered in its composition and characters with time under the influence of variable environmental and non-environmental factors. Such instability or degradation of a drug substance is of a great concern in pharmaceutical industry as can lead to partial or complete loss of its efficacy as a therapeutic agent or even a possible transformation into a hazardous substance. This fact made the stability of drug substances and drug products to be considered as an important subject in the field of pharmaceutical industry.

Measurement of drug stability is as essential as defining its composition, method of manufacture as well as the drug specifications and ingredients. Drug stability usually requires vigorous work and lengthy studies to identify by drug companies. Studying and measuring of drug stability is one of the steps involved in the development process of any new product in the pharmaceutical industry which basically includes drug discovery, laboratory development, animal studies, clinical trials, and regulatory registration.

Since the drug stability is a means of proving safety and efficacy, approval of any drug product by the regulatory agencies requires that the stability has to be provided prior to its release for sale.

1.1 Drug Stability

Stability of a drug is defined as "the capacity of a drug substance or drug product to remain within established specifications to maintain its identity, strength, quality, and purity throughout the retest or expiration dating period" (1). It is crucial to maintain drug stability over an adequate period of time after its synthesis until its use to ensure achievement of the desired pharmacological effects. Instability of a drug may cause undesired change in its performance or leads to its failure. Given the fact that drug can degrade over time into a toxic substance, it is important along with measuring how much of the drug is lost to always identify the degradant. For example, tetracycline was found to degrade into epianhydrotetracycline which can cause Fanconi syndrome (2).

1.2 Types of Drug Stability

In fact, there are three modes of stability for any drug product: physical, chemical, and microbiological. These are briefly described as follows:

1.2.1 Physical Stability

This implies that the drug product remains unchanged throughout its shelf life with no alteration in its physical properties that include its appearance, organoleptic properties, hardness, brittleness, and particle size. This stability is essential to ensure drug efficacy and safety and should be maintained during all the stages of the drug product formulation, manufacturing, packaging and storage and closely monitored and evaluated via special tests that are decided according to the property of interest.

1.2.2 Chemical Stability

This refers to the lack of any alteration in the chemical composition of the drug formulation. Generally, with time, most of the drug products can undergo degradation via chemical reactions such as hydrolysis, oxidation, and photolysis. Such reactions can lead to decrease in the active ingredient concentrations of the drug as well as formation of undesired by-products. This in turn, can cause the drug to have lower or no therapeutic effect or even to contain a harmful or toxic substance. The chemical degradation can also happen to preservatives and excipients contained in the drug products as well as their packages leading to the same unwanted chemical instability. It has been noticed that the solid dosage forms are more stable than liquid dosage forms since they undergo a slower chemical degradation.

1.2.3 Microbiological stability

This refers to the sterility of the drug formulation and lack of contamination by different types of microorganisms (e.g. fungi and bacteria). Obviously, microbial growth in a drug product can compromise its safety and lead to serious effects. Because of their high moisture content, solutions and water based semi-solids drugs are more liable to suffer from microbial contamination. This makes addition of antimicrobial preservatives to those drug dosage forms essential to ensure their sterility. Moreover, to prevent contamination of the formulation during the storage, the container should be suitably designed preferably using a single dose container.

1.3 Drug Shelf Life

Shelf life or expiration date of a drug refers to the time interval that the average drug

characteristics such as strength and purity of the drug is expected to remain within the approved specifications after manufacture (3).

Because of the existence of batch to batch variation, the true shelf life of different batches might differ and must be treated as a random variable, which depends on drug stability, temperature, humidity, exposure to light, and class of container among other possible variables. Thus, the design of any stability study should establish a shelf life that is applicable to all future batches of the drug manufactured under similar circumstances (4, 5).

1.4 Food and Drug Administration Stability Guidelines

The Food and Drug Administration (FDA) is the USA federal agency that is supervising the drug manufacturing and marketing. Through closely monitoring and validating the drug development process, FDA ensures that the drug product is safe and that it would remain stable and of no harm to customers throughout the whole period of the indicated shelf life (4).

The FDA first required stability study testing in 1984. However, specific requirements on statistical design and analysis of stability studies for human drugs and biologics were not established until 1987 when the FDA guideline was issued. This was revised and expanded in 1998.

For every drug in the market, FDA requires that a shelf-life must be indicated on the container label. Moreover, the drug companies are required by the FDA to submit data that ensure the drug products will retain their identity, strength, potency and purity and demonstrate that the average drug characteristics can meet the approved specifications during the claimed shelf life period.

As per the FDA stability gridline, the expiration date period is the time at which 95% one sided lower confidence bound for the mean degradation curve intersects the approved lower

specification limit. This applies for the drugs of which the degradation is expected to decrease with time in a linear fashion, See Figure [1.1]. If the time degradation relationship is not linear, it may be made so using an appropriate transformation (e.g. a linear, quadratic, or cubic function on an arithmic or logarithmic scale) (6).

To overcome the problem of batch to batch variability in determining the expiration date, FDA stability guideline requires that three batches or more should be studied in order to estimate a more accurate single expiration dating period that can be applied to all future batches of the drug product. FDA also suggests that every drug product should be tested for stability at 3- month intervals during the first year, 6-months intervals during the second year and yearly thereafter (4). Since the containers as substances are in close contact with drugs, they can adversely affect the stability of the drug products. This fact made the FDA guideline suggest that containers such as bottles packages and vials should be included in the stability studies (7).
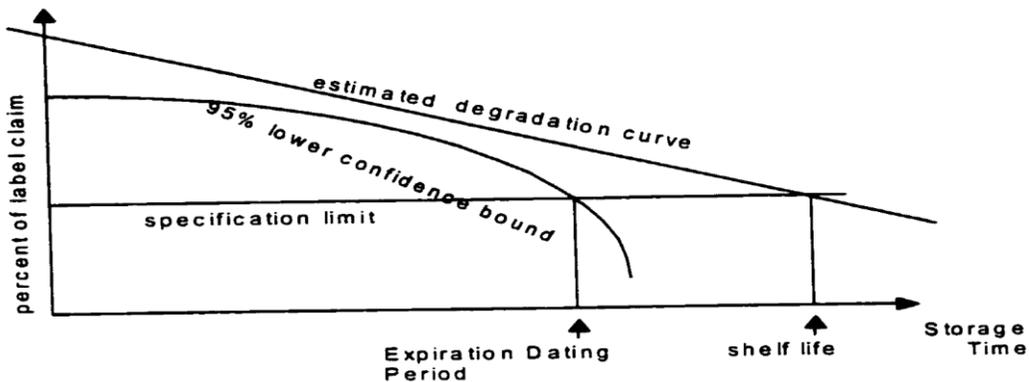
Figure [1.1]: Drug Shelf Life Determinations (6).

2. Research Objectives and Approach

Drug stability is one of the most important aspects of any drug product in pharmaceutical industry. It is the drug stability that can decide its efficacy and pharmacological benefit to the consumers as well as the time after which it would lose its desired quality or even change into a toxic agent.

As mentioned in the FDA guidelines, the purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light. Several drug stability testing methods are used in practice to study the rate of drug degradation in order to estimate its expiration date. Most of these methods are based on analytical laboratory experiments which are usually expensive and time consuming.

A newer and a more efficient approach that is currently adopted for measuring the behaviour of the active ingredient through time is the use of statistical analysis. This implies formulation of statistical techniques and mathematical models where specific equations are used to study the drug stability and estimate its shelf life or expiration date.
The mathematical models that are commonly used nowadays are the empirical models such as linear regression, artificial neural network, and the mechanistic models.

In order to evaluate the applicability and efficiency of statistical modeling in studying drug stability and estimating its degradation with time and to know whether statistical modeling can efficiently substitute the ordinary analytical methods in studying drug stability several models were applied on three different drugs in this research. These drugs were chosen randomly from

different articles that were published in the pharmaceutical literature. The experimental data of these drugs were collected and all the three mathematical models (linear regression, artificial neural network, and mechanistic models) were applied on the data set of each of the three drugs to determine the best model to predict its stability. A comparison among these mathematical models was made at the end of this dissertation to conclude the best statistical method should be adopted in practice to achieve the most accurate shelf life.

CHAPTER 2

BACKGROUND AND LITERATURE REVIEW

2.1 Shelf Life Estimation

The goal of shelf life estimation is to predict the time when the drug stability is no longer within the approved specification limit. Estimation of the expiration date of every newly released drug product in the market is one of the essential stages required by law to prove its safety in order to be licensed and released to the market. This stage is quite compromising because of the ethical implications and operational costs involved.

Since the shelf life determines the length of time during which the drug product can be safely used, it should be estimated accurately. Labelling with an overestimated shelf life (expiry date) can lead to the use of product with an altered chemical composition which can cause unwanted or serious side effects to the patient. On the other hand, underestimation of the shelf life carries no risk to the consumers' health. However, can lead to premature withdrawal of the drug product from the market and this can increase its cost and lead to financial losses (8).

The expiration date period must be obtained through a rigorous experimental analysis with several batches of the product. The analytical procedures and conclusions derived from the analysis have to be closely monitored and well supervised.

To predict the shelf life, accelerated studies are used for estimating the rate of chemical and physical degradation. To do this, the order of reaction has to be determined. For example, a zero order equation represents a linear relationship between the drug characteristic and time, whereas

in the first order the logarithmic transformation of the drug characteristic which is a linear function of time. After the order of the reaction is determined, the Arrhenius equation is then applied to decide the relationship between the rate of degradation and the temperature, as Arrhenius equation describes the linear relationship between log (degradation) and reciprocal of the absolute temperature (6).

2.2 Statistical Models in Literature

Statistical modeling as a new approach for drug stability studying in the pharmaceutical industry has attracted many researchers. Many articles have been published with regards to the use of statistical models in studying the drug product behaviour in terms of the chemical and physical decomposition as well as estimating the shelf life.

In this section, several articles about drug stability study involving the use of mathematical modeling are summarized;

Yang and Macdonald (9) applied both experimental and mathematical methods to determine the optimum pH that gives the highest stability for two of the factor Xa inhibitors (MLN1021 and CT54004). The rate of degradation of drug in solution formulation is mainly pH-dependent. Experiments after being prepared in buffer solution of widely variable pH (1-10) were first conducted on both compounds. Then the rate constants were obtained under different pH conditions while the drugs were stored at 60°C. It was found that the two compounds were most stable in water (i.e. at pH=1) and least stable under alkaline media. The stability then was decided by calculating the % drug remaining using HPLC analysis.

The experimental rate constants were then used in setting up a kinetic model equation to determine the optimum pH. The optimum pH for each compound was predicted by plotting the

log degradation rate (logK) of each compound against the pH. The optimum pH for MLN1021 and CT54004 were 6.18 and 5.92, respectively. These two values are very close to the experimental results which were 6.1 and 6.0 for MLN1021 and CT54004 respectively. The overall conclusion was that both experimental methods and mathematical models can give almost the same results and can be quite similar in assessing drug stability.

As one of the commonly used mechanistic models in the pharmaceutical industry is Arrhenius theory. It has the advantage of rapidly assessing the stability and estimating the shelf life of the drug products under stress storage conditions. Gil-Alegri et al (10) applied Arrhenius theory to in stability testing of Mitonafide drug during development of both tablet and parenteral formulations. The degradation rate constant (k) under different storage temperatures was first determined. Then the correlation between the K_T and the inverse of the storage temperature was evaluated and found to be statistically significant. This work showed that the stability prediction for this drug product can be reliably made by statistical methods.

Anderson And Scott (11) performed an accelerated stability testing on 5 drugs of abuse (morphine, delta-9-THCC, amphetamine, phencyclidine, and benzoylecgonine) in order to estimate their shelf lives. The products were stressed by exposing them to variable degrees of high temperature and relative humidity. The degradation rate constants for each agent were calculated by using regression analysis. This allowed calculation of the activation energy; the amount of heat required for degradation to start by applying Arrhenius equation.

Oliva et al (12) applied mechanistic modeling to study the stability of cholecystokinin fragment CCK-4 solution. HPLC method was used and experimental data were analysed by both isothermal and non-isothermal assays. For the non-isothermal studies, both the derivative and

integral parameters were used to estimate the Arrhenius parameters, whereas 2-step linear regression was used in the isothermal studies. The best model was then decided according to multiple statistical criteria and it was found that non-isothermal studies are faster and can give more reliable information about drug stability.

2.3. Drug Dosage Forms and Stability

The stability of drug product with respect to physical, chemical, microbiological, therapeutic and toxicological characteristics is a dosage form specific that differs from one dosage form to another.

FDA guideline requires specific tests of stability depending on the dosage form of the drug, that is, for every specific dosage form certain characteristics need be maintained throughout its shelf life to ensure its efficacy and safety. Hardness, brittleness and dissolution are examples of such characteristics that are mainly specific to solid dosage forms. Special tests are available to test these characteristics and are usually conducted during the drug manufacture process. Table [2.1] shows the different important drug characteristics for different dosage forms.

Hardness is one of the important physical properties of the solid dosage forms. It is commonly used as a measurement for the tablet strength. The tablet strength should be evaluated during its formulation and manufacturing process to ensure its stability and resistance to breakdown throughout the different steps of packaging, shipping, and dispensing as well as the possible abuse by the consumer. Hardness in pharmaceutical industry is defined as *the force required to break the tablet in diametrical compression test.* This test implies placing the tablet between two anvils where one of the anvils is moved against the tablet until it breaks and the force at which the tablet breaks is the hardness (13, 14)

Table [2. 1]: Drug Characteristics for Different Dosage Forms

Dosage form	Drug characteristics
Tablets	Appearance, friability, hardness, color, odour, moisture, strength, dissolution,
Capsules	Strength, moisture, color, appearance, shape, brittleness, dissolution,
Emulsions	Appearance, colour, odour, pH, viscosity, strength
Oral solution and suspensions	Appearance, strength, pH, colour, odour, redispersibility, dissolution, clarity
Oral powder	Appearance, pH, dispersibility, strength
MDI aerosols	Strength, delivered dose per actuation, number of metered doses, colour, clarity, particle size, lose of propellant, pressure, valve corrosion, spray pattern
Topical and ophthalmic preparations	Appearance, clarity, colour, homogeneity, odour, pH, resuspendibility, consistency, particle size distribution, strength, weight loss
Small-volume parenterals	Strength, appearance, colour, particulate matter, pH, sterility, pyrogenicity
Large-volume parenterals	Strength, appearance, colour, clarity, particulate matter, pH, volume, extractables, sterility, pyrogenicity
Suppositories	Strength, softening range, appearance, dissolution

Shelf life in most of the stability studies is related to the primary drug characteristics such as the strength. The strength of a drug product is defined as either the concentration of the drug substance or its potency which refers to the therapeutic activity of the drug product. FDA guideline considers the drug strength as a quantitative measure of the active ingredient of a drug product as well as other ingredients that require quantitation, such as alcohol and preservatives.

If a drug is designed to be used as an additive to another drug product such as parenterals or aerosols, FDA requires that stability study should be conducted on the dosage form made of the mixture of both drugs rather than each component alone.

The stability of the characteristics of a drug product for a particular dosage form may be influenced by storage conditions, such as temperature, humidity, light, or air, and by package types such as high-density polyethylene (HDPE) (6).

2.4. Factors Affecting Drug Stability

To maintain drug stability, it is important to completely understand the drug structure and its characteristics along with the impact of different physical, chemical, microbiological, toxicological, and environmental factors upon the drug formulation (16). This ensures providing optimal storage conditions and modes of transportation of the drug products and identifying the precautions that should be taken to prevent or minimize the loss of activity. For example, knowing the effect of temperature on a certain drug can help avoiding its damage by keeping it under a suitable temperature during its storage and shipment (17).

Basically, both environmental factors such as heat, moisture, light, and oxygen and product related factors such as formulation composition, manufacturing, and packaging can influence the drug product stability by inducing alterations of its physicochemical properties. Such influence can expedite the degradation of drug products (18). The degradation usually varies depending on the dosage form although different formulations can be influenced by the same factor quite similarly. Factors that commonly affect the stability of different dosage forms are summarized below:

2.4.1. Liquid Dosage forms

Liquid dosage forms stability is influenced by the following factors:

2.4.1.1. PH

PH is the most important factor that can influence the rate of hydrolysis in liquid form. Some drugs undergo hydrolysis at a rapid rate in the presence of strong acids and bases and that is why it is important to determine the pH at which the stability is greatest.

2.4.1.2. Temperature

It was found that as temperature increases, the hydrolysis rate of drugs in solution profoundly increases. Some drugs would not be stable even at room temperature which makes it necessary to provide cool storage conditions to maintain drug stability. Examples of these drugs include injecting penicillin and insulin.

2.4.1.3 Ionic Strength

Some drug solutions require that electrolytes be added to adjust their tonicity. But this was found to have an impact on the stability.

2.4.1.4. Solvent

To avoid hydrolysis in some drugs, its water content can be replaced with a solvent such as alcohol or propylene glycol. However, if such a procedure is applied to other drugs, it can actually increase the rate of degradation.

2.4.1.5. Oxygen

Since the presence of oxygen can induce oxidation, proper packing keeping the oxygen content of the solution less and leaving very little head space in the bottle above the drug products are methods to fight against oxidation. In some cases, oxygen can be replaced by nitrogen or carbon dioxide in the storage containers.

2.4.1.6. Surfactant

Nonionic, cationic and anionic surfactants when added to solution-containing drugs form micelle and the drug particles become trapped in the micelle. The hydrolytic groups such as OH cannot penetrate this micelle cover to reach the drug particles, and hence hydrolysis rate is decreased.

2.4.2. Solid dosage form

Solid dosage forms stability is influenced by the following factors:

2.4.2.1. Moisture

When water soluble solid drugs get exposed to moisture, they undergo decomposition in a similar way as the liquid dosage forms. For example, moisture can induce hydrolytic cleavage of ester or amide linkages in the drugs. Hence, the moisture should be avoided during manufacture and storage and packaging should be selected carefully.

2.4.2.2 Excipients

The decomposition of the drug is found to be proportionally related to the water content of its excipients. Starch, povidone, and magnesium trisilicate are examples of excipients of high moisture content. Hydrolysis of aspirin in tablet form is increased because of magnesium

trisilicate. Also, the chemical interactions between the excipients in solid dosage forms can increase the degradation rate. Incorporation of polyoxyethylene glycols into aspirin suppository bases leads to its decomposition.

2.4.2.3 Temperature

Temperature can directly affect the drug or its excipients either directly by inducing melting or polymorphism. Temperature can also indirectly influence the drug decomposition through changing the relative humidity.

2.4.2.4 Light and Oxygen

Many drugs can undergo photodecomposition or oxidation and hence, such drugs should be kept away from light and oxygen. Since water contains oxygen, moisture should be avoided by storing the drugs under dry conditions.

2.4.3. Semisolid Dosage Form

The chemical stability of ointments and creams is mainly based on the base in their formulation. For example, hydrocortisone decomposes quickly in polyethylene glycol base making its shelf life to be only 6 months. Moreover, dilution of some highly potent ointments such as steroids to make them safer was found to influence their stability and hence, the choice of diluents should be made carefully. Also, the stability of these drugs can be influenced by their incorporation into gel structures by causing increased degradation. However, the viscosity has only a minimum influence on the drug stability (17).

2.5 Mechanism of Drug Degradation

Drug products of different dosage forms such as liquid, solid, and semi solid dosage forms can usually undergo some form of chemical degradation or breakdown with time. Such change in the dosage form may change either the drug physical appearance such as discoloration or its chemical structure with consequent change in its potency or safety. Several modes of degradation have been identified ainclude; hydrolysis, oxidation, isomerisation, photochemical decomposition and polymerization.

The mode of degradation that will take place is determined by the type of the chemical groups that are present in the drug molecules. Some drugs can undergo more than one mode of degradation.

2.5.1 Hydrolysis

Hydrolysis as a term means splitting in water. Hydrolytic degradation occurs for a drug that is a derivative of carboxylic acid or contains a molecular group that is based on this moiety such as an ester, amide, lactone, lactam, imide, or carbamate. Examples of such drugs include acetylsalicylic acid, physostigmine, and methyldopa.

Hydrolysis can be catalyzed by Hydrogen ions (specific acid-catalysis) or hydroxyl ions (specific base-catalysis) and also by buffer that contain acidic or basic species.

To stabilise the drug against acid-base catalyzed hydrolysis several ways are available. The most commonly one is to formulate the drug at a pH of maximum stability that is determined values. Hydrolysis can be also by the addition of non aqueous solvents such as alcohol, glycerine or propylene glycol. Making the drug less soluble is another method to suppress degradation and

this is done by using additives such as citrates, dextrose, sorbitol, and gluconate. Adding a compound that forms a complex with the drug or solubilisation of a drug by surfactants can increase stability of many drugs (17, 19).

2.5.2 Oxidation

This is another very common way of drug degradation. It can occur simultaneously with hydrolysis. Oxidation occurs by either loss of an electropositive atom, radical, or electron or addition of an electronegative atom or radical. Oxidation process usually involves combining oxygen with free radicals via quite slow chain reactions. These free radicals usually result from organic compounds by the action of light, heat or trace metals.

Drugs that can degrade by oxidation include phenolic compounds such as morphine, phenyl

epinephrine and catecholamine.

One method that is commonly used to prevent oxidation and stabilize the drugs is to store them under anaerobic condition. This requires replacing oxygen in the containers with nitrogen or carbon dioxide. Also, since heavy metals such as iron, cobalt, and nickel can act as catalysts for oxidation process, avoiding the use of containers made from these metals during their storage or manufacture can be a protective method against oxidation. Other methods involve reducing the storage temperature and adding small amounts of antioxidants or reducing agents which were proven to be helpful in many cases. (17, 19, 20)

2.5.3 Isomerisation

Isomerisation refers to the process of changing of drug into its optical or geometric isomers. Such isomers are usually of no therapeutic efficacy. As an example, adrenaline can undergo

racemisation where it converts from its levo-rotary form into less active isomer. Isomerisation can be catalyzed by low pH. Also, vitamin A can isomerise from its active form of all-trans into the less active cis-isomer.

2.5.4 Photochemical degradation

Light exposure can initiate chemical degradation of some drug products. The photochemical reactions can be either oxygen dependent (photo-oxidation) or independent on oxygen (such as dehydrogenation, rearrangement, and dimerization). Sodium nitroprusside which is a treatment of acute hypertension has to be protected from light. If the solution is protected from light, it is stable for at least 1 year; if exposed to normal room light, it has a shelf life of only 4 hours. Other examples of drugs that can rapidly degrade by ultraviolet light are phenothiazines, hydrocortisone, and ascorbic acid compounds. In some cases photochemical degradation can be manifested as discoloration of the drug.

An efficient way to prevent photo-degradation of drugs is by the use of amber coloured glass containers for solution dosage forms. Other means include the use of cardboard outers, aluminum foil over wraps and film coating that can absorb light for tablets as well as storage of photosensitive drug products in the dark (17).

2.6. Stability Study

Drug stability studies are conducted by drug companies to ensure that a drug can meet the approved specification prior to its expiration date printed on the package. Such studies are designed to define the degradation of drug product over time and its shelf life based on the degradation curves.

Generally, drug stability studies consist of a random sample of dosage units (e.g. tablets, capsules, vials) from a given batch or several batches placed in a storage room with controlled temperature and humidity conditions.

Stability study involves taking a sample from several batches of a certain drug product and storing it under controlled condition then degradation is measured at fixed intervals such as 0, 3, 6, 12, 18, 24 months and yearly afterwards, up to 5 years. The samples are not usually stored for more than 5 years because the maximum shelf life allowed by FDA is 5 years (1, 21).

Even though the stability studies mainly aim at determining the shelf life, it usually helps towards providing evidence on how the quality of a drug substance varies with time under the influence of variety of environmental factors such as temperature, humidity, light, etc. Such information is used in determining the recommended storage conditions and retest periods as well (15).

There are two classes of stability studies: accelerated and long term studies.

2.6.1 Accelerated Stability Studies

These are also known as short term studies or stress testing. The accelerated testing is considered as a practical and economic approach as it speeds up the rate of chemical or physical degradation of the drug product. And this can define the drug properties and estimate the tentative shelf time in a short term. This is conducted through exposing the drug to stressed condition by using a high level of special temperature (e.g., 50, and 75°C) and relative humility conditions (e.g., 75% or greater). Hence, the pharmaceutical companies are usually conducting such type of stability study especially in the early stage of a product development. The main purpose of accelerated stability testing is to estimate essential kinetic parameters, establish the relationship between

decomposition and storage conditions, and to establish a tentative drug shelf life for preparation and design of a long term stability study. The data obtained from accelerated testing are referred to as supportive stability data as opposed to the data resulting from the long term stability study which is known as the primary stability data.

Accelerated testing should be carried out over a 6 month period and at a temperature that is at least 15 °C above the designated long term storage temperature as shown in Table [2.2] (6).

Arrhenius regression model is the most commonly used statistical models for estimation of drug stability parameters in accelerated drug stability studies.

2.6.2 Long Term Stability Studies

The long term studies is carried out over a minimum of 12 months duration on at least three batches at the time of submission and should be continued for a period of time that is equivalent to the presumed shelf life. In long term studies, the drug product is stored at a room temperature, and humidity conditions, and stability testing is performed under regular environmental conditions. In practice, there is usually a batch to batch variation leading to variation in the estimated shelf life of different batches. For this reason, to estimate a single shelf life of the drug, combining data from different batches is preferred.

Table [2. 2]: Temperature and Humidity in Stability Testing (6)

Study	Storage condition	Minimum time period of submission
Long term testing	25°C ±2°C/60%RH±5%	12 months
Accelerated testing	40°C±2°C/75%RH±5%	6 months

2.7 Stability Testing

Stability testing as a requirement by regulatory agencies is developed to determine the active ingredients of every newly made drug product in order to evaluate and provide assurance on detection of changes in its identity, strength, quality, potency and purity.

2.7.1 Stability-Indicating Assay Methods

In pharmaceutical industry, such testing procedures or assays are known as stability indicating assay methods (SIAMs) which are analytical methods that are employed for the analysis of the

samples collected from stability studies (15).

According to 1987 FDA guidelines, the stability indicating methods were defined as the "*quantitative analytical methods that are based on the characteristic structural, chemical or biological properties of each active ingredient of a drug product and that will distinguish each active ingredient from its degradation products so that the active ingredient content can be accurately measured*".

For a stability-indicating assay to be able to provide reliable data along with assurance on detection of changes in the drug products, guidelines require that SIAMs should be validated and suitable for the detection and quantitation of degradation products. To achieve such purpose, SIAMs are required to involve forced decomposition studies (stress testing) where the drug would be degraded under all types of severe stress conditions, such as extremes of PH, and light, oxidation, dry heat, etc. and separation of intact drug from degradation products. Moreover, the SIAMs are supposed to be able to analyze the degradation products (22, 23).

Bakshi and Singh (22) identified two classes of the stability indicating assay methods: i) The specific stability-indicating assay method, which refers to the method that is able to measure unequivocally the drug in the presence of all degradation products, excipients, and additives expected to be present in the formulation. ii) The selective stability indicating assay method which refers to the method that is able to measure unequivocally the drug and all degradation products in the presence of excipients, and additives expected to be present in the formulation.

2.7.2 Techniques of Stability-Indicating Assay

A review of literature shows that over the past 4 decades a large number of techniques have been commonly used in analysis of stability samples. These techniques mainly include titrimetric, spectrophotometric, and chromatographic as well as other miscellaneous methods

1-titrimetric and Spectrophotometric

These methods aims at the analysis of the drug under study alone in the matrix of excipients, and additives, degradation products, impurities, etc., and also other drugs in case of the combination products. They are not expensive and simple to perform but rarely used because of limited sensitivity and specificity.

2- Chromatographic

This technique is widely used in the analysis of stability samples thanks to its advantage of separating multiple components as well as its high accuracy and sensitivity for even small quantities of degradation products produced. Chromatographic methods that are commonly used include: thin-layer chromatography (TLC), high performance thin layer chromatography (HPTLC), gas chromatography (GC), high performance liquid chromatography (HPLC), and newer technique like capillary electrophoresis (22).

As an example of HPLC application; Mohammadi et al (24) developed a validated stability-indicating HPLC analytical method for the determination of Oristat in API and dosage forms. The results of stress testing undertaken according to the International Conference on Harmonization (ICH) guidelines reveal that the method is selective and stability-indicating. The proposed method is simple, accurate, precise, specific, and has the ability to separate the drug from degradation products and excipients found in the capsule dosage forms. The method is suitable for use for the routine analysis of Orlistat in either bulk API powder or in pharmaceutical dosage forms. In addition, the HPLC procedure can be applied to the analysis of samples obtained during accelerated stability experiments to predict expiry dates of pharmaceuticals.

3- Miscellaneous

The famous example is proton nuclear magnetic resonance (NMR) spectroscopy.

Bakshi and Singh (22) proposed a systematic approach for the development of a stability-indicating method by following these steps:

Step 1: Critical study of the drug structure to assess the likely decomposition route(s)

Step 2: Collection of information on physicochemical properties.

Step 3: Stress (forced decomposition) studies

Step 4: Preliminary separation studies on stress samples

Step 5: Final method development and optimization

Step 6: Identification and characterization of degradation products and preparation of standard

Step 7: Validation of the stability-indicating assay method.

2.7.3 Analytical Method Validation

Validating the stability-indicating assay methods is a vital step in stability testing. Without validation, analytical method can fail to accurately study the degradation of the drug product over time. Also, it may not be able to accurately estimate the drug expiration dating period. Hence, the regulatory agencies required that any new assay methods to be validated and documented with sufficient laboratory data and information. Thus validation is considered as the process by which the analysis methods are proven through laboratory studies to have the performance features that comply with the intended analytical applications.

A set of analytical validated parameters were suggested by the USP-NF (United States Pharmacopeia –National Formulary) which are: accuracy, precision, limit of detection, limit of quantitation, selectivity, range, linearity, and ruggedness (15, 25).

CHAPTER 3

STABILITY MODELLING AND TECHNIQUES

The main aim of this chapter is to introduce the statistical techniques that will be used in modelling of drug stability studies.

Process controls in pharmaceutical industry is mainly based on statistics as a means of drug behaviour under variable environmental conditions. Several statistical techniques (process models) are applied in the analysis of different process variables and estimation of unknown parameters.

It is crucial to use a suitable process model depending on the complexity of the process control and the number of equations involved. Ideally, the model should take into consideration all the important dynamic behaviour with a reasonable number of equations, variables, and parameters. Two types of models are widely used in practice: empirical and theoretical models. These models differ in their applications advantages and process of their development.

3.1 Empirical Models

Empirical models are developed from experimental data. They are the ideal models to use for complex processes that involve large number of equations and process variables and many unknown parameters such as physical and chemical properties. Compared to theoretical models, empirical models are simpler and easier to develop. Another advantage is that the computational time needed for the model solution is much shorter than the actual process response time. However, one disadvantage of empirical models is their inability to extrapolate well.

Estimation of unknown model parameters and Comparison between several mathematical models can be achieved by statistical analysis (26).

3.1.1. Regression Analysis

Regression analysis is a statistical procedure for estimating the average relationship between the dependent variable, such as label claim and one or more independent variables like, time, pH, temperature etc. (27). Regression analysis is one of the most commonly and extensively used statistical methods in wide spread areas including chemicals and pharmaceuticals (28, 29). Regression analysis attempts to find a line of best fit so that the squared deviations of the observed points from that line are minimized. Thus, this general procedure is referred to as least squares estimation (29).

3.1.1.1 Simple linear Regression

Linear regression is concerned with models linear in parameters. Simple linear regression involves only one independent variable, like temperature and the linear relationship equation is given by

$$y = \beta_0 + \beta_1 x_1 + \varepsilon \qquad \text{[eq3.1]}$$

Where the intercept β_0 & slope β_1 are unknown linear parameters, called regression coefficients, y and x are dependent and independent variables respectively.

3.1.1.2 Multiple Linear Regressions

Multiple regressions involve two or more independent variables. A multiple linear regression model that might describe the relationship is:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + ... + \beta_k x_k + \varepsilon \qquad \text{[eq3.2]}$$

where y denotes the response (dependent variable) and x_1 to x_k represents the regressors (independent known variables).

The random error term ε is assumed to follow the normal distribution with a mean of 0 and a variance of σ^2 and the errors are not correlated, that is, statistically independent.

The term linear is used because the equation [eq3.2] is a linear function with respect to unknown parameters ($\beta_0, \beta_1, ..., \beta_k$).

The unknown parameters of the model, Eq. [3.2], are estimated by minimizing the sum of squares of the differences between the observed (experimental) values y and the fitted or the predicted value $\hat{y}$ from the model to provide a good fit. So, the least-squares estimator of β is:

$$\hat{\beta} = (X'X)^{-1}X'y \qquad \text{[eq3.3]}$$

Where;

$$X = \begin{bmatrix} 1 & x_{11} & x_{12} & ... & x_{1k} \\ 1 & x_{21} & x_{22} & & x_{2k} \\ & \vdots & & \ddots & \vdots \\ 1 & x_{n1} & x_{n2} & ... & x_{nk} \end{bmatrix}$$

And

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

Since the matrix $(\acute{X}X)$ is non-singular, its inverse is present. This matrix $(\acute{X}X)$ will present if the regressors are linearly independent, that is, a column of the X matrix is not in linear combination with the other columns.

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

$$\hat{y} = X\hat{\beta}$$
[eq3.4]

And the model predictions are given by:

$$\hat{y} = \hat{\beta}_1 + \hat{\beta}_2 x_1 + \dots + \hat{\beta}_k x_k$$
[eq3.5]

Multiple linear regression models are often used to fit empirical models (predictive models) which are obtained by fitting observed (experimental) data set of y and X values. After developing such a model, if an additional value of X is then given without its accompanying value of y, the fitted model can be used to make a prediction of the value of y. (26).

3.1.2. Regression Diagnostics

3.1.2.1 Residual Analysis

Residuals e_i are defined as the difference between actual observation value of the dependent variable y_i and the predicted value $\hat{y}_i$ computed from a regression model.

$e_i = y_i - \hat{y}_i, i = 1, 2, \dots, n,$
[eq3.6]

Residual analysis is helpful to assess the adequacy of the fitted regression model and assess the assumption that the errors are uncorrelated random variables and normally distributed with mean equal to 0 and constant variance σ^2 (29).

3.1.2.2 Residual Plots:

Plot the residuals to check whether the regression model represents the data correctly. If so, the residuals should be normally distributed with a mean equal to zero. If the residuals are distributed with a trend or pattern, the model is then considered inappropriate.

For assessment of regression models by residual plots, the following steps are to be followed:

1- A miss-specified model can be identified by plotting the residuals $(y_i - \hat{y}_i)$ against each independent variable (x) in the model. When a plot has a curvilinear trend, this indicates that the model adequacy could be improved by presence of a quadratic term.

2- An unequal variance can be identified by plotting the residuals $(y_i - \hat{y}_i)$ against the predicted values $(\hat{y}_i)$.

3- Non normal errors can be identified by constructing a histogram for the residuals. Extreme skewness in the histogram can imply presence of outliers.

4- Outliers can be identified by plotting the residuals $(y_i - \hat{y}_i)$ against the predicted values $(\hat{y}_i)$ to locate the ones that are located at 3x standard deviation (or more above or below 0. Outliers can be due to coding or recoding errors. i.e. error while recording the data or measrument. Usually, if they don't influence the model, they can be ignored.

5- Correlated errors are determined by plotting the residuals in time order (30).

3.1.2.3 Coefficient of Determination (R^2)

Coefficient of determination R^2 refers to the ratio between the regression sum of squares (SSR) and the total sum of squares (SST) and it indicates what percentage of the variation present in the data is being provided by the model (29).

$$R^2 = \frac{SSR}{SST} = \frac{SST-SSE}{SST} = 1 - \frac{SSE}{SST} \qquad \text{[eq3.7]}$$

Where SSE is the error sum of squares, SST is the total sum of squares and SSR is the regression sum of squares.

It is also defined as the fraction of the variation in the data that is explained by all the regressors in the model (31)

$$R^2 = \frac{SS\ explained\ by\ all\ the\ regressors}{total\ SS} \qquad \text{[eq3.8]}$$

Coefficient of determination values range between 0 and 1. The value approaching 1 indicates that the regression model explains the data very well (29). If the regression line passes exactly through every point on the scatter plot of data, it would be able to explain all of the variations. The further the regression line is away from the points, the less it is able to explain a variation among data.

It is well known that it is possible to make R^2 equal to one by adding more terms to the fitted polynomial. The statistic R^2 cannot be used to judge the adequacy of the fitted model when nested models are compared. Two models are nested if both contain the same terms and one has at least one additional term. Instead, one should use the mean square errors of the two compared models to choose the model with best predictability. However, one can use R^2 to discriminate between non nested models (26).

3.1.3. Artificial Neural Network (ANN)

Neural network as a term has derived originally from artificial intelligence (AI) research. The main goal of AI was to develop computers that are capable of solving problems through the use

of symbolic non algorithmic methods. Neural network is one of the main major AI- based techniques that is widely used in computer engineering as well as chemical engineering reactors, and also it has non-engineering applications such as medical diagnosis, stock market analysis, and cheese strategies.

The great significance of neural networks is attributed to its applicability to model complex processes with large input-output data set when the real model is not known, as well as its high efficiency in time and effort saving. Moreover, NN plays an important role in modelling non-linear systems (26).

Robert Hecht-Nielsen (1990) defines a neural network as "*a computing system made up of a number of simple, highly interconnected nodes or processing elements, which process information by its dynamic state response to external inputs*".

The neural network works in a similar way as the human brain where the nervous system performs its function through interaction between multiple components and complex pathways. The process starts at special receptors in human body which receives the stimuli (input) and then transmit them to the brain via neurons. In the brain, other neurons process this information and produce output that is usually based on the brains past experience. Similarly, ANNs function through setting up fixed systems by which certain outputs are produced from specific inputs (32).

To ensure accuracy of the resultant output, every neural network should undergo a training process during which a set of data are tried first several times to confirm the presence of real relationship between the process input and output. The next step will be then to make sure that the desired relationship was learned (33).

3.1.3.1 Artificial Neural Network Structure

The ANN, as it is shown in Figure [3.1] is composed of three different layers: inner layer, hidden layer, and an output layer along with highly interconnected network of nodes. All these layers are composed of one or more neurons (32). One of the common types of NNs is a feed-forward network in which a neuron in each layer is connected to all neurons in the next layer. (33).
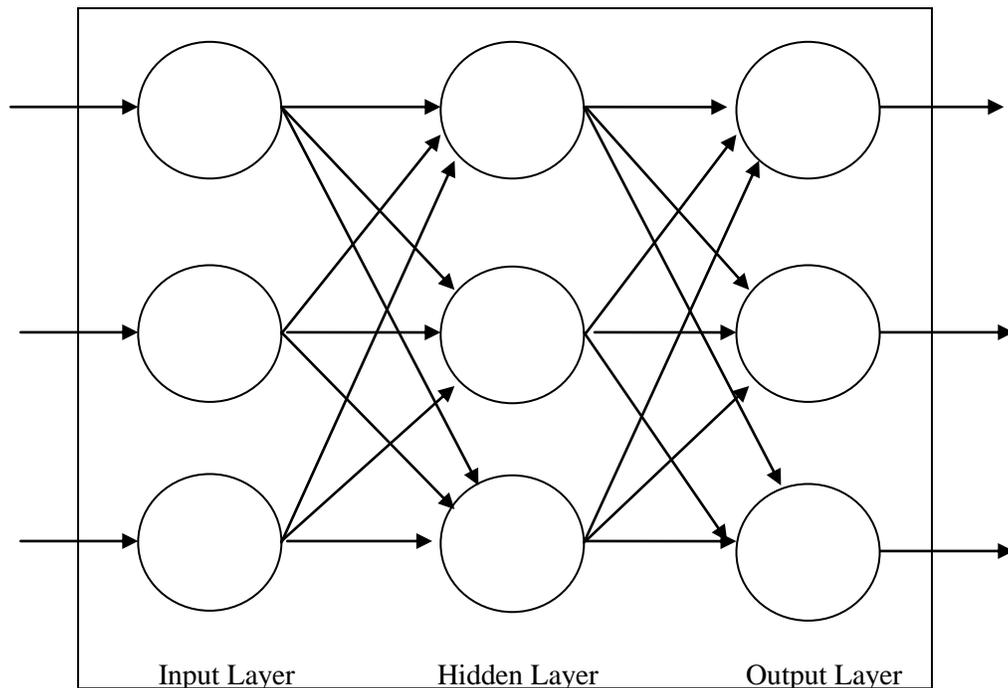
Figure [3. 1]: A typical Neural Network Formed by an Interconnection of Nodes (32)

The node is the basic structure of NN that functions as the processing element which performs most of the calculations. The node receives and manipulates the NN inputs to give the output. There are three factors that determine the output: weight factors W_j, internal thresholds T_j, and transfer function f . The Figure [3.2] shows the structure of neuron that transfers the input a_i to the jth output b_j through a weight factor W_j and a transfer function $f(x_j)$. T_j is the internal threshold.
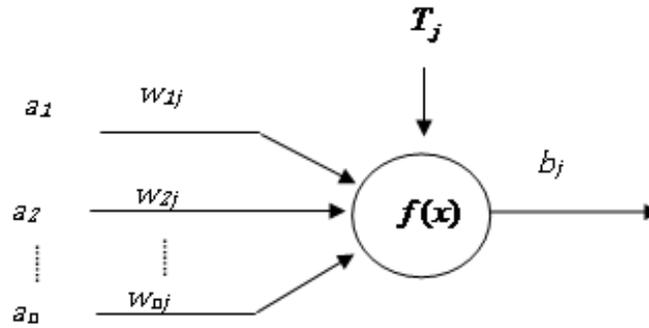
Figure [3. 2]: Schematic Display of Neuron (26)

Every link between two neurons has a specific weight that represents the strength of their connection. These weights are unknown parameters that need to be estimated out of the modelling process by using input and output data set (26). As shown in the following equations, when such weights are multiplied by their inputs and summed together followed by subtracting the internal threshold, the total activation will result. This total activation is then applied to a transfer function to calculate the output. The following equations are given below

$$\text{Total activation} = x_j = \sum_i w_{ij} a_i - T_j \qquad \text{[eq3.9]}$$

The output of the neuron can be shown as:

$$z = f(x_i) = f\left(\sum_i w_{ij} a_i - T_j\right) \qquad \text{[eq3.10]}$$

Where; f is the transfer function. The most commonly used functions are sigmoid, hyperbolic tangent and the radial basis functions. Mathematicians and computer scientists found the sigmoid, hyperbolic tangent to be very useful for prediction purposes, whereas the radial basis functions were considered to be the most efficient in classification networks. However, any functional form can be used such as square root, log, e^x, etc. (32).

34

It is very important to normalize the inputs and the outputs to avoid any false influence of factors with higher magnitude when they are not of the same order of magnitude.

The data can be normalized by using the following formula

$$\hat{x} = \frac{x - x_{min}}{x_{max} - x_{min}}$$

[eq3.11]

Where $\hat{x}$ is the normalized value, and x_{min} and x_{max} are the minimum and maximum values of x, respectively. x can be either input or output variable.

Two steps are considered in the developing of artificial neural network model. The first step is a training stage, where the network is exposed to training set of inputs and outputs to learn the relationship between input and output variables. The second step is considered as a testing stage, where the function of the network is tested on patterns that the network has not been subjected during the training stage. So, the trained network can be used to predict the output for data points that were not used in training the network (34).

3.1.3.2 Back Propagation Algorithm

Different algorithms can be implemented to adjust the weights in order to map input-output correctly. One algorithm which has greatly contributed to neural network notoriety is the back-propagation algorithm. This algorithm involves an iteration search where the weights from output layer are adjusted back to input layer in each run. This process is repeated until no further improvement in the mean squares error (MSE) value is found.

Feed-forward interlayer connections are required for back propagation where each layer feeds into the following layer subsequently. The back propagation algorithm relies on the error-correction learning that is an optimization process using steepest gradient descent principle (35).

3.2. Theoretical (Mechanistic) Models

Theoretical models are established by using the principles of chemistry, physics, and biology. The advantages of these models represent in their applicability in many different conditions. They can lead to a great deal of understanding of the physical behaviours. However, the fact that their development can be expensive and time-consuming is considered as a disadvantage. Moreover, in case of complex processes, some theoretical model parameters such as reaction rate coefficients, physical properties or heat transfer coefficients are usually unknown.

Generally, theoretical models of chemical processes follow ordinary differential equation (ODE) and/or partial differential equation (PDE) along with algebraic equations. Through these equations, these models allow a reasonable extrapolation and estimation of unmeasured variables that can't be achieved. Theoretical models are formulated by using unsteady-state conservation laws such as the conservation of mass and energy.

- **Conservation of mass**

{Rate of mass accumulation} = {rate of mass in} − {rate of mass out}

- **Conservation of component i**

{Rate of component i accumulation}= {rate of component i in} − {rate of component i out} ± { rate of component i produced or consumed}

- **Conservation of energy**

{Rate of energy accumulation} = {rate of energy in by convection and conduction} − {rate of energy out by convection} + {net rate of heat addition to the system from the surrounding} + {net rate of work performed on the system by the surrounding} (26)

3.2.1 Kinetics of Chemical Reactions

Understanding the kinetics of the drug decomposition is important for the shelf life prediction (17). The rate of change in the concentrations of the reactants and products can be used to characterize the rate of a chemical reaction.

General chemical reaction equation $\qquad A+B \longrightarrow C+D$

The rate of disappearance of reactant A, $-r_A$, is dependent on concentration and temperature.

$$\frac{dC_A}{dt} = - K_{(\alpha+\beta)} C_A{}^{\alpha} C_B{}^{\beta} \qquad\qquad [eq.3.12]$$

where;

$\frac{dC_A}{dt}$ is the rate of chemical decomposition A with respect to the time.

k is the reaction rate constant and its unit depends on the order of the chemical reaction.

C_A & C_B are the concentrations of the reactants A and B, respectively.

α & β are the partial orders of the reaction and they are determined experimentally.

The overall reaction rate order is $\alpha + \beta$.

3.2.2 The Order of the Reaction

The decomposition reaction of the drugs can be a simple that falls under the category of one of the well known reaction orders namely; zero, first, second and third order reactions. However, most of the drugs decompose through multiple steps or complex reactions.

If the general degradation reaction of a drug product is $A \longrightarrow B$

The reaction rate is $\dfrac{dC}{dt} = -k \, C_A{}^{\alpha}$ [eq3.13]

3.2.2.1 Zero-Order Reaction:

The rate of the drug product degradation $\dfrac{dC}{dt}$ is independent on the initial concentration of the reactant.

The reaction rate equation is given by the following;

the differential equation form is $\dfrac{dC_A}{dt} = -k_0$, and [eq3.14]

the integrated form is $C_A = C_{A0} - k_0 t$ [eq3.15]

where;

C_{A0} & C_A are the concentrations of the drug at time zero and time t, respectively.

k_0 is the zero-order rate constant and its unit is, for example, $\dfrac{mole}{liter.second}$

The linear relationship between the concentration C_A and time t is obtained in the zero-order reactions. Acetyl salicylic acid hydrolysis follows zero order kinetics (17, 36).

38

3.2.2.2 First-Order Reaction

The rate of the degradation of a drug product $\frac{dC}{dt}$ is dependent on the initial concentration of the reactant.

The reaction rate equation is given by;

The differential equation form is $\frac{dCA}{dt} = - k_1 C_A$ [eq3.16]

The integrated form is $C_A = C_{A0} \exp(-k_1 t)$ [eq3.17]

The first order equation can be written in a linearized form;

$\ln C_A = \ln C_{A0} - kt$ [eq3.18]

Where;

$\ln C_{A0}$ and $\ln C_A$ are the natural logarithm of the concentration at time zero and time t, respectively.

k_1 is the first-order rate constant and its unit, for example, is $\frac{1}{Second}$

The linear relationship between the logarithmic concentration $\ln C_A$ and the time t is obtained in the first-order reaction. Drugs that hydrolyse in solution usually follow first order reaction (17)

3.2.2.3 Second Order Reaction
The reaction rate equation is given by;

The differential equation form is $\frac{dC}{dt} = - k_2 C_A^2$ [eq3.19]

The integral form is $\frac{1}{CA} - \frac{1}{CA0} = k_2 t$ [eq3.20]

where;

C_{A0} & C_A are the drug concentrations at time zero and time t, respectively.

K is the second-order rate constant and its unit, for example, is $\dfrac{liter}{mole.second}$

The linear relationship between the reciprocal concentration $\dfrac{1}{CA}$ and time t is obtained in the second-order reaction (36)

3.2.3 Arrhenius Regression Method

Arrhenius regression model is the most commonly used statistical models for estimation of drug stability in accelerated drug stability studies. Its function is to develop a relation between rate constant and temperature to estimate the potential shelf life of a drug product during the early stages of its pharmaceutical development. By this method, the effect of temperature on chemical stability as well as the effect of a catalyst on decomposition can be predicted. The Arrhenius method is expressed mathematically by the following equation:

$k(T) = A \times \exp(-Ea/[R \times T])$ [eq3.21]

$\ln K = \ln A - Ea/[R \times T]$ [eq3.22]

where:

T = Temperature (Kelvin)

R = 1.987 (Rate Gas Constant, cal/mol.k)

E_a = Activation Energy (cal/mole)

A = Frequency Factor

k(T) = Rate Constant of any order as a function of absolute temperature

Arrhenius method has been traditionally used to describe the temperature dependency for various chemical reactions by regarding E_a and A to be independent of temperature.

One of the most important applications of Arrhenius equation is to predict the drug stability at room-temperature or any lower or slightly upper temperature from accelerated data. This can be accomplished by; firstly, determining the corrected order of the degradation reaction experimentally in order to describe the functional relationship between the drug concentration and time and obtain the degradation rate constants (k) at different temperatures; secondly, setting up the Arrhenius equation relates the logarithm of reaction rate linearly against reciprocal of the elevated temperature to determine the activation energy and the frequency factors.

This method has been successfully applied to estimate the shelf life and predicting the stability of various pharmaceutical drug products (2).

The next chapters 4, 5, and 6, will present three case studies related to three different drug products that were taken from pharmaceutical articles. On each drug, different empirical and kinetic mathematical models were applied. The predicted values obtained from these models were then compared with the observed values available from experiments.
The smaller the differences (or the so called errors) between these two values, the more reliable the predicted values. Hence, depending on the value of these errors, one can firstly decide which model is the best among the three and secondly, whether statistical modelling techniques can reliably substitute the experimental methods in practice.

CHAPTER 4

STABILITY OF ASPIRIN TABLETS

In this case study, the experimental data for the stability of acetyl salicylic acid (ASA) tablets were taken from the article "Stability of Aspirin in A Moisture Containing Direct Compression Tablet Formulation" by Michael, et al (37).

In this experiment, ASA was mixed with a mixed-sugar diluent containing about 8% moisture for 20 minutes and then blended after adding stearic acid for 3 minutes. A part of the blend was compressed into tablets. Both tablets and uncompressed powder blend were packaged into separate glass bottle containers. Stress stability studies were conducted on the compressed tablets (10 tablets per bottle) and the powder blend (3 grams per bottle).

The bottles of tablets and powder blend were stored under different temperatures 35, 40, 45, and 50 $^{\circ}$C) for different time intervals then the stability of these drug samples was assessed according to the rate of decomposition. Each time, the content of each bottle rinsed and ground with acetonitril and then filtered and assayed for ASA remaining by using stability indicating simultaneous UV assay.

It was concluded by this experiment that aspirin degradation is accelerated with time and with temperature increment. The rate of degradation was found to be following the emperical equation: $y = 100 - Kt^n$; where y is the % ASA remaining, t is the time, and k and n are constants. The formulation tested proved good stability with only $< 1\%$ degradation after 1.75 years at room temperature. Moreover, the moisture content in this formulation had no influence on the decomposition rate without an obvious explanation.

4.1 Experimental Data:

Experimental data on Aspirin stability were collected and all the three mathematical models (linear regression, artificial neural network, and mechanistic models) were conducted on these data to determine the model that best predicts its stability. A comparison among these three mathematical models was made at the end to conclude the best statistical method that should be adopted in practice to achieve the most accurate shelf life.

Table [4.1] shows both independent variables (temperature and time) and dependent variables (percentage of ASA remaining).

Table [4. 1]: Effect of Temperature and Time on the %ASA Remaining

Independent Variables			Dependent Variable
Temperature (Celsius)	Temperature (Kelvin) X_1	Time (Weeks) X_2	%ASA Remaining Y
50	323	1.19023	98.5603
50	323	4.12644	96.5046
50	323	7.13936	92.0614
50	323	10.1548	86.7537
50	323	13.8791	77.577
45	318	1.25856	99.0543
45	318	7.2619	97.9072
45	318	14.1113	93.7145
45	318	20.188	91.3324
45	318	26.2672	88.0858

45	318	30.1097	86.278
40	313	1.25749	99.4248
40	313	9.14346	98.6499
40	313	15.2181	96.9676
40	313	20.1743	96.0253
40	313	44.1901	90.6133
35	308	1.25749	99.4248
35	308	14.235	99.1485
35	308	20.2368	98.5365
35	308	35.1707	97.274
35	308	45.9181	96.1724

4.2 RESULTS AND DISCUSSION:

4.2.1 Linear Regression

As it is known, the linear regression model is concerned with models that are linear in parameters. In this experiment, the values of the independent variables are perfectly known and a linear model was created to estimate the unknown parameters.

The coefficient of determination was found to be 84.67% and the standard error was 2.51 as it is shown in the table [4.3].

Table [4. 2]: Statistical Results of Regression Model

Regression	Statistics
Multiple R	0.920195
R square	0.846759
Adjusted R square	0.808449
Standard Error	2.51667
Observations	21

Table [4. 3]: ANOVA Table

Source of variance	DF	SS	MS	F critical	F Significance
Regression	4	559.9597	139.9899	22.10265	2.36E-06
Residual	16	101.338	6.333626		
Total	20	661.2977			

The underlying model:

The following table [4.4] represents the parameters that have significant effects as has been found from ANOVA table.

Table [4. 4]: Parameters Estimations

	Parameters Values	Standard error	t observed	P-Value	Lower 95%	Upper 95%
Intercept (β_0)	-8803.22	2618.405	-3.36205	0.003965	-14354	-3252.45
Temp K (β_1)	56.55087	16.53652	3.419757	0.003511	21.49502	91.60672
Time (β_2)	17.26145	3.327954	5.186805	9E-05	10.2065	24.31639

Temp*Time (β_3)	-0.05608	0.010653	-5.26406	7.71E-05	-0.07866	-0.03349
Temp square (β_4)	-0.08977	0.026101	-3.43942	0.003368	-0.14511	-0.03444

$t_{critical} = 2.05183$

General regression model

$$\hat{\gamma} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \beta_4 X_2^2 + \varepsilon \qquad \text{where } \varepsilon \sim N(0,\sigma^2)$$

Fitted linear regression model

$$\hat{\gamma} = -8803.22 + 56.55087 X_1 + 17.26145 X_2 - 0.05608 X_1 X_2 - 0.08977 X_2^2 + \varepsilon$$

Model evaluation:

To test the validity of the underlying assumption (error $\sim N(0,\sigma^2)$ as a diagnostic check, we are going to look at the residuals and normal probability plots from the fitted model. Also determining the coefficient of determination to measure how well the linear regression model represents the data.

❖ **Residual plots**

Using the residual plots is to investigate whether the errors are scattered, independent and normally distributed. See Figure [4.1].
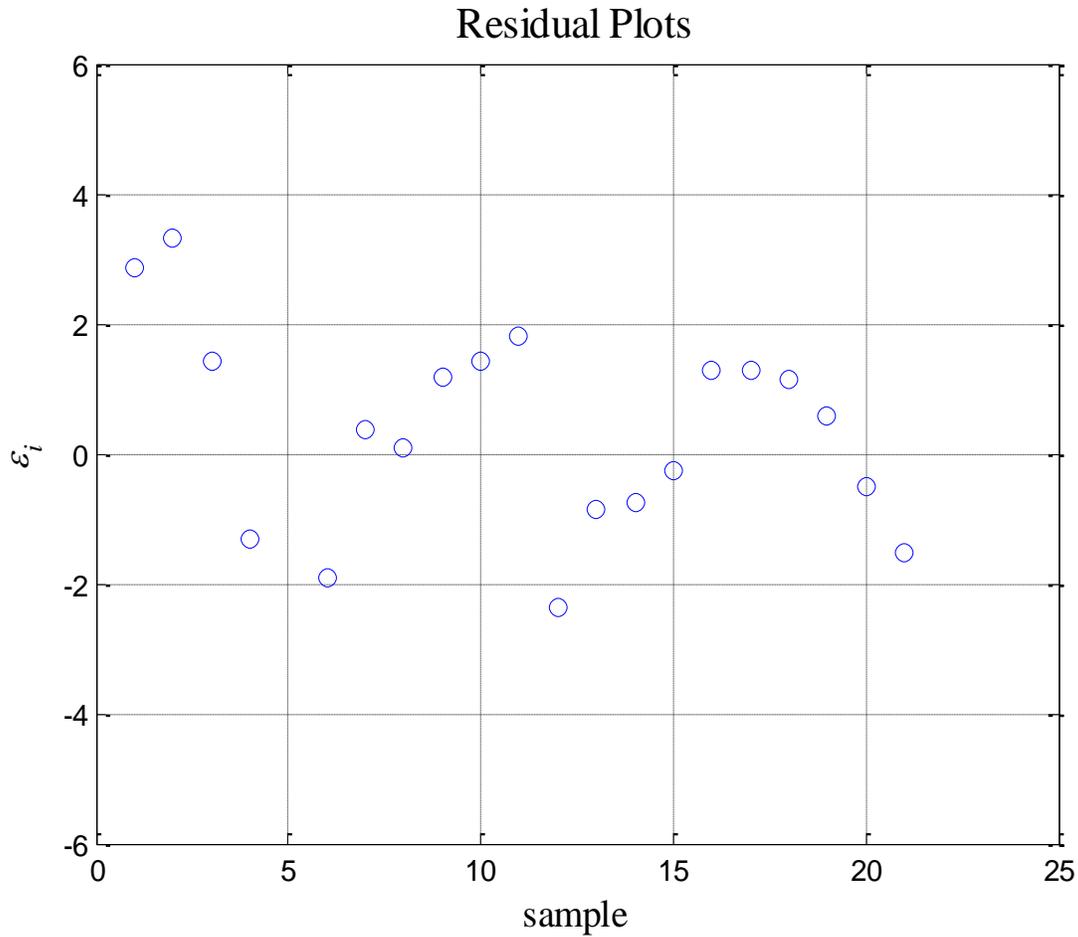
Figure [4. 1]: Aspirin Residual Plots

We can see from the Figure 4.1 that the residual has a random pattern.

❖ **Normal probability plot:**

Using normal probability plots to investigate whether the error are normally distributed. See Figure [4.2].

Normal Probability Plot
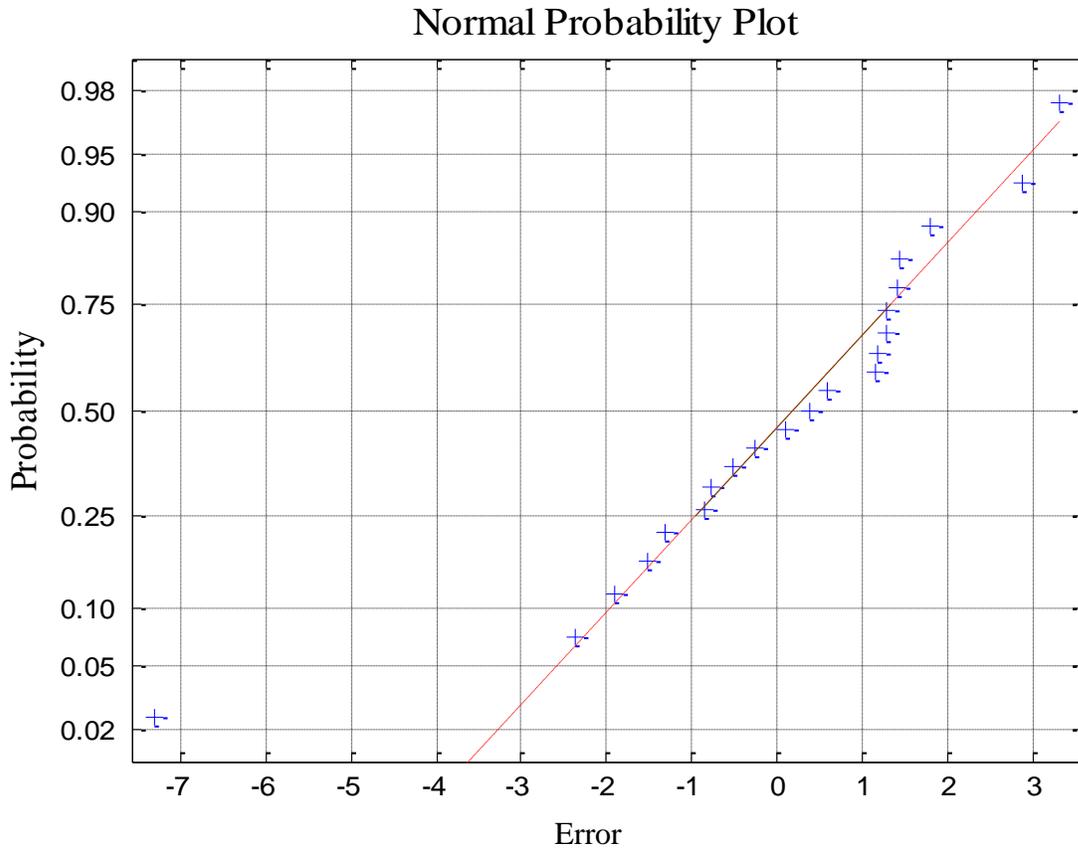
Figure [4. 2]: Aspirin Normal Probability Plot

Shown in Figure [4.2] is that the normal probability plot shows a straight line. So, we can conclude that the observed sample comes from a normal distribution.

❖ **Coefficient of Determination (R^2)**

Using the coefficient of determination as a diagnostic check is to measure whether the regression line represents the data.

From the results, it is shown that $R^2 = 0.8467$, which means that 84.67% of the total variation in observed sample can be explained by the linear relationship between the regressors and observed response (as described by the regression model). The other 15.3241% of the total variation in the observed sample are unexplained.

4.2.2 Artificial Neural Network (ANN)

Back propagation feed forward based on Levenberg-Marquadt training algorithm was employed to train the network. MATLAB® neural network toolbox was used to train and simulate the network model. Log sigmoid was found to give reasonable response.

The data was randomly divided into a 15 training set and a 6 testing set as shown in Tables [4.6] and [4.7]. About 10 – 15 % of the data was selected randomly to be the testing data set. Then before training, the data was normalized by using the following formula $\hat{x} = \dfrac{x - x_{min}}{x_{max} - x_{min}}$

So that, the inputs and outputs are belong to the interval [0, 1].

Table [4. 5]: A Training Data Set of Aspirin

Training Data Set		
Temperature(K)	**Time (Weeks)**	**ASA remaining %**
323	7.1394	92.0614
318	20.1880	91.3324
318	1.2586	99.0543
318	7.2619	97.9072
313	20.1743	96.0253
313	9.1435	98.6499
318	30.1097	86.2780
323	13.8791	77.5770
308	35.1707	97.2740
308	20.2368	98.5365
318	14.1113	93.7145

313	44.1901	90.6133
308	14.2350	99.1485
308	45.9181	96.1724
308	1.2575	99.4248

Table [4. 6]: A Testing Data Set of Aspirin

Testing Data Set		
Temperature(K)	**Time (weeks)**	**ASA remaining %**
323	1.1902	98.5603
323	4.1264	96.5046
323	10.1548	86.7537
318	26.2672	88.0858
313	1.2575	99.4248
313	15.2181	96.9676

The result was acceptable by using 300 epochs of training, with the goal 10^{-4} and 10 neurons and one hidden layer. Tan sigmoid functions were applied in this set of data.

The coefficient of determination (R^2) was 72.016% for training data set and 98.756% for the testing data set as shown in Tables [4.8] and [4.9], respectively. Since R^2 approaches 1, it indicates an excellent predictive ability of the neural network model.

Table [4. 7]: Statistical Properties for the Training Data Set

Average error	1.94692
Maximum error	10.87060
Minimum error	0.00541
R^2	0.72016

Table [4. 8]: Statistical Properties for the Testing Data Set

Average error	3.88989
Maximum error	10.87060
Minimum error	0.00541
R^2	0.94009
SSE	9.1008
MSE	1.5168

As it is shown in Figure [4.3], the parity line passes through almost all the observed and predicted values on the scatter plot. This indicates high accuracy of the predicted values with close proximity to the observed data.
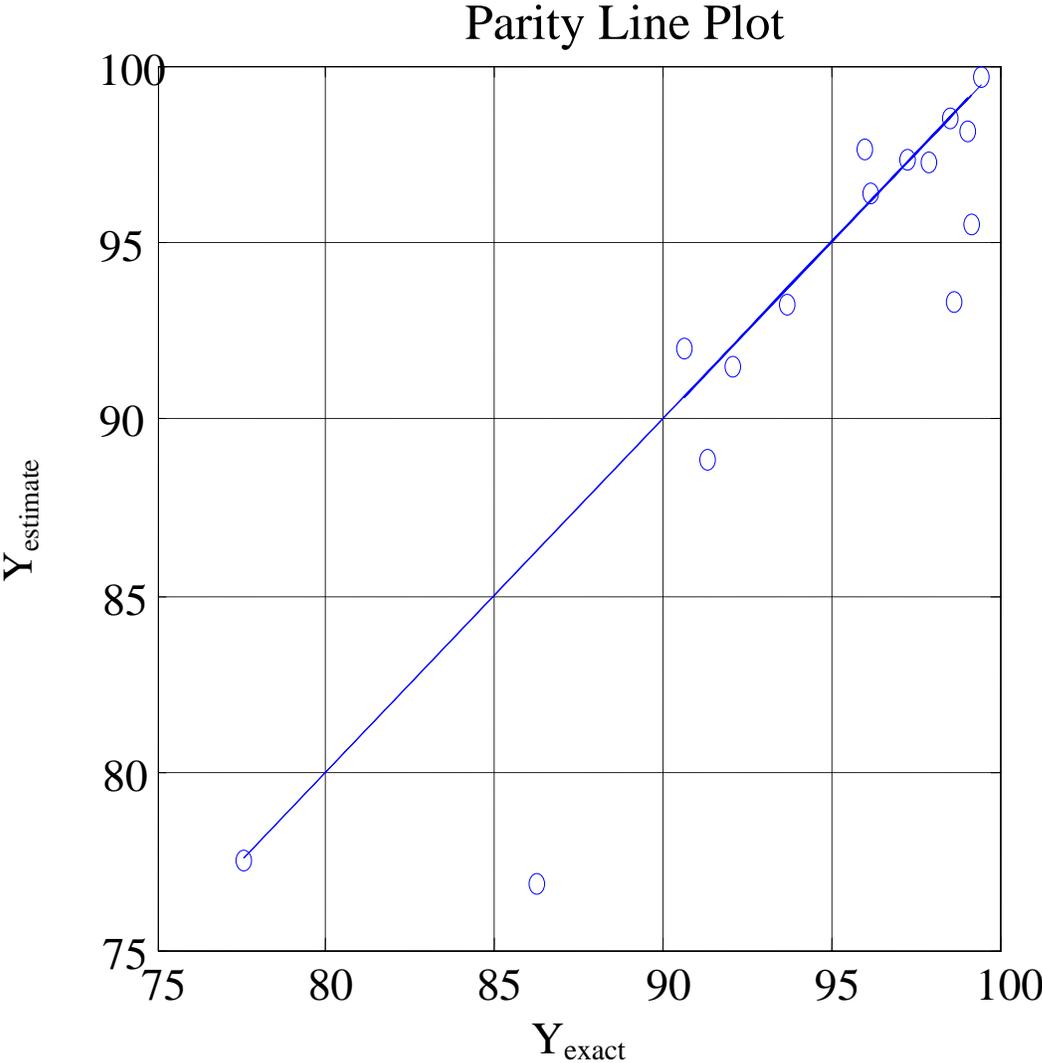
Figure [4. 3]: Parity Line Plot for Aspirin Training Datasets

Since there was no enogh data to be used both for training and testing, it wasn't possible to clearly show how close the predicted values are to the observed. This can be seen in the Figure [4.4] where the straight line does not pass through all the points. However, the points fall close to a parity line.
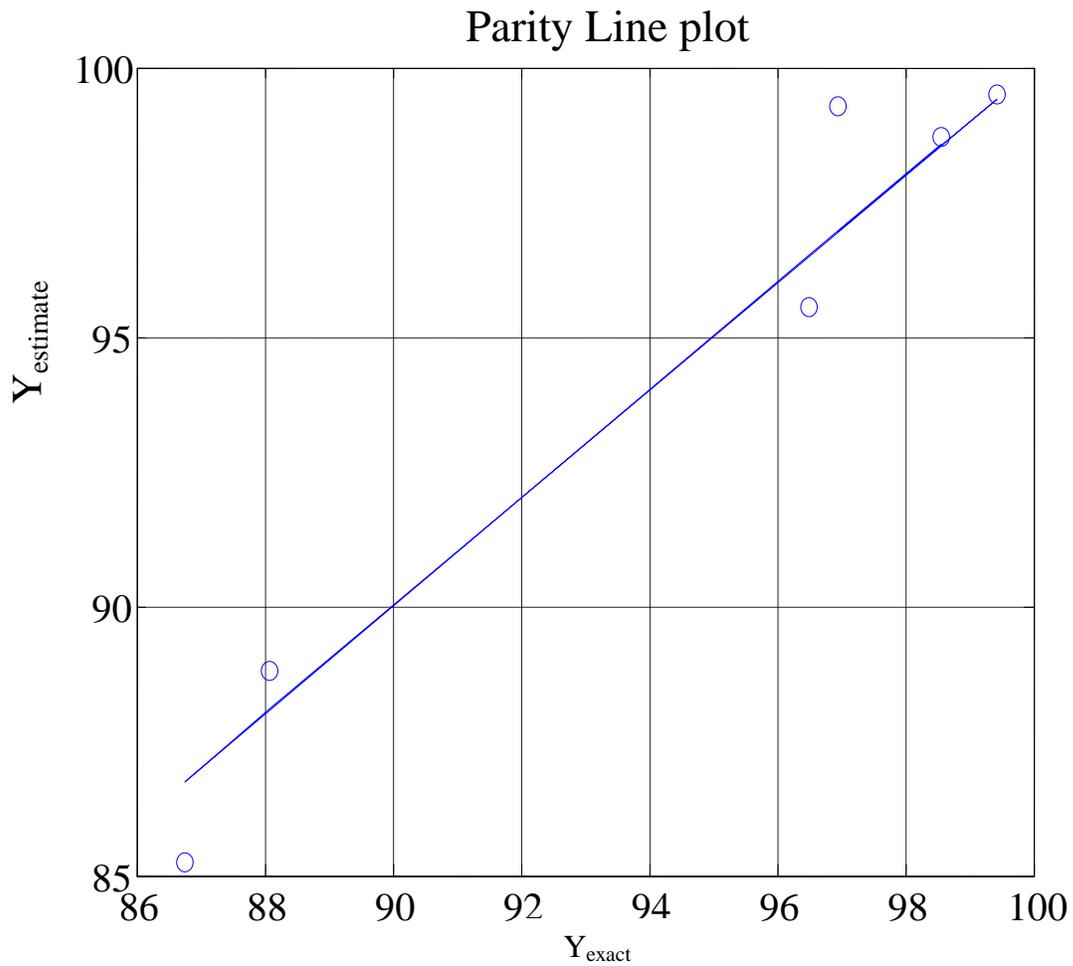
Figure [4. 4]: Parity Line Plot for Aspirin Testing Datasets

Figure [4.5] shows the distribution of the errors. Most of the errors lie in the range between 0-1%.
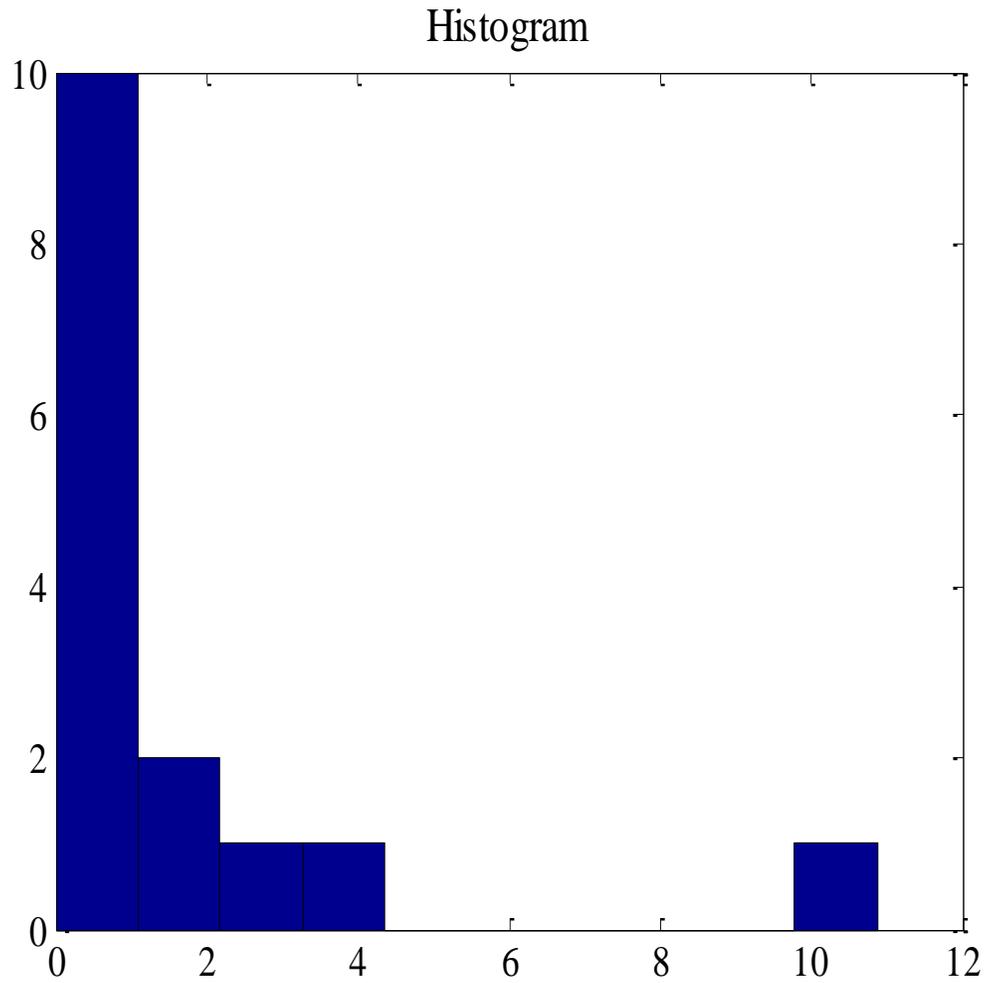
Figure [4. 5]: Aspirin Histogram

4.2.3 Mechanistic Model

Microsoft Office Excel© version 2007 was used for mechanistic model calculations in this research. The determination of degradation kinetic orders as well as the reaction rate constants was attempted. A mechanistic model for Aspirin was propose as shown in the Equation [4.1] and both of the rate constant and the rate order were determined at different temperatures using a linear regression method.

$$-r_a = k_A C_A{}^\alpha \hspace{8cm} [eq4.1]$$

Equation [4.1] was linearized as:

$$\ln(-r_a) = \ln(k_A) + \alpha \ln C_A \hspace{6cm} [eq4.2]$$

The results are summarized in the following Table [4.9].

Table [4. 9]: The Reaction Rate Constant (k) for Aspirin Degradation at Various Temperatures

Temperature(K)	Reaction rate constant k_A (Week^{-1})	The reaction order (α)
323	0.197484	0.9784
318	0.053547	0.9428
313	0.027645	0.9681
308	0.003763	0.92

The hydrolysis process of aspirin was found to follow first order kinetics. The mean of squared error was found to be 2.92×10^{-7} and the coefficient of determination was 96.48%.

The natural logarithm of observed rate constants over the temperature range of 40-63°C were plotted versus reciprocal of temperature according to the Arrhenius equation to calculate the frequency factor and the activation energy. The activation energy was calculated from the slope of the straight line obtained by linear regression method and found to be approximately 49 Kcal/mole and the frequency factor was approximately 75 week^{-1}.

CHAPTER 5

STABILITY OF AMOEBICIDE DILOXANIDE FURATE DRUG

Diloxanide Furoate (DF) is a luminal Amoebicide. After being administered orally, it gets hydrolyzed to produce active ingredient; diloxanide in the gastrointestinal tract. Hence, its function requires it to remain stable in the gut where different PH media is encountered.

A study was performed by Gadkariem, et al (38) on DF stability at different temperature in alkaline media using a stress stability study and Arrhenius regression equation for prediction of its shelf life and in order to obtain a drug with a better formulation and design.

In this experiment, the standard solution was prepared by diluting 200 µg ml^{-1} of DF solution in methanol with phosphate and alkaline borate buffers and the kinetic of drug degradation was controlled by HPLC method. Initially, the pH-rate profile for the hydrolysis of DF at 40°C (pH range 7.0-10.0) was studied by plotting the log of remaining drug versus time. The effect of temperature on DF solution stability was then studied. Four 50-ml flasks were each filled with 5ml of DF solution (200 µg ml^{-1}) and an alkaline borate buffer (pH=8.0). The rate of drug hydrolysis was studied at various temperatures (40, 50, 55 and 63°C) using HPLC method. When they plotted the log % remaining drug versus time (0-100 minutes), the hydrolysis process was found to follow first order kinetic with an estimated activation energy of hydrolysis of 18.25 Kcal.mol^{-1}.

This study concluded that the DF drug decomposes in solutions by hydrolysis process that is temperature and pH dependent. The three DF decomposition products are diloxanide (the active drug), furoic acid, and methylfuroate. They were identified by using HPLC, TLC, mass

spectrometry, and infrared methods. In addition, the effect of simulated gastric and intestinal fluid on DF drug was studied and it was found that this drug undergoes an enzyme-catalyzed hydrolysis in the intestines to its active form (diloxanide) and furic acid.

5.1 Experimental Data:

Experimental data on Diloxanide Furoate stability were collected and all the three mathematical models (linear regression, artificial neural network, and mechanistic models) were conducted on these data to determine the model that best predicts its stability. A comparison among these three mathematical models was made at the end to conclude the best statistical method that should be adopted in practice to achieve the most accurate shelf life.

Table [5.1] shows both independent variables (temperature and time) at pH=8.0 and dependent variables (percentage of Diloxanide Furoate remaining).

Table [5. 1]: Effect of Temperature and Time on the %DF Remaining

Independent Variable		Dependent Variable
Temperature (Kelvin) X_1	Time (minutes) X_2	%DF Remaining Y
336	0	100
336	5.4095	79.5463
336	10.305	63.7309
336	15.2084	46.5246
336	20.4931	35.456
336	25.6549	25.3355
336	30.1576	22.2756

336	35.3242	15.0321
336	41	11.1322
336	45.8973	8.363
336	50.409	6.605
328	0	100
328	10.6751	76.2062
328	20.7097	57.248
328	30.6184	41.7946
328	40.6548	30.7312
328	50.6906	22.7583
328	70.5163	10.9742
323	0	100
323	15.5551	76.9946
323	30.4649	56.4352
323	45.8881	43.3162
323	60.6726	32.0782
323	75.072	23.7564
323	90	17.4678
313	0	100
313	20.801	89.6004
313	40.1926	77.4819
313	60.6146	64.1874
313	80.0062	55.634
313	100	47.3053

5.2 RESULTS AND DISCUSSION:

5.2.1 Linear Regression

As it is known, the linear regression is concerned with models that are linear in parameters. In this experiment, the values of the independent variables are perfectly known and we want to create a model to estimate the unknown parameters.

The coefficient of determination was found to be 93.1266% and the standard error was 8.421122 as shown in Table [5.2]. This result indicates that the model highly represents the data.

Table [5. 2]: Statistical Results of Regression Model

Regression	Statistics
Multiple R	0.965073
R square	0.931366
Adjusted R square	0.92374
Standard Error	8.421122
Observations	31

Table [5. 3]: ANOVA TABLE

Source of variance	DF	SS	MS	F critical	F Significance
Regression	3	25982.78	8660.926	122.1306	8.1E-16
Residual	27	1914.713	70.91529		
Total	30	27897.49			

The underlying model:

Table [5.4] represents the ANOVA table and show the parameters that have significant effects in

the regression.

Table [5. 4]: Parameters Estimations

Parameters		Parameters values	Standard error	t observed	P-Value	Lower 95%	Upper 95%
Intercept	(β_0)	136.6907	17.04501	8.019395	1.28E-08	101.7172	171.6642
Temp.	(β_1)	1.412406	0.359157	3.932557	0.000529	0.675477	2.149336
Time	(β_2)	-0.89834	0.312237	-2.8771	0.007748	-1.53899	-0.25768
Temp*time	(β_3)	-0.04816	0.00718	-6.70726	3.36E-07	-0.06289	-0.03342

$\mathbf{t_{critical}} = 2.776445$

General regression model

$$\hat{\gamma} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \varepsilon \qquad \text{where } \varepsilon \sim N(0, \sigma^2)$$

Fitted linear regression model

$$\hat{\gamma} = 136.6907 - 1.412406X_1 - 0.89834X_2 - 0.04816X_1X_2 + \varepsilon$$

Model Evaluation:

To test the validity of the underlying assumption (error $\sim N(0,\sigma^2)$ as a diagnostic check we are

going to look at the residuals, normal probability plots from the fitted model. Also, it is

important to determining the coefficient of determination to measure how well the linear

regression model represents the data.

❖ **Residual Plots**

Using the residual plots is to investigate whether the errors are scattered, independent and normally distributed.
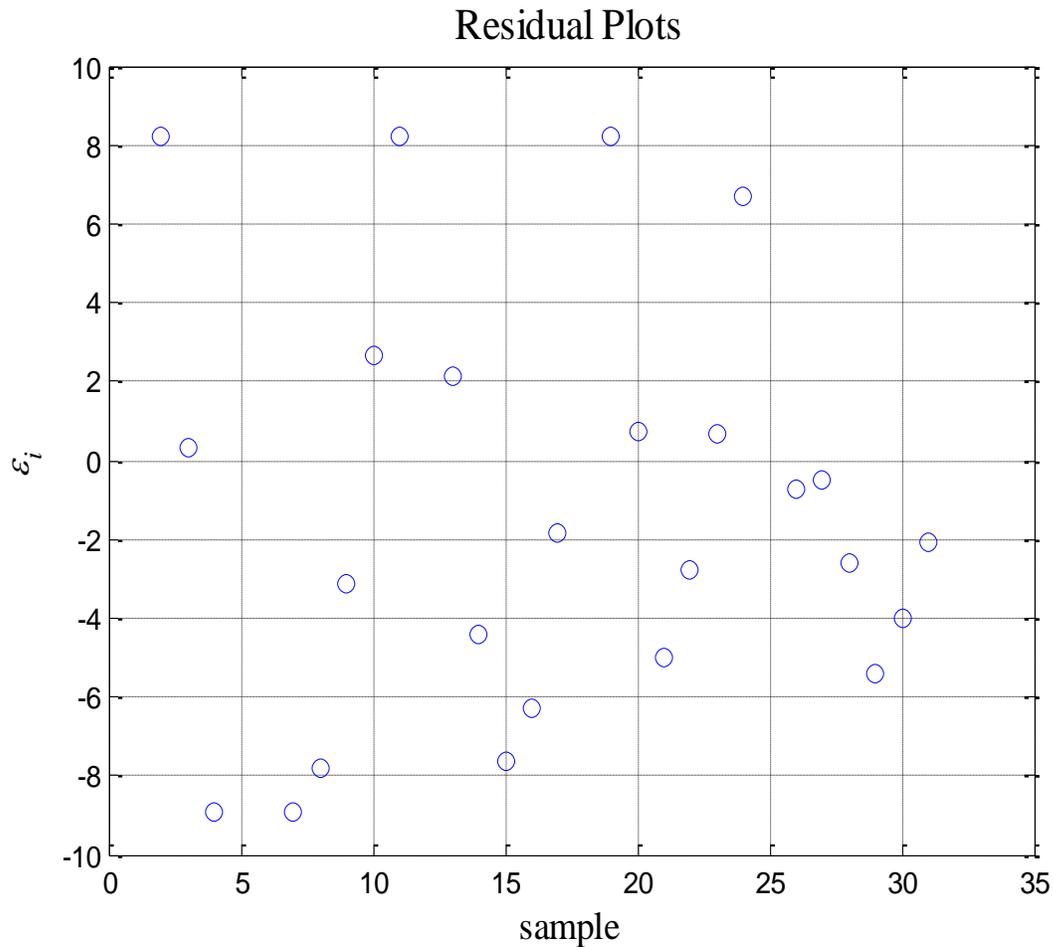
Figure [5. 1]: Diloxanide furoate Residual Plot

We can see from the figure 4 that the residual has a random pattern, and can be assumed that are from a normal distribution. As a result, the statistical approach is valid.

❖ **Normal probability plot:**

Using normal probability plots to investigate whether the error are normally distributed.
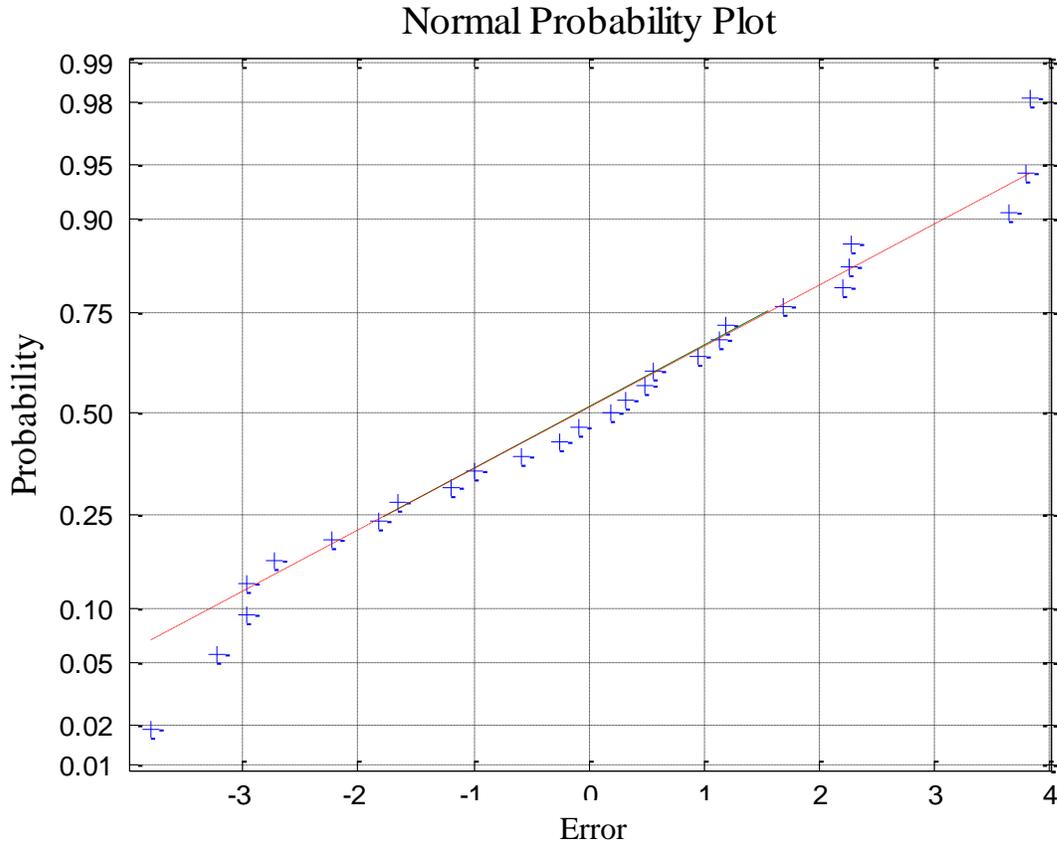
Figure [5. 2]: Diloxanide furoate Normal Probability Plot

What is shown in Figure [5.2] is that the normal probability plot shows a straight line. So, we can assume that the observed sample comes from a normal distribution.

❖ **Coefficient of Determination (R^2)**

Using the coefficient of determination as a diagnostic check is to measure whether the regression line represents the data.

From the results, it is shown that $R^2 = 0.93$, which means that 93.1366% of the total variation in observed sample can be explained by the linear relationship between the regressors and observed response y as it is described by the regression model. Only 6.86 % of the total variation in the observed sample is unexplained.

5.2.2 Artificial Neural Network (ANN)

Back propagation feed forward based on Levenberg-Marquadt training algorithm was employed to train the network. MATLAB® neural network toolbox was used to train and simulate the network model. Log sigmoid was found to give reasonable response.

The data was randomly divided into a 25 training set and a 6 testing set as shown in Tables [5.5] and [5.6]. About $10 - 15$ % of the data was selected randomly to be the testing data set. Then before training, the data was normalized by using the following formula $\hat{x} = \dfrac{x - x_{min}}{x_{max} - x_{min}}$

,so that, the inputs and outputs are belong to the interval [0, 1].

Table [5. 5]: A Training Data Set

Training Data Set		
Temperature(K)	**Time (minutes)**	**%DF Remaining**
313	60.6146	64.1874
336	5.4095	79.5463
328	50.4090	6.6050
323	60.6726	32.0782
336	0	100
336	41	11.1322
328	0	100
313	20.8010	89.6004
323	70.5163	10.9742
336	20.4931	35.4560
336	25.6549	25.3355

328	40.6548	30.7312
336	35.3242	15.0321
313	100	47.3053
336	30.1567	22.2756
328	10.6751	76.2062
336	10.3050	63.7309
313	40.1926	77.4819
323	75.0720	23.7564
328	20.7097	57.2480
323	50.6906	22.7583
323	30.4649	56.4352
323	45.8881	43.3162
336	15.2084	46.5246
328	30.6184	41.7946

Table [5. 6]: A Testing Data Set

Testing set data		
Temperature(K)	**Time (minutes)**	**%DF Remaining**
336	45.8973	8.3630
323	0	100
323	15.5551	76.9946
323	90	17.4678
313	0	100
313	80.0062	55.6340

The result was acceptable by using 500 epochs of training, with the goal 10^{-5} and 5 neurons and one hidden layer. The log sigmoid function was applied on this set of data.

The coefficient of determination (R^2) was 99.214% for training data set and 98.756% for the testing data set as shown in the Tables [5.7] and [5.8] respectively. Since the coefficient approaches 1, it indicates an excellent predictive ability of the neural network model.

Table [5. 7]: Statistical Properties for Training Data Set

Average error	5.55825
Maximum error	32.13496
Minimum error	0.0
R^2	0.99214

Table [5. 8]: Statistical Properties for Testing Data Set

Average error	12.72280
Maximum error	52.39252
Minimum error	2.06339
R^2	0.98756
SSE	99.2874
MSE	16.5479

As it is shown in Figure [5.3], most of the observed and predicted values are located on the parity line on the scatter plot which means that the predicted values of y are very close to the observed data and hence, considered highly accurate.
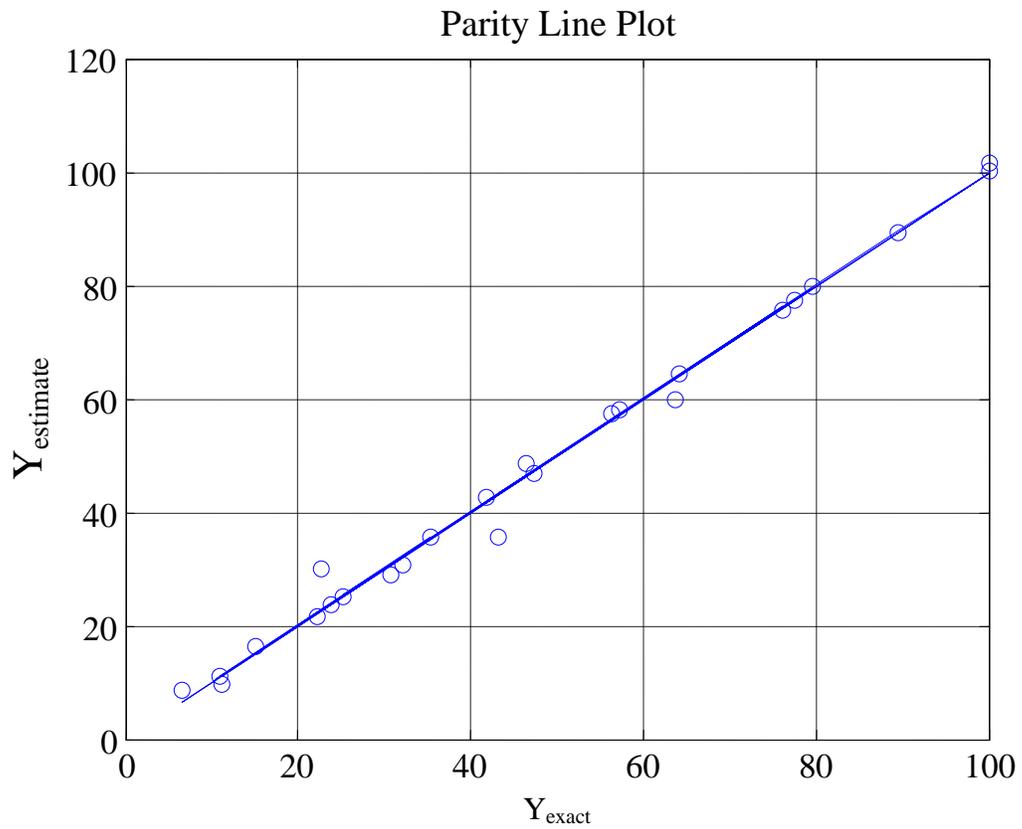
Figure [5. 3]: Parity Line Plot for Diloxanide Furoate Training Datasets

Although there was no enogh data to be used for both training and testing as it can be seen in the Figure [5.4], still the closeness of the few points to the parity line implying that the ANN techniques has functioned well.
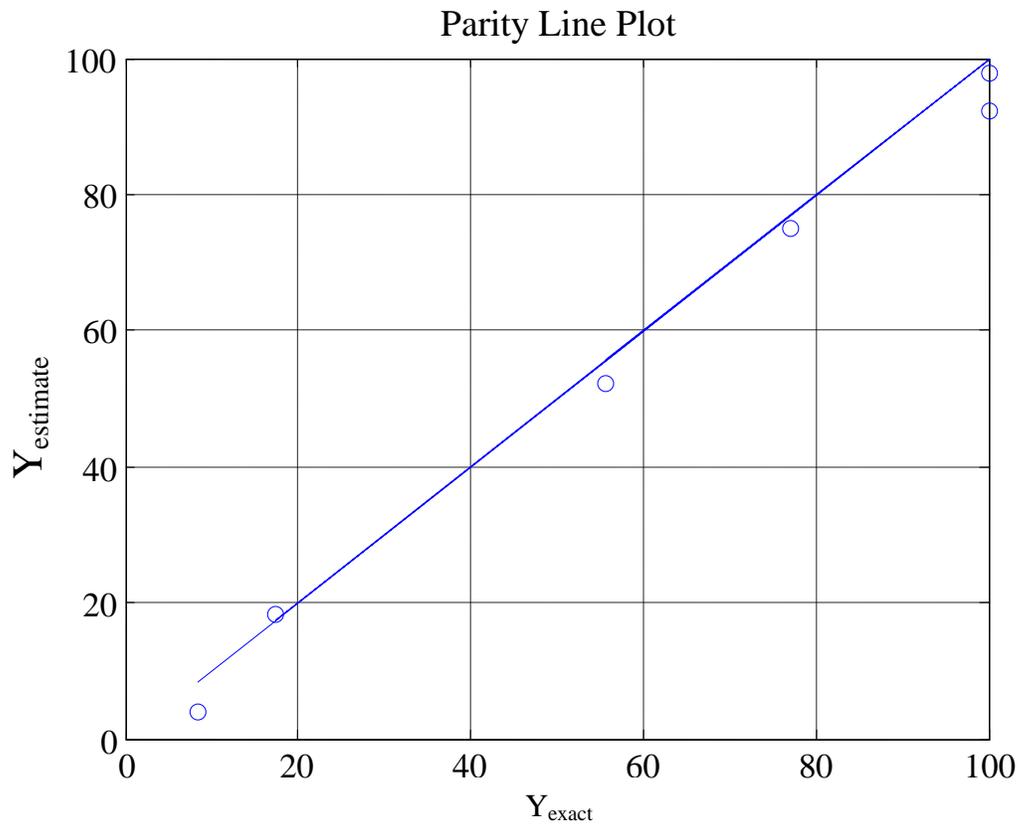
Parity Line Plot

Figure [5. 4]: Parity Line Plot for Diloxanide Furoate Testing Datasets

The Figure [5.5] shows the distribution of the errors. It shows a major part of the errors are between 1% and 10%.
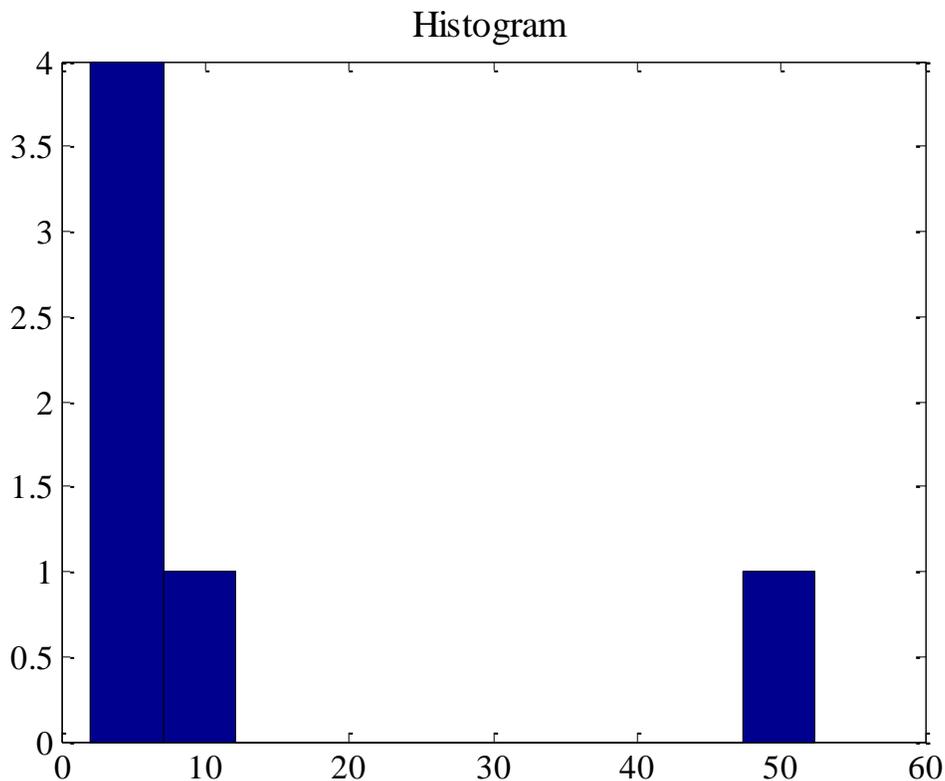
Figure [5. 5]: Diloxanide Furoate Histogram

5.2.3 Mechanistic Model

Microsoft Office Excel© version 2007 was used for mechanistic model calculations in this research. A mechanistic model for diloxanide furoate was proposed as shown in Equation [5.1] and both of the rate constant and the rate order were determined at different temperatures using a linear regression methods.

$-r_a = k_A C_A^{\alpha}$ [eq5.1]

The Equation [5.1] was linearized as:

$$\ln(-r_a) = \ln(k_A) + \alpha \ln C_A \qquad \text{[eq5.2]}$$

The results are summarized in Table [5.9].

Table [5. 9]: Reaction Rate Constants (k) for Diloxanide Furoate Degradation at Various Temperatures

Temperature(K)	Reaction Rate Constant k_A (min.$^{-1}$)	Reaction Order (α)
336	0.054201	0.9878
328	0.006682	1.0811
323	0.013702	1.0163
313	0.001583	1.0214

It was found that the mean of squared errors is 1.7×10^{-5} and the coefficient of determination was 95.40%.

The rate of the reaction follows a first order kinetics. The natural logarithm of observed rate constants over the temperature range of 40-63°C (313-338K) were plotted versus reciprocal of temperature according to the Arrhenius equation to calculate the frequency factor and the activation energy. The activation energy was calculated from the slope of the straight line obtained by linear regression method was found to be 16.304043 Kcal/mole and the frequency factor was approximately 35 minutes $^{-1}$.

CHAPTER 6

HARDNESS OF UNCOATED TABLET (X)

Hardness of tablet drug products is one of the most important physical characteristics that need to be maintained throughout the process of packaging and storage until used. Hardness in is defined as *the force required to break the tablet in diametrical compression test,* and hence it is used in pharmaceutical industry as a measurement of the solid dosage forms stability and resistance to breakdown by any of the stress factors. Hardness has to be tested by exposing the drug tablets to variable degrees of temperature and relative humidity over a defined period of time to determine the best environmental conditions to be provided so that the tablets remain stable and strong enough until their use by consumers.

In the following case study, the experimental data for the hardness of uncoated drug tablets were taken from the thesis "Designing A Package For Pharmaceutical Tablets in Relation to Moisture and Dissolution" by Yoon Seungyil (39).

This experiment was carried out to investigate the compressive strength (effect of intermolecular forces among the ingredients on tablets) and detecting tablet fracture. Tablets were stored at different relative humidity, two different temperatures (25°C and 40°C). Then the hardness of tablets was measured at different time intervals by using Strength Testing Machines.

6.1 Experimental Data:

Experimental data on hardness of uncoated drug tablets were collected and all the three mathematical models (linear regression, artificial neural network, and mechanistic models) were

conducted on these data to determine the model that best predicts its stability. A comparison among these three mathematical models was made at the end to conclude the best statistical method that should be adopted in practice to achieve the most accurate shelf life.

Table [6.1] shows independent variables (temperature, time, relative humidity) and dependent variable (hardness of tablets).

Table [6. 1]: Effect of Temperature, Relative Humidity and Time on Hardness

Independent Variables			Dependent Variable
Temperature (K °)	Relative Humidity (RH%)	Time (Days)	Hardness (Kp)
298	90	0	9.2
298	90	6	2.4
298	90	18	1.8
298	90	70	8.1
298	90	100	1.6
298	90	130	1.9
298	90	170	1.9
298	90	190	1.9
298	75	0	9.2
298	75	6	7.6
298	75	18	7.3
298	75	70	8
298	75	100	7.5
298	75	130	7.9
298	75	170	7.9
298	75	190	8.4
298	65	0	9.2
298	65	6	9.2
298	65	18	8

298	65	70	8.2
298	65	100	9.5
298	65	130	9.2
298	65	170	9.7
298	65	190	9.6
298	50	0	9.2
298	50	6	8.2
298	50	18	8.2
298	50	70	8.8
298	50	100	9
298	50	130	9.3
298	50	170	9.2
298	50	190	9.2
298	0	0	9.2
298	0	6	8.4
298	0	18	8.2
298	0	70	8.3
298	0	100	8
298	0	130	8.4
298	0	170	8
298	0	190	8
313	90	0	9.2
313	90	6	2.5
313	90	18	2.6
313	90	70	2.5
313	90	100	2.6
313	90	130	2.8
313	90	170	3
313	90	190	3
313	75	0	9.2
313	75	6	7.5

313	75	18	8
313	75	70	8.4
313	75	100	8.7
313	75	130	9
313	75	170	9.2
313	75	190	9.3
313	65	0	9.2
313	65	6	8.6
313	65	18	9.1
313	65	70	9.7
313	65	100	9.9
313	65	130	10
313	65	170	10.9
313	65	190	10.3
313	50	0	9.2
313	50	6	8.9
313	50	18	10.2
313	50	70	10.6
313	50	100	10.3
313	50	130	10.8
313	50	170	11.2
313	50	190	11.2
313	0	0	9.2
313	0	6	8.2
313	0	18	8.4
313	0	70	8.4
313	0	100	8.3
313	0	130	8.6
313	0	170	8.4
313	0	190	8.6

6.2 RESULT AND DISCUSSION

6.2.1 Linear Regression

As it is known, the linear regression is concerned with models that are linear in parameters. In this experiment, the values of the independent variables are perfectly known and we want to create a model to estimate the unknown parameters.

Tentatively, a linear regression model has been fitted to the experimental data. However, it has been found that the linear model is not adequate since it failed the diagnostic test such as residual plots and normal probability plots as seen in Figure [6.1] and [6.2], respectively. Therefore, nonlinear empirical models such as a neural network have been investigated.
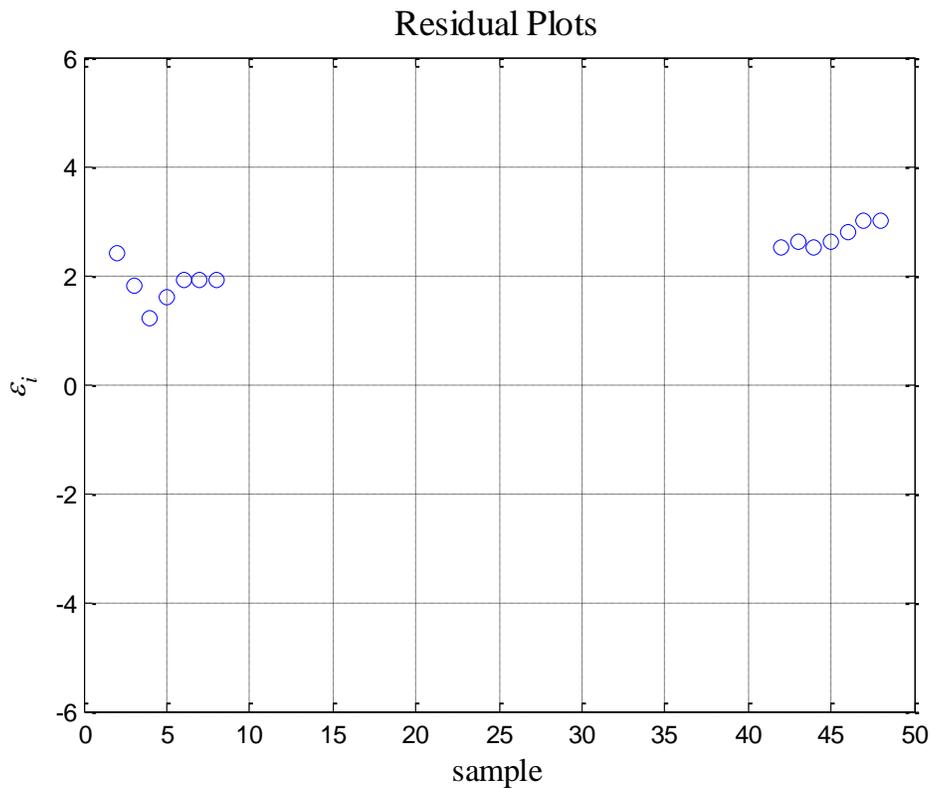
Figure [6. 1]: Uncoated Tablets Residual Plot

75
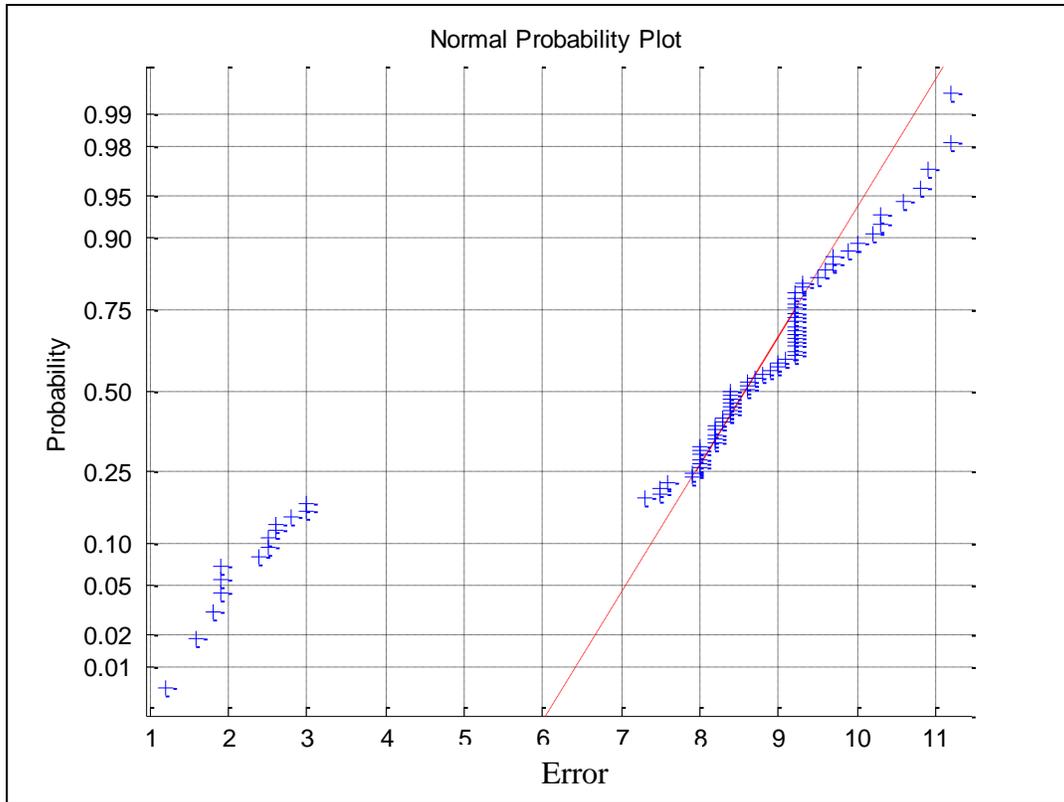
Figure [6. 2]: Uncoated Tablets Normal Probability Plot

6.2.2 Artificial Neural Network (ANN)

Back propagation feed forward based on Levenberg-Marquadt training algorithm was employed to train the network. MATLAB® neural network toolbox was used to train and simulate the network model. Log sigmoid was found to give reasonable response by trying different activation functions.

The data was randomly divided into a 70 training set and a 10 testing set as shown in tables [6.2] and [6.3]. About $10 - 15$ % of the data was selected randomly to be the testing data set. Then before training, the data was normalized by using the following formula $\hat{x} = \dfrac{x - x_{min}}{x_{max} - x_{min}}$

So that, the inputs and outputs are belong to the interval [0, 1].

Table [6. 2]: A Training Data Set of Uncoated Tablet

Training Data Set			
Temperature (K)	Relative Humidity (RH%)	Time (Days)	Hardness (kp)
298	90	0	9.2
298	90	6	2.4
298	90	18	1.8
298	90	70	8.1
298	90	100	1.6
298	90	130	1.9
298	90	170	1.9
298	75	0	9.2
298	75	6	7.6
298	75	18	7.3
298	75	70	8
298	75	130	7.9
298	75	170	7.9
298	75	190	8.4
298	65	0	9.2
298	65	6	9.2
298	65	18	8
298	65	70	8.2
298	65	100	9.5
298	65	130	9.2
298	65	190	9.6
298	50	0	9.2
298	50	6	8.2
298	50	18	8.2
298	50	70	8.8

298	50	130	9.3
298	50	170	9.2
298	50	190	9.2
298	0	0	9.2
298	0	6	8.4
298	0	18	8.2
298	0	100	8
298	0	130	8.4
298	0	170	8
298	0	190	8
313	90	0	9.2
313	90	6	2.5
313	90	18	2.6
313	90	70	2.5
313	90	130	2.8
313	90	170	3
313	90	190	3
313	75	0	9.2
313	75	6	7.5
313	75	18	8
313	75	70	8.4
313	75	100	8.7
313	75	170	9.2
313	75	190	9.3
313	65	0	9.2
313	65	6	8.6
313	65	18	9.1
313	65	70	9.7
313	65	130	10
313	65	170	10.9

313	65	190	10.3
313	50	0	9.2
313	50	6	8.9
313	50	18	10.2
313	50	100	10.3
313	50	130	10.8
313	50	170	11.2
313	50	190	11.2
313	0	0	9.2
313	0	6	8.2
313	0	18	8.4
313	0	70	8.4
313	0	100	8.3
313	0	170	8.4
313	0	190	8.6

Table [6. 3]: A Testing Data Set of Uncoated Tablet

Training Data Set			
Temperature (K)	Relative Humidity (RH%)	Time (Days)	Hardness (kp)
298	90	130	1.9
298	75	100	7.5
298	65	170	9.7
298	50	100	9
298	0	100	8
313	90	100	2.6
313	75	130	9
313	65	100	9.9

313	50	70	10.6
313	0	130	8.6

The result was acceptable by using 5000 epochs of training, with the goal 10^{-5} and 9 neurons and two hidden layers. The log sigmoid function was applied in this set of data.

Table [6. 4]: Statistical Properties for Training Data Set

Average error	3.25830
Maximum error	24.40375
Minimum error	0.00040
R^2	0.98407

Table [6. 5]: Statistical Properties for Testing Data Set

Average error	8.08678
Maximum error	17.32032
Minimum error	0.37100
R^2	0.92806
SSE	5.8424
MSE	0.5842

The scatter plot in Figure [6.3] shows that the parity line passes through a great number of the observed and predicted values. This indicates a strong predictive ability of the neural network.
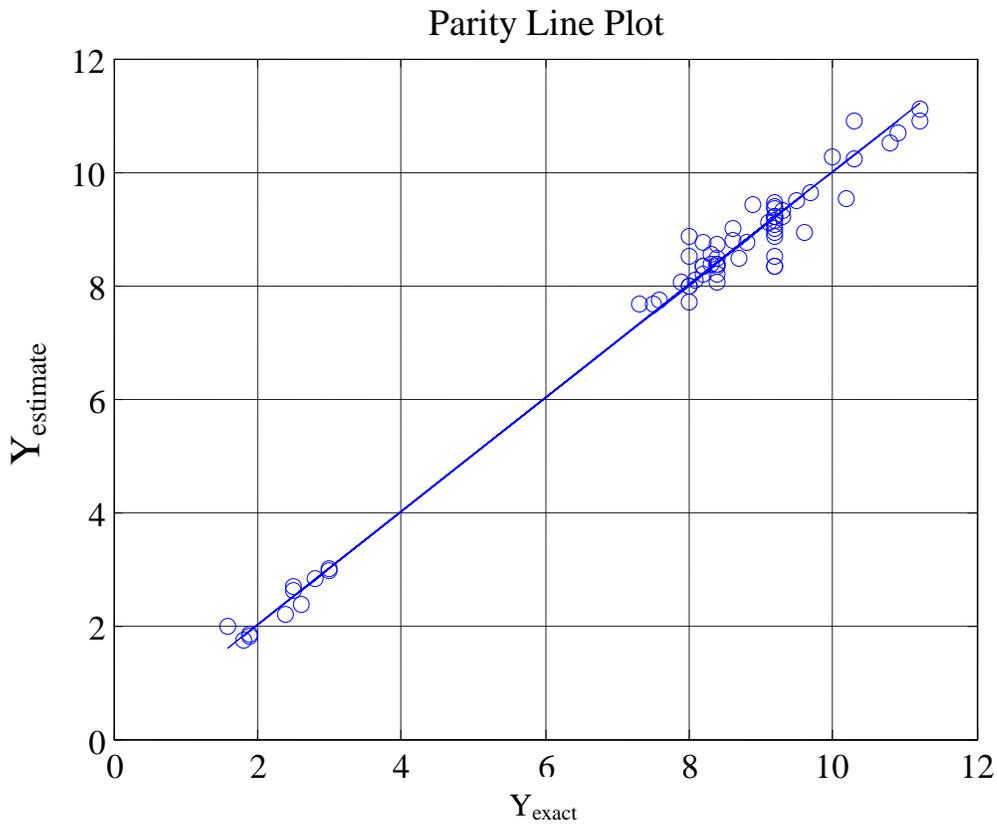

Figure [6. 3]: Parity Line Plot for Uncoated Tablets Training Datasets

Because of lack of data points used for testing the trained neural network model, the model was

not able to capture a clear relationship between the inputs and the output in the testing datasets

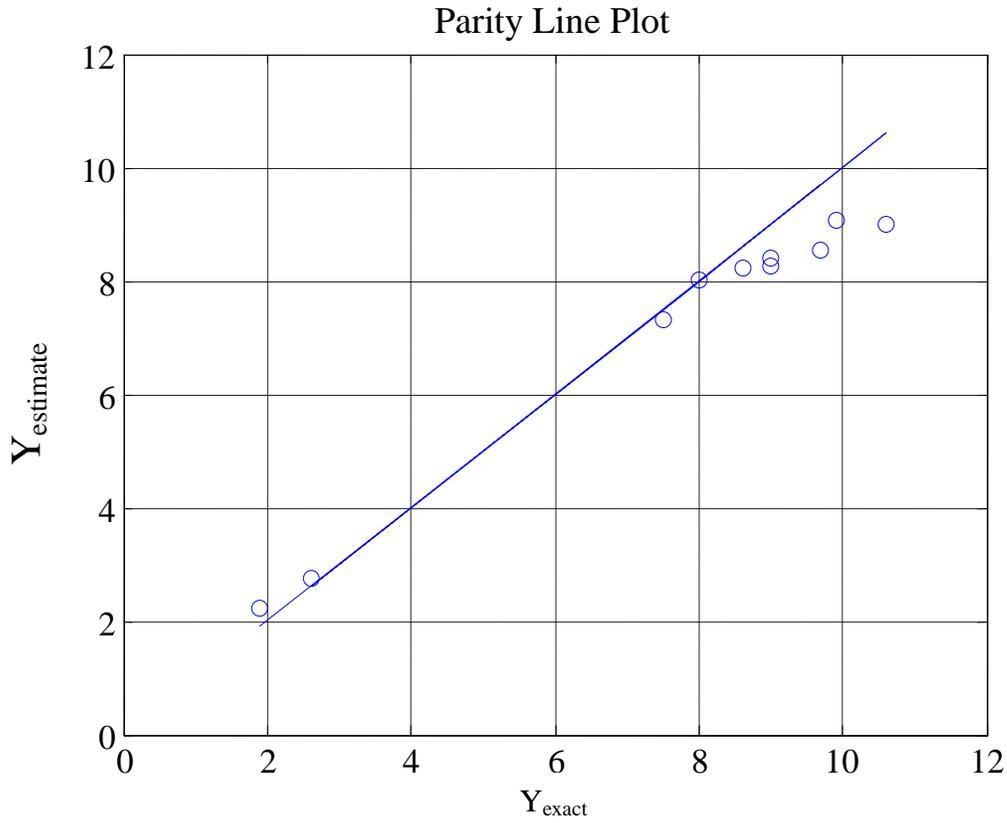as seen in Figure [6.4].This made it hard to judge the predictive ability of the trained network.

Figure [6. 4]: Parity Line Plot for Uncoated Tablets Testing Data

It was found that the frequency of the error distribution lies mostly in the range between 5-9% as shown in figure [6.5]. Also, there are substantial errors elsewhere.
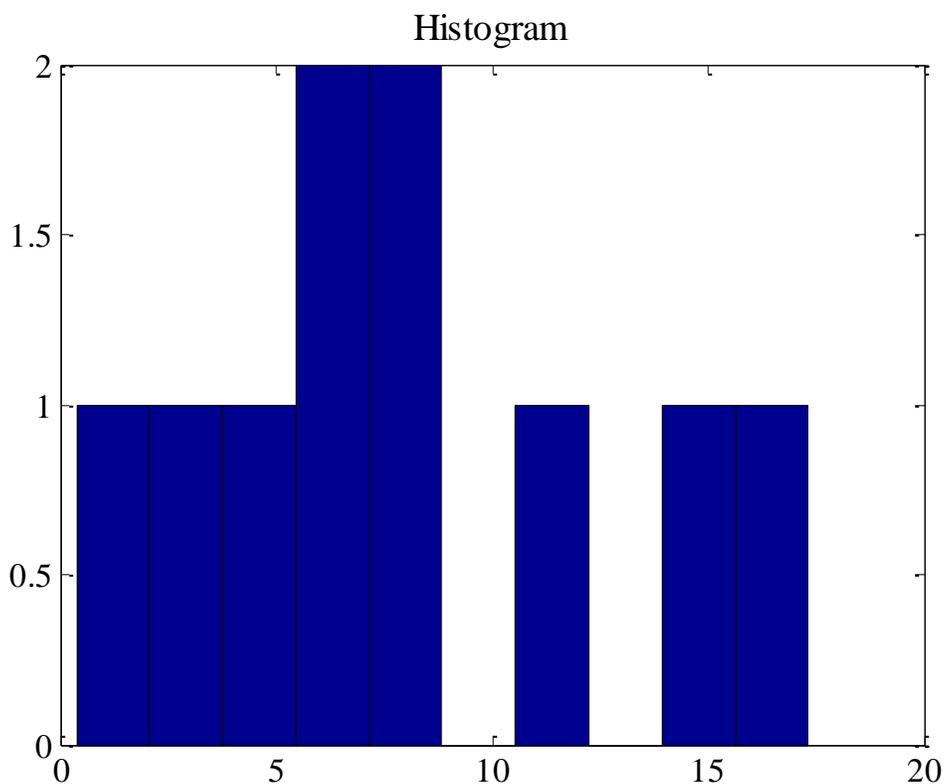

Figure [6. 5]: Uncoated Tablets Histogram

6.2.3 Mechanistic Model

In order to determine the predicted values with regards to the hardness of the uncoated tablets, the mechanistic model of Nakabayashi, K. et al (40) was used. This model relied on applying an iterative procedure where the influence of temperature and relative humidity over a time interval of several days is evaluated.

The following equations were used for creating the mechanistic model:

$$P_{i,j} = P_{0,i} . \exp\left(-\frac{E_i}{R.T_j}\right)$$ [eq6.1]

$$V_j = V_0 . \exp\left(-\Delta H/(R.T_j)\right)$$ [eq6.2]

$$\Delta P_j = V_j . (RH_{1,j} - 75.0)/100$$ [eq6.3]

$$\Delta q_j = \frac{\Delta P_j}{\Sigma((N_i.L_i)/(P_{i,j}.S_i))}$$ [eq6.4]

$$\Delta m_j = \Delta q_j/W$$ [eq6.5]

$$m_j = m_{j-1} + \Delta m_j$$ [eq6.6]

$$m_{a,j} = (m_{j-1} + m_j)/2$$ [eq6.7]

$$k_j = A . \exp\left(-\frac{B}{T_j}\right.$$ [eq6.8]

$$k_{j'} = k_j . m_{a,j}$$ [eq6.9]

$$\Delta C_j = k_{j'} . (\Delta t)$$ [eq6.10]

$$C_j = C_{j-1} + \Delta C_j$$ [eq6.11]

Where; the subscript j denotes to the each time intervals, P is the permeability constant, E is the activation energy of moisture permeation, V_j is the saturated vapour pressure for the j-th interval, R is the rate constant, ΔH is the heat of vaporization of water, ΔP_j is the vapour pressure difference across the package, T_j is an absolute temperature, W is the weight of the tablets, S is the surface area of the tablet, L is the thickness of the tablet, N is the number of tablets per package, k is the rate constant, $k_{j'}$ is the apparent rate constant, $m_{a,j}$ is the moisture content of the tablet for the j-th interval.

The MSE was found to be 17.36. This is a quite large value which obviously indicates that there is no agreement between the actual observed data and the predicted values.

CHAPTER 7

CONCLUSION AND RECOMMENDATIONS

7.1. Conclusion

Drug products can easily degrade with change in their composition and chemical and physical characters over time under the influence of variable environmental factors such as temperature, light and moisture. Such instability or degradation can lead to partial or complete loss of drug efficacy as a therapeutic agent or even a transformation into a toxic substance. This fact made the stability of drug substances and drug products to be considered as an important subject in pharmaceutical industry.

Measuring the drug stability usually is as important as any of the other steps involved in the development process of any new product in pharmaceutical industry such as drug discovery, laboratory development, animal studies, clinical trials, and regulatory registration. Since the drug stability is a means of proving an evidence for its safety and efficacy, approval of any drug product by the regulatory agencies requires that the stability has to be provided prior to its release for public use.

Stability testing requires vigorous work and lengthy studies. Analytical techniques have been the commonly used methods in drug stability testing. These techniques include high performance liquid chromatography (HLPC) or thin layer chromatography (HPTLC), gas chromatography (GC) and electrophoresis. Recently, there has been a new trend towards the use of statistical modelling techniques. The modelling techniques commonly used nowadays are the empirical models such as linear regression, artificial neural network, and the mechanistic models. Such

techniques are thought to be reliable and economic alternatives with the advantage of time saving.

To assess the reliability and applicability of statistical modelling in studying drug stability and estimating its degradation with time and to know whether statistical modeling can efficiently substitute the ordinary analytical methods, several models were applied in this research on three different drugs. These drugs were chosen from the pharmaceutical literature. The concentration or hardness of these drugs over time was used as indicators of the drug stability. Three different models (linear regression, artificial neural network and mechanistic models) were implemented on each drug to predict the drug concentration over time. These predicted values were compared to the observed values and then evaluated by calculating the mean of square errors (MSE) to determine how close those values were to the exact values resulted experimentally. Moreover, the coefficient of determination (R^2) was calculated to further evaluate the model accuracy as well.

The first case study addressed the stability of acetyl salicylic acid (ASA) tablets. The concentration of ASA at variable temperatures (35, 40, 45, 50 ^{o} C) was determined experimentally at different time intervals using HPLC. Firstly, a linear regression model was proposed and the unknown parameters were estimated and found to be significant as concluded from ANOVA table. The errors were found to be normally distributed with a mean equal to zero and the residuals residual had a random pattern as seen in normal probability and the residual plots, respectively. The MSE was 6.33, and R^2 was 84.67%. This meant that the regression model represents the data very well. Secondly, the artificial neural network was applied and it was able to capture the relationships between the inputs and the output in the testing datasets in spite of the limited number of the experimental data (21 data points). The MSE was 1.5168

whereas the R^2 was 94% indicating a high accuracy for artificial neural network model as well. The third model applied was the mechanistic model. This model was linearized to determine the decomposition rate constants (k_{App}) at various temperatures and the order of the reaction (α). The hydrolysis process was found to follow first order kinetic dependent only on the concentration of one reactant; i.e. aspirin. The apparent activation energy of aspirin hydrolysis of 49 Kcal/mole was calculated by using the Arrhenius equation method. MSE was found to be 2.92833×10^{-7} and the R^2 was 96.48% which indicates that the predicted values were very close to the observed values which in turn indicated that the mechanistic model is reliable.

The second case studied the stability of diloxanide furoate (DF) in solution. The concentration of DF at variable temperatures (40, 50, 55, 63 $^{\circ}$C) was determined experimentally at different time intervals at pH 8 using HPLC. Firstly, a linear regression model was proposed and the unknown parameters were estimated and found to be significant as concluded from ANOVA table. The errors were found to be normally distributed with a mean equal to zero and the residuals had a random pattern as seen in normal probability and the residual plots, respectively. The MSE was 70.91, and R^2 was 93.13%. This meant that the regression model represents the data quite well. Secondly, the artificial neural network was implemented and it was able to capture the relationships between the inputs and the output in the testing datasets in spite of the limited number of the experimental data (31 data points). The MSE was 16.54 whereas the R^2 was 98.75% indicating a high accuracy for artificial neural network model as well. The third model applied was the mechanistic model. This model was linearized to determine the decomposition rate constants (k_{App}) at various temperatures and the order of the reaction (α). The hydrolysis process was found to follow first order kinetic. The apparent activation energy of DF hydrolysis of 16.30 Kcal/mole was calculated by using the Arrhenius equation method. This value of

activation energy lied within the accepted limits for hydrolysis of esters (8-20 Kcal/mole). MSE was found to be 1.7×10^{-5} and the R^2 was 95.78% which indicates that the predicted values were very close to the observed values which in turn indicated that the mechanistic model is reliable.

In the third case, the hardness of an uncoated drug tablet was tested experimentally by exposing the drug tablets to variable degrees of temperature and relative humidity over a defined period of time. When linear regression model was proposed, it was found to be inadequate as it failed the diagnostic tests (residual and normal probability plots) along with having R^2 of 10%. This meant that the regression model could not represent the data. Therefore, a nonlinear empirical model which is the artificial neural network was applied. This model was able to capture the relationships between the inputs and the output in the testing datasets. The MSE was 0.5842 whereas the R^2 was 92.806% indicating reliable predictions of the performance of the process for artificial neural network model.

When mechanistic model was applied, the MSE was found to be 17.36 which revealed a significant disagreement between the observed values and the predicted ones. This thought to be attributed to the sever fluctuation of the actual data.

With regards to the advantages of mathematical models, they are clearly economic and time saving and mostly quite easy to perform. However, mechanistic models sometimes require a great deal of expertise as compared to the empirical models.

One conclusion is that when linear regression model fails to achieve accurate predicted values, the non linear regression models such as artificial neural network could be reliably alternative thanks to its generalization ability whence the learning of network is successful. However, artificial neural network has the following disadvantages:

1) There is no systematic way to obtain an optimal architecture.

2) It requires sufficiently large number of data points for training.

3) There is no guarantee to obtain a global of optima which may require global optimization techniques to improve the solution (e.g. genetic algorithm).

4) Over learning (analogues to over fitting).

7.2. Recommendation and Future Works

In general, prediction models can be classified into three types: first-principles models, empirical models and semi-empirical models. Each of these models is further characterized, based on the nature of the correlation between variables and linear or nonlinear models. In this project we used first principles and empirical models to predict the drug stability. More specifically, linear, nonlinear and kinetics models have been used. Each of these model structures has its own advantages and disadvantages. From model development point of view, a first principle-based model (kinetics) is usually difficult to be developed compared to the empirical-based models (e.g. linear regression and neural networks). Future woks on the drug stability can be geared into two directions:

1) Investigating different empirical based models (linear/nonlinear)

As a continuation of this project, other types of models should be examined. For examples, subspace models, such as Partial Least Squares (PLS) has many attractive properties. It is characterized by the ability to model multi response variables (e.g. active ingredient concentration, hardness) as a function of the input variables by maximizing the covariance between the input and output matrix (41). This is necessary and important especially when inputs and outputs are cross-correlated. The latter is typical in most of practical systems. In addition, subspace models are characterized by their filtering capabilities which can give more accurate predictions. While PLS is an example of linear models, Support Vector Machine (SVM) arises as a strong competitive to Neural Networks based modeling. The main advantageous of the SVM based models is the convexity of the regression problem compared to the NN-based modeling, where only suboptimal solutions can be found. PLS and SVM have not

been investigated in the context of drug stability and exploring their capabilities would be of advantage.

2) Implement the mathematical models on variables other than temperature and relative humidity to test the applicability of these models. For example, study the effect of pH and light on the drug stability. This can provide stronger evidence on the reliability of the models.

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