ASSAY OF IBUPROFEN AND EVALUATION OF STABILITY USING ISOTHERMAL AND NON-ISOTHERMAL METHODS

BY

DINTLETSE RAMATLAPENG

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ASSAY OF IBUPROFEN AND EVALUATION OF STABILITY USING ISOTHERMAL AND NON-ISOTHERMAL METHODS

BY

DINTLETSE RAMATLAPENG

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

ABSTRACT

Shelf-life of pharmaceutical products is usually obtained by a technique known as the classical isothermal method. In the classical method, the degradation rate constants are determined at different elevated constant temperatures. In the present studies, an improved HPLC method was developed for ibuprofen analysis. This analytical method was used to test the new nonisothermal methods with ibuprofen tablet formulations. The research was designed as a feasibility study for application of the new nonisothermal methods. Since most research with these new methods has been on liquid dosage forms, the intention of this project was to test the applicability of nonisothermal method to a solid dosage form. In nonisothermal methods, temperature is increased with time, thus the temperature changes throughout the experiment. There is a major focus on these new methods at present because they are time saving and cost effective. Shelf-life of liquid pharmaceutical products can be obtained much more quickly with these new methods than conventional methods, and non-isothermal methods could be valuable for routine use with ibuprofen tablets. However, results did not support the application of the method tested for ibuprofen tablets.

ACKNOWLEDGEMENTS

I would like to thank my advisor Dr. C. Briggs for his invaluable encouragement and contributions not only with the success of this work but also my academic experience in this university. I would also like to thank Dennis Cote of St. Boniface Research Centre for his assistance and for allowing the use of laboratory facilities, chemicals and supplies throughout this research. Finally Canadian International Development Agency for the financial support. EDUPLUS for thier good management of the BLSM programme.

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$$\text{Log } K_t = \text{Log } K_o + (Eb/R)\text{Log}(1+t) \quad \ldots\ldots(13)$$

$$K_t = K_o(1+t)^{Eb/R} \quad \ldots\ldots(14)$$

$$\text{Log}[2.303\ \text{Log}(C_o/C_t)] = \text{Log } K_o - \text{Log}(1+Eb/R) + (1+Eb/R)\text{Log}(1+t) + \text{Log}[1-(K_o/K_t)^{1+R/Eb}] \quad \ldots\ldots(15)$$

$$\text{Log } F = \text{Log } K_o - \text{Log}(1+Eb/R) + (1+Eb/R)\text{Log}(1+t) \quad \ldots\ldots(16)$$

$$dC/dt = -Ae^{-E/RT}C \quad \ldots\ldots(17)$$

$$\text{Log}(C_o/C_t) = (RK_o/aE)e^{-aEC/R} - RK_o/aE) \quad \ldots\ldots(18)$$

$$\text{Log}(C_o/C_t) = \text{Log}(RK_o/aE) + (aE/2.303R)t \quad \ldots\ldots(19)$$

$$\text{Log}(C_1/C_2) = \text{Log}[(e^{aEC/R} - 1)(RK_o/aE)] + (aE/2.303R)t \quad \ldots\ldots(20)$$

$$f(a)-f(b)/(b-a) = (K_1 + K_2 + K_3 + \ldots + K_n)/n \quad \ldots\ldots(21)$$

$$K_1 = Ae^{-E/RT1} \quad \ldots\ldots(22)$$

$$K_{n+1} = Ae^{-E/RT1+1} \quad \ldots\ldots(23)$$

$$K_{n+1} = K_1 e^{-E/(R[T1+1-T11/1T1T1+1])} \quad \ldots\ldots(24)$$

$$\text{Ln } C - \text{Ln } C_o = t/n(K_1 + K_2 + K_3 + \ldots\ldots + K_n) \quad \ldots\ldots(25)$$

$$\text{Ln } C - \text{Ln } C_o = K_1\, t/n\{1+\Sigma e^{[K/R\ AT\ \Sigma(T)\Sigma T]-1}\} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (26)$$

CHAPTER 1

1. INTRODUCTION

1.0 OBJECTIVE OF STUDY

Expiration dating of pharmaceutical products is usually based on data obtained from accelerated decomposition studies. The shelf-life is predicted by monitoring the decomposition rate at several elevated temperatures and the data extrapolated to room temperature using the Arrhenius equation. This method is normally referred to as the classical isothermal method [1-6].

A stability testing programme for pharmaceutical products usually utilizes the classical isothermal method. However this method is often too burdensome and time consuming to incorporate in the early stages of product development.

Alternative short-term methods have been developed. These involve heating a product, using a temperature profile which is accurately controlled. It is vital that the temperature and the potency of a drug product are accurately determined as a function of time. In nonisothermal methods, kinetic parameters such as heat of activation and reaction rate constant can be determined in a single experiment [7-24].

An example of a nonisothermal temperature programme is the one used in this study, known as the linear temperature programme [11,13,14,19].

The objective was to evaluate the stability of some ibuprofen tablet formulations and determine if shelf-life of ibuprofen in a tablet form can be determined by using this new method. Other nonisothermal temperature programmes include logarithmic, hyperbolic and stepped temperature programmes.

Most studies with nonisothermal methods in the literature involve extensive work on liquid dosage forms [7-24]. It would be useful to determine the applicability of the linear temperature programme method to the stability testing of solid dosage forms and whether there are drug specific complications.

It is important to note that the conventional method is the only method that is acceptable to most drug regulatory agencies for drug registration purposes. The data obtained from accelerated studies are normally submitted to drug regulatory agencies together with data obtained from normal storage conditions. It was thus appropriate to include shelf-life determinations of ibuprofen using the conventional method in the present study as controls.

The research was divided into five different phases:

1. A short compatibility study of ibuprofen and several excipients
2. Development of four different formulations of ibuprofen tablets.
3. Isothermal stability studies: Incubation of formulations at four different elevated temperatures followed by analysis of each sample and determination of shelf-life of ibuprofen under normal

storage conditions.

4. The nonisothermal testing programme: incubation of tablets under programmed temperature conditions, followed by sample analysis and shelf-life determination at normal storage conditions.

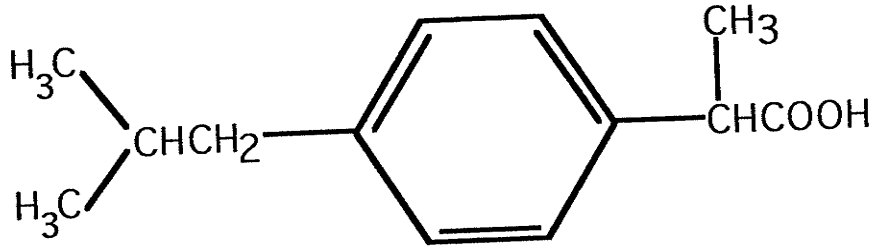
5. Data analysis of the two stability testing programmes.

1.1 DRUG USED IN THE RESEARCH

A stability profile of ibuprofen was studied because ibuprofen is a drug that is used extensively. Ibuprofen was used as a model to assess the feasibility or applicability of these new stability methods to solid dosage forms.

1.1.0 Chemical Structure of Ibuprofen

Ibuprofen, [(RS)-2-(4-isobutylphenyl)propionic acid] is a well known nonsteroidal anti-inflammatory drug, whose spectrum of pharmacological properties and toxicity have been widely investigated.



1.1.1 Physical properties

A white crystalline powder or a white fine powder

Melting point: 75° to 78°C

Solubility: Practically insoluble in water, but very soluble in organic solvents, 1 part in 1.5 parts of ethanol, 1 part in 1 part of chloroform, and 1 part in 2 parts of ether.

Dissociation constant: pK. 4.4, 5.2 [25]

1.2 STABILITY OF PHARMACEUTICAL PRODUCTS

The stability of a drug product is defined as compliance with the quality, as defined in the specification of a product, up to the end of the shelf-life as laid down by the manufacturer [4]. The quality of a drug product is determined by its content of active ingredient, its purity and its organoleptic, physicochemical, microbiological and toxicological properties.

The content of the active ingredient of a drug product should not fall below 90% of the labelled claim up to the end of the shelf-life [4]. This tolerance limit applies to stored products and must be confirmed by results of suitable tests. All other properties, should remain unaltered through the shelf-life of a drug product.

To ensure that a drug product meets appropriate standards of identity, quality, and purity at the time of use, it has an expiration date determined by appropriate stability tests.

It is mandatory for drug manufacturers to print the expiration date of all manufactured drugs on the label of the container.

1.2.0 STABILITY TESTING

The classical isothermal method for stability testing is normally used for pharmaceuticals. This is usually referred to as the "accelerated" stability testing method, which may be defined as the validated method or methods by which product stability may be predicted by storage of the product under conditions that accelerate change in a predictable manner [4].

These conditions usually affect some properties of the drug, for example, the organoleptic properties, but most importantly, the conditions affect the rate at which the drug decomposes. The rate of decomposition of a drug is a useful tool in stability testing and shelf-life determination of a drug.

Major conditions that accelerate change are temperature, humidity, light, and agitation. The pH is important if a product is a solution [3,5]. This is also true for solids where solid state interactions which are analogous to those in solution may occur in localised micro-environments. In this study only the effect of modifying temperature is studied, while other conditions are kept constant. In accelerated stability testing where the drug product is stressed at temperatures higher than those expected in normal storage conditions, drug decomposition is more rapid than at room temperature.

In a relatively short period of time, much information is obtained about the stability of a drug product.

1.2.1 ANALYTICAL METHODS

Drug analysis and assay play important roles in the development, manufacture and therapeutic use of drugs. Analysis involves physical or chemical tests as well chromatographic procedures. These procedures are used to determine the physical properties of a drug such as melting point, chemical properties such as reactivity towards acids or bases, or chemical constitution such as the different functional groups present.

Assay methods involving titrimetric and gravimetric analysis were the mainstay of drug analyses during the first 60 years of this century. During the past 25 years, chromatographic and spectrometric methods of analysis have been widely developed and approved by drug regulatory agencies [43,49].

Chromatographic procedures, including thin layer chromatography (TLC), gas chromatography (GC), and high performance liquid chromatography (HPLC) are desirable because they provide direct measurement of active ingredient. Furthermore, the outstanding separation capacity of chromatographic procedures provides methods that permit simultaneous monitoring of drug purity.

Spectrometric methods of analysis UV/Vis, IR, NMR and MS vary in their capacity to detect impurities directly. However, coupling spectrometric and chromatographic procedures, powerful tools have been developed for quantification and identification of drugs.

Other assay methods include biological, electrochemical and thermal methods. Biologic analyses involve quantitation of the biologic behaviour of drugs. Typical analyses of this type are microbiologic assay of antibiotics. Inhibition of the growth of nonpathogenic organisms can be measured in solid or liquid cultures as a function of antibiotic concentration and thus provide active drug concentration.

1.2.2 KINETICS

The emphasis will be on first order kinetics as the rate of elimination for most drugs is a first order process. The rate of degradation is one of the chemical kinetic parameters of a drug essential in the determination of the stability and hence the shelf-life of a product.

The relationship between temperature and reaction rate constant is given by the equation (1), first suggested by Arrhenius,

$$K = A\, e^{-E/RT} \qquad (1)$$

or

$$\log K = \log A - E/2.303(RT) \quad (2)$$

where

K is the specific reaction rate

A is the frequency factor

E is the energy of activation

R is the gas constant (1.9872 Cal deg^{-1} $mole^{-1}$)

T is the absolute temperature

For a drug that degrades by first order kinetics, the rate of degradation is proportional to concentration and this relationship can be shown as follows

$$dC/dt = -K\,C \quad (3)$$

Integrating equation (3) and taking the natural logarithm of the equation yields the following equation

$$\text{Ln } C_t - \text{Ln } C_o = -Kt \quad (4)$$

or

$$\text{Log } C_t - \text{Log } C_o = -Kt/2.303 \quad (5)$$

where,

C_0 is the initial concentration

C_t is the concentration at time t

K is the degradation rate constant

A plot of Log C_t vs t yields a linear plot, where the slope of the graph is - K/2.303 and K the rate constant is calculated from the slope. The graph in figure 1 is obtained when plotting the log concentration of the drug that remained undecomposed versus time.

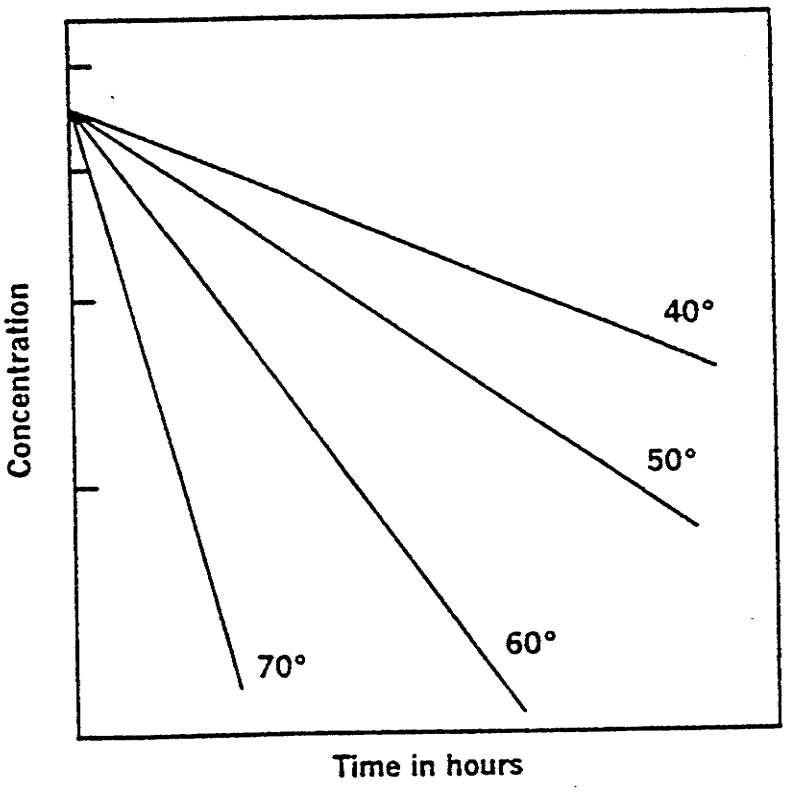


Fig.1 Model for first order accelerated breakdown of a drug in aqueous solution at elevated temperatures [3].

Each temperature has its own linear plot and hence its own slope, calculated as -K/2.303. A plot of the log of the specific rates of decomposition against the reciprocals of the absolute temperature result in a linear plot according to figure 2. From this graph, rate of decomposition at room temperature is obtained through extrapolation of the line to the reciprocal of room temperature. E(activation energy) is obtained from the slope of the line, and Log A is the Y intercept of the line.

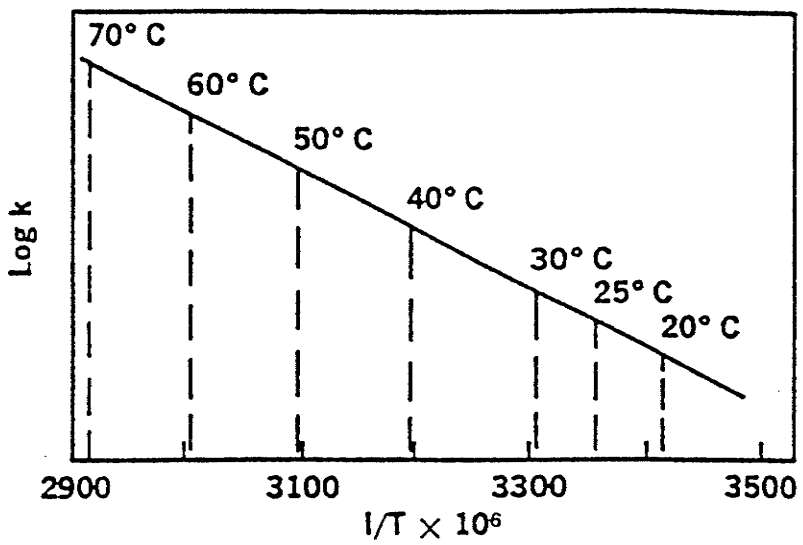


Fig.2 Model for Arrhenius plot of log of the specific rate of decomposition versus the reciprocal of the absolute temperature [3].

K_{rt} obtained is then used to calculate shelf-life of the drug under ordinary storage conditions.

Shelf-life of a drug can be calculated according to equation (6).

$t_{90\%} = 0.105/K_{25}$ (6)

where $t_{90\%}$ is the time taken for a drug to degrade to 90% of the labelled claim, the normally accepted shelf-life for most drug products.

In general equation (6) can be written as shown below

$t = (2.303/K_{25}) \log(C_0/C_t)$ (7)

where

t is the shelf-life of a drug

C_0 is the initial concentration of the drug

C_t is the concentration at time t

K_{25} is the rate of the decomposition of drug at room temperature

The use of equation (8) usually eliminates the need for graphing the data.

$\text{Log}(K_1/K_2) = E/2.303R(1/T_1 - 1/T_2)$ (8)

CHAPTER 2

2. LITERATURE REVIEW

2.0 ISOTHERMAL METHOD

Traditionally it has been normal practice of many pharmaceutical companies to evaluate the stability of pharmaceutical products by observing them for a year or more in order to establish their stability and hence the shelf-life. These methods are time consuming and uneconomical.

When stability studies at higher temperatures were introduced, the criteria used by most companies were often arbitrary and were not based on fundamental kinetic principles.

For example, some companies used the rule that the storage of liquids at 37°C accelerated the decomposition at twice the room temperature rate, while other manufacturers claimed that the decomposition was accelerated to 20 times the room temperature rate [3].

The isothermal (sometimes referred to as the classical) accelerated stability testing method was first introduced by Garrett in the 1950s [26] as a testing method for the prediction of shelf-life of pharmaceutical products. Unlike the previous methods, the isothermal method is based on chemical kinetics.

The isothermal method uses two sequential steps of linear regression involving

(a) A function of drug content versus time to obtain the rate constants (K) at several elevated temperatures.

(b) The relationship of logarithm of rate constants (K) versus reciprocal temperature to predict the room temperature rate constant and hence the shelf-life of a drug. Since Garrett introduced this method, much pharmaceutical literature has been published about stability testing and shelf-life determination of drugs [26-39]. There are several papers which show how the shelf-life can be obtained by slightly modifying some of the Arrhenius calculations from the normal isothermal method [31-34].

In the mid 1960s, predictive techniques based on the validity of the Arrhenius equation, which involve curve-fitting operations that can be carried out graphically or through least mean square calculations, did not have wide spread application in the pharmaceutical industry, mainly for economic reasons. Application of these new methods meant that new pharmaceutical products were researched for a much longer time before they were released to the market.

2.0.0 EARLY TECHNIQUES

In view of the cost of classical predictive stability studies, Lordi et al (1965) [39], designed stability charts, using nomographic representation of the Arrhenius equation for

computing estimates of the shelf-life of pharmaceutical products directly from the assay data.

The nomogram or stability chart in figure 3 shows the relationship between the room temperature stability, heat of activation and the specific rate of degradation at two elevated temperatures. This method has since been proven not to be reliable.

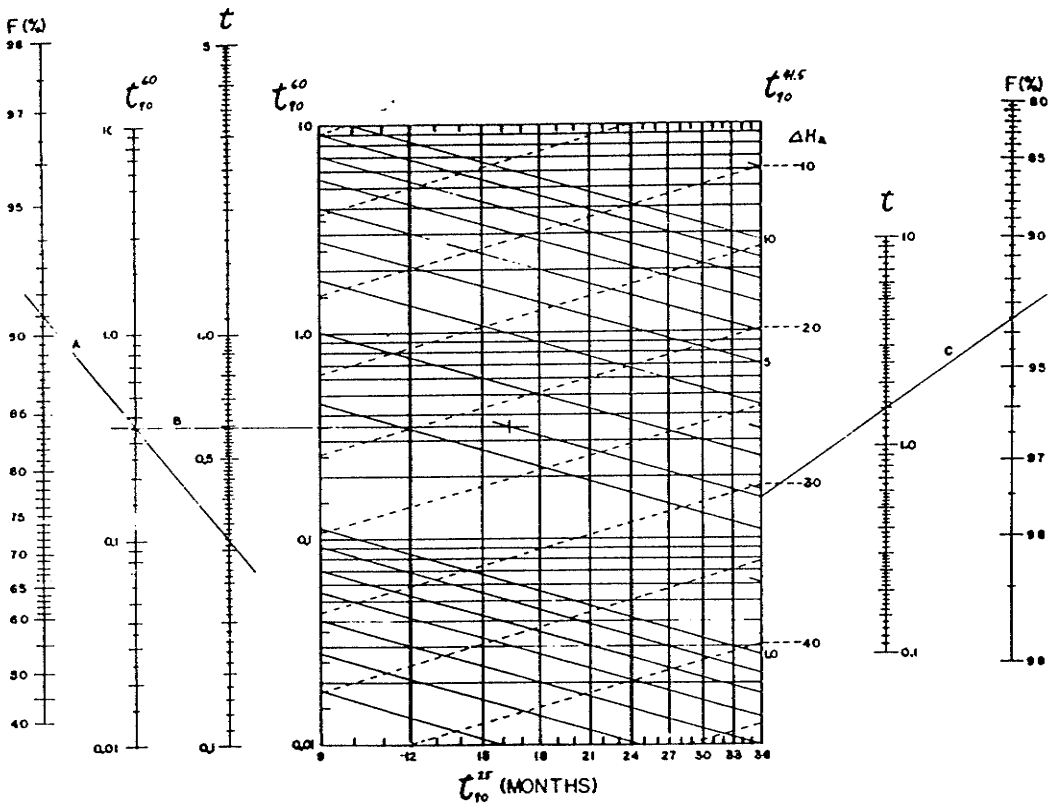


Fig. 3 Stability chart constructed for a first order degradation and the elevated temperatures of 60° and 41.5°C. [39]

2.0.1 STATISTICAL TECHNIQUES

Many statistical evaluation papers on stability determination have been published [28,32,38]. Some key references are cited here. Ertel et al. [34] modified the Arrhenius relationship for pharmaceutical stability prediction. In most cases, the Arrhenius law holds reasonably well, although there are instances when modifications of this law are necessary, for example where limited amounts of stability data are available. The problem in this case is that the calculated confidence interval for an extrapolated rate constant at storage conditions is very wide and the rate constant therefore may be meaningless.

Several statistical techniques have been introduced or proposed in order to improve stability prediction using accelerated, elevated temperature data. The improved prediction also provides a reliable confidence interval of an extrapolated rate constant. Several authors have recommended use of the nonlinear [28] and linear [34] modifications of the Arrhenius equation which often yield narrower confidence intervals for extrapolated degradation rates.

L. Bently [32] suggested the method of weighted least-square analysis as opposed to the simple least-square analysis for the treatment of data, especially when the degradation pathway of a drug follows a non zero order path. He pointed out that the benefits of a weighted least-square analysis are many, and most importantly, estimates of parameters of the Arrhenius equation

meet statistical requirements as the most accurate which can be obtained. Also a confidence interval can easily be constructed around the rate constant for any desired temperature, such as room temperature.

Multiresponse estimation in drug stability studies [36] is the simultaneous analysis of data collected on the parent compound and its degradation products. Papers on stability studies of pharmaceuticals rarely consider this kind of approach. According to Kowalski et al. [35], a method of multiresponse estimation can be used to obtain estimates of kinetic rate constants which relate the degradation of the parent compound to the formation of the degradation products. The multiresponse model can be further used to treat data obtained from accelerated testing of drug stability using the Arrhenius relationship.

2.0.2 OTHER PARAMETERS

Scher et al. [33] suggested a new parameter, the kinetic ratio, which is used for estimating product potency during storage conditions under ambient warehouse temperatures. Actual warehouse temperature data are integrated using Arrhenius kinetics, and the resulting potency errors are evaluated. Kinetic ratio is a ratio of a rate constant determined at a temperature that changes with time compared to the rate constant at a fixed reference temperature.

2.1 EVOLUTION OF NONISOTHERMAL METHODS

In chemical kinetics, degradation of a chemical compound is usually studied under isothermal conditions and the reaction rate constant is determined for a series of different temperatures. Nonisothermal techniques involve the continuous measurement of change of a physical property such as weight, volume and heat capacity, as temperature is increased, usually at a predetermined rate [40-43].

As early as 1957 Borchardt and Daniels [41] worked on the decomposition of benzene diazonium chloride using differential thermal analysis (DTA). From the results they were able to obtain the rate of reaction and activation energy of the reaction.

In the Borchardt method, the amount of heat produced by the reaction was compared with a solvent blank while the entire system was heated continuously. The kinetic parameters of the reaction were determined by an analysis of the slope, height and area of the DTA curve. All the kinetic parameters of the chemical reaction were obtained in a single, rapid experiment.
Figure 4 is an example of the DTA curve that Borchardt and Daniel obtained from the decomposition of benzene diazonium chloride.

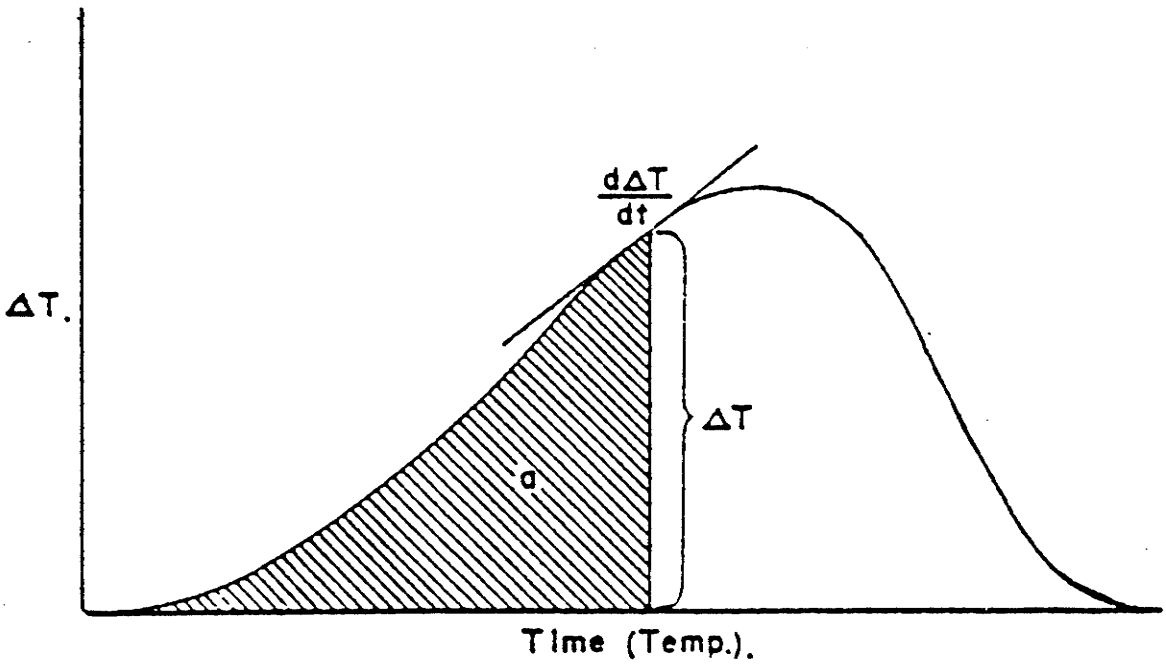


Fig.4 DTA curve showing the quantities which are measured in order to evaluate the rate constant for the reaction giving rise to the curve [41].

2.2 NONISOTHERMAL METHODS

Nonisothermal methods for the prediction of shelf-life of pharmaceutical products are attractive alternatives to the classical isothermal method.

In nonisothermal kinetics, the rate constant K, in equation (3), $dC/dt = -K\,C$ is not constant as in the usual rate equation. K varies throughout the experiment as the temperature is altered.

The use of nonisothermal methods on pharmaceutical products was introduced in 1963 by Rogers [7]. Even though much work had been done in applied chemistry, there has been very little or no exchange of information between researchers in the chemical and the pharmaceutical industries.

Rogers introduced a non-isothermal temperature programme, in which the rise of temperature is programmed so that the reciprocal of the temperature varies logarithmically with time. The equation that describes the temperature programme is as follows:

$$1/T_0 - 1/T_t = 2.303\ b\ \log(1+t) \qquad (9)$$

where

T_0 is the absolute temperature at the start of the experiment.

T_t is the absolute temperature at time t.

b is the heating rate constant.

With this accelerated test the rate constant at a specified temperature and the energy of activation of the degradation reaction may be estimated from a single experiment. Rogers applied the technique to the first order decomposition of solutions of riboflavin and sucrose [7].

Several other nonisothermal temperature programmes have also been developed; e.g., Eriksen and Stelmach [28] described the use of a reciprocal heating device and derived the following

relationship:

$$1/T = 1/T_o - at \qquad (10)$$

where

a is a reciprocal heating constant

Linear nonisothermal kinetics have been used to follow first order degradation of N-acetyl-p aminophenol and procainamide hydrochloride according to the following temperature time programme Zoglio et al. [13,14,19]:

$$T_t = T_o + bt \qquad (11)$$

Other temperature programmes include the stepped temperature rise, the uncontrolled temperature rise, the polynomial temperature programme and the cyclic temperature programme [11-22]. Since little work has been done on the last three temperature programmes, only the first four temperature programmes will be discussed in detail.

2.2.0 LOGARITHMIC TEMPERATURE PROGRAMME

This system is based on the following equation:

$$1/T_o - 1/T_t = 2.303\ b \log(1 + t) \qquad (9)$$

Sometimes this programme is referred to as the logarithmic increasing temperature programme. The temperature increase is programmed so that the reciprocal of the temperature varies logarithmically with time [7-10,12].

THEORY

The relationship between rate constant K and absolute temperature can be described by the Arrhenius equation in the following manner,

$$\log K_t = \log K_0 + (E/2.303\ R)(1/T_0 - 1/T_t) \qquad (12)$$

Substituting 2.303 b log(1 + t) from equation (9) into equation (12) gives the following equation

$$\log K_t = \log K_0 + (E\ b/R)\ \log(1 + t) \qquad (13)$$

or

$$K_t = K_0\ (1 + t)^{E/R} \qquad (14)$$

Assuming first order kinetics, the rate of decomposition of a drug is proportional to concentration, ie

$$-dC/dt = KC \qquad (3)$$

The mathematics involved in these calculations are very complex. Only the final term after integration of the equation will be shown.

Substituting equation (14) for K into equation (3) and integrating, the final equation becomes,

$$\log[2.303 \log(C_o/C_t)] = \log K_o - \log(1 - E\,b/R) + (1 + E\,b/R) \log(1 + t) + \log[1 - (K_t/K_o)^{\cdots}] \qquad (15)$$

The final term on the right-hand side of equation (15) varies with time. Just after the start of the experiment, it is large and negative, but it rapidly tends to zero as the reaction proceeds. It becomes negligible as K_t becomes substantially greater than K_o.

In general this equation can be written as shown below,

$$\log F = \log K_o - \log(1+Eb/R) + (1+Eb/R)\log(1+t) \qquad (16)$$

where

$$F = 2.303 \log(C_o/C_t)$$

A plot of log F against log(1 + t) in figure 5 will be a straight line with a slope of the line being (1 + E b/R) and the intercept at log(1 + t) = 0 is equal to log K_o - log(1 + E b/R).

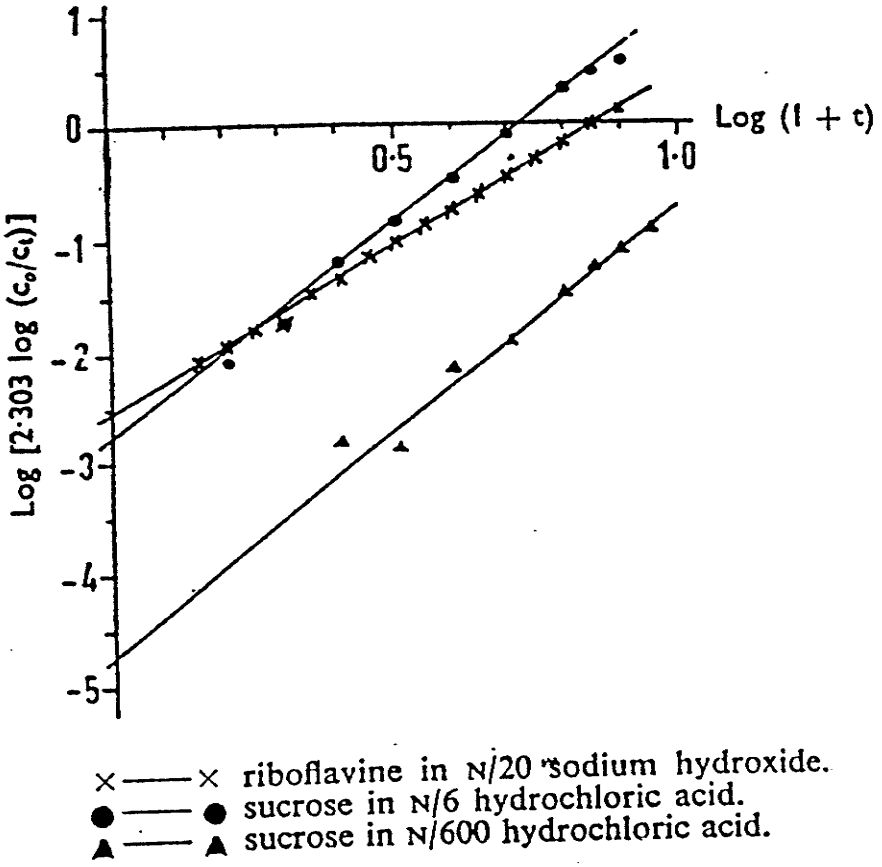


Fig.5 Decomposition of sucrose and riboflavin during a temperature rise according to the logarithmic programme. [7]

Since b, the programme heating constant and R the gas constant are known, the activation energy E can be calculated from the slope. Also the rate constant K_0 at temperature T_0 can be calculated from the intercept. From K_0 and E, the rate constant at any other temperature can be calculated by the use of Arrhenius equation (8).

Thus the rate constant at ordinary storage conditions, as well as the shelf-life of a drug product can be calculated using the following equation:

$$t_{90} = 0.105/K_{90} \qquad (6)$$

or a more general equation,

$$t = (2.303/K_{90})\log(C_0/C_t) \qquad (7)$$

A value of $2.171(10^{-4})$/deg was selected for the programme heating constant b, with the initial temperature $1/T_0 = 0.0035$ deg so that the temperature was programmed to rise from 12.5°C to 55°C in 7 hours. A water bath with a thermostat attached was used to control the temperature.

One disadvantage of this method is that the order of reaction cannot be determined unless more than 20% decomposition has occurred. Also the manually controlled thermostat is bound to incorporate some errors, especially if the experiment has to run for a few days.

2.2.1 HYPERBOLIC TEMPERATURE PROGRAMME

$$1/T = 1/T_o - at \qquad (10)$$

The approach used in this system, samples are taken at intervals chosen to give pairs of readings at time t and $(t + \delta t)$.

THEORY

Equation (3), the rate of degradation of a drug by first order kinetics,

$$dC/dt = -KC \qquad (3)$$

can be written in the following form by substituting K, the rate constant from equation (1) into equation (3).

$$dc/dt = -A\, e^{(-E/RT)}C \qquad (17)$$

This programme allows the integration of equation (17), to give the following equation, for first order reaction,

$$\text{Log } C_o/C_t = (R\, K_o/a\, E)e^{aE/RT} - R\, K_o/a\, E \qquad (18)$$

When the temperature is sufficiently greater than T_o the equation reduces to

$$\text{Log}(C_o/C_t) = \log\,(R\, K_o/a\, E) + (a\, E/R\ 2.303)t \qquad (19)$$

A plot of the logarithm of the concentration function against time should give a linear plot, so that E energy of activation and K_o can be estimated from the slope and the intercept. This equation holds only after an increase in temperature to about 40 degrees Celsius and when E is 20 kcal/mol.
This implies that the usable data cover only a small percentage of decomposition [11,17,21].

The problem was overcome by pairing equi-spaced data points so that the following equation was obtained,

$$\text{Log}(C_1/C_2) = \log[(e^{\delta t/\tau} - 1)(R\,K_o/a\,E)] + (a\,E/2.303\,R)t \qquad (20)$$

where C_1 and C_2 are concentrations at time t and $t+\delta t$ respectively. A plot of the log of the concentration function versus time is linear. The energy of activation can be calculated from the slope and k_o which is the rate constant at the start of experiment can be calculated from the intercept. If T_o is the desired shelf temperature, then K_o is the reaction rate constant. But if T_o is not the shelf temperature, then E and K_o may be used to determine the appropriate reaction rate constant by extrapolation to shelf temperature by the use of equation (8), and hence the shelf-life can be calculated. Eriksen et al.
used their reciprocal heating device to determine the kinetic parameters of ethyl acetate and para nitrophenol acetate.

The equipment comprised a reaction vessel and stirrer, which was immersed in a water bath whose temperature was controlled by a variable speed programmer. Temperature inside the reaction vessel was monitored using an iron-constantan thermocouple having ice water as its reference temperature. This temperature was recorded on a calibrated potentiometric recorder. The major source of error in this temperature programme appeared to be in the imperfections of the heating programme.
This limitation could easily be overcome by more sophisticated instrumentation. Another source of error is if δt is not held constant [11].

2.2.2 LINEAR TEMPERATURE PROGRAMME

This temperature programme is sometimes referred to as linear-up temperature programme or linear increasing temperature programme. With this system, the temperature is increased at a set constant rate over the entire temperature span. The temperature programme is described by the following equation,

$$T_t = T_s + bt \qquad (11)$$

Zoglio et al. [11,13,14,19] used this temperature programme when studying the decomposition of N-acetyl-p-amino phenol and procainamide hydrochloride.

THEORY

The rate constant for the reaction is expressed according to the following equation

$$K = A\, e^{-E/RT} \qquad (1)$$

The rate of change of drug concentration with time,

$$dc/dt = -kC \qquad (3)$$

and substituting k in equation (1) into equation (3) yields the following equation,

$$dc/dt = -A\, e^{-E/RT}C \qquad (17)$$

This equation can not be integrated for the case where temperature is changing linearly with time. In this case it is not possible to obtain a concentration-time equation that would yield the activation energy and reaction rate directly.

This problem can be overcome by using differential methods. This involves the estimation of the derivatives dc/dt at various times and hence at corresponding temperatures. Figure 6 below shows a first order decomposition profile of a drug that was determined using a nonisothermal linear temperature program of 6°C per week.

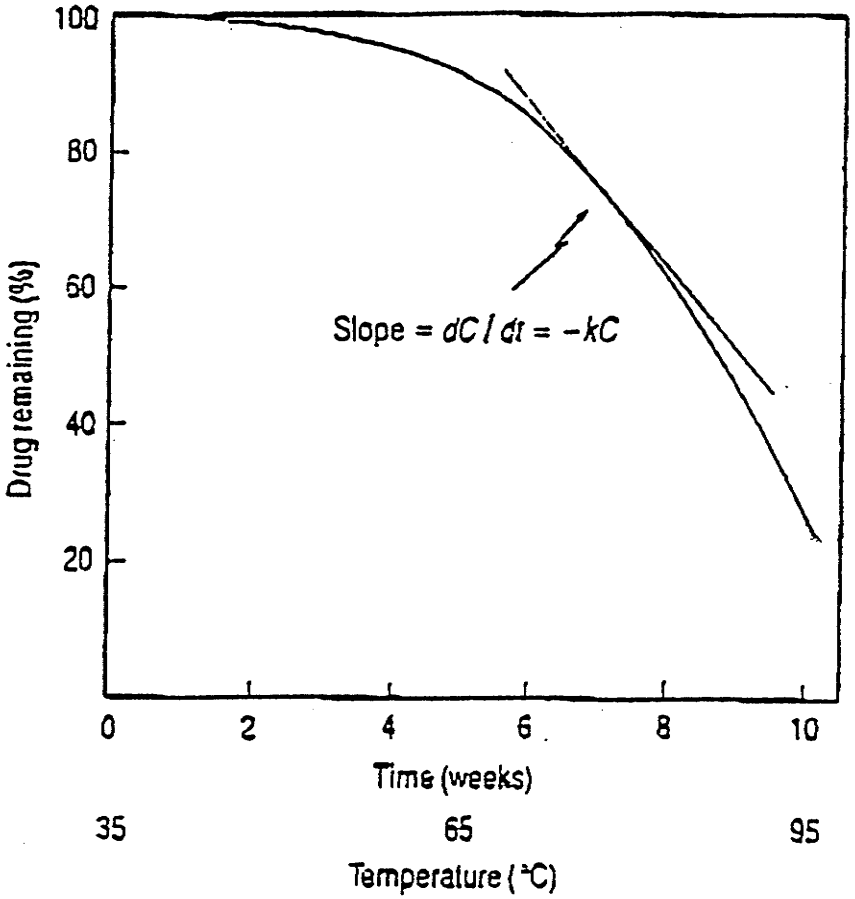


Fig.6 Decomposition profile for a drug subjected to a linear temperature programme of 6°C/week. [11]

The slope of the curve at each time and temperature is equivalent to (dc/dt)/C = k. where k is the rate constant at the chosen temperature.

Derivatives can be estimated in various ways, but the disadvantage of these derivative methods include the need for an almost continuous record of temperature and concentration

changes, and the sensitivity of the derivatives to errors in the measured concentration.

Zoglio et al. utilized a polynomial regression for the treatment of data from the linear time temperature relationship study. The theory is based on the following: consider the hypothetical degradation of a drug during a linear nonisothermal study. In this degradation the reaction order remains unchanged throughout the experiment, and the temperature changes linearly as a function of time, according to equation (11). In figure 7 the curve for the degradation, f(C), can be described by a series of slopes or rate constants each representing the instantaneous rate of change of a drug concentration with time at some temperature.

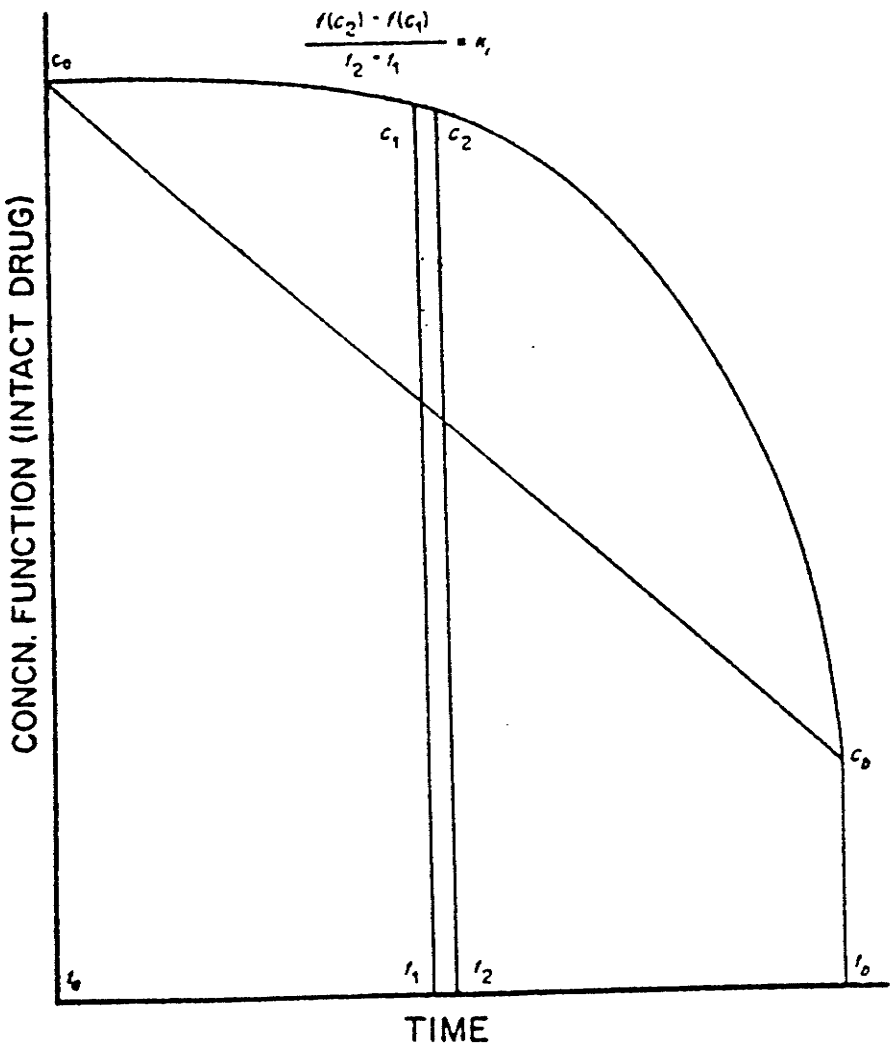


Fig.7 Hypothetical degradation of a drug during a linear nonisothermal stability study. [13]

The linear method is based on the assumption that the arithmetic mean of these rate constants is equal to the total degradation of the drug during the experiment divided by the time span required for the experiment.

The appropriate equation is:

$$f(a)-f(b)/(b-a) = (k_1 + k_2 + k_3 + \ldots\ldots + k_n)/n \quad (21)$$

Solving for individual rate constants, by the use of the Arrhenius equation, k_1 can be written as follows, using equation (1),

$$k_1 = Ae^{-E/T1} \quad (22)$$

$$K_{i+1} = Ae^{-E/RTi+1} \quad (23)$$

divide equation(23) by equation(22)

$$k_{i+1} = k_i e^{-E/(R(Ti+1-Ti)/(TiTi+1))} \quad (24)$$

Thus each rate constant can be expressed in terms of k_1. Since the quantity f(a) - f(b) /b-a is known through the experiment, the rate constants k_1 to K_n can be calculated for a particular activation energy. The calculations are so cumbersome that a digital computer is used to calculate the rate constants k_1 to k_n.

For their study Zoglio et al. used a digital computer to obtain 384 slopes or rate constants. These were used to generate a series of degradation curves, figure 8, corresponding to various activation energies. The analytical data were then superimposed over the model curves to obtain the activation energy for the reaction.

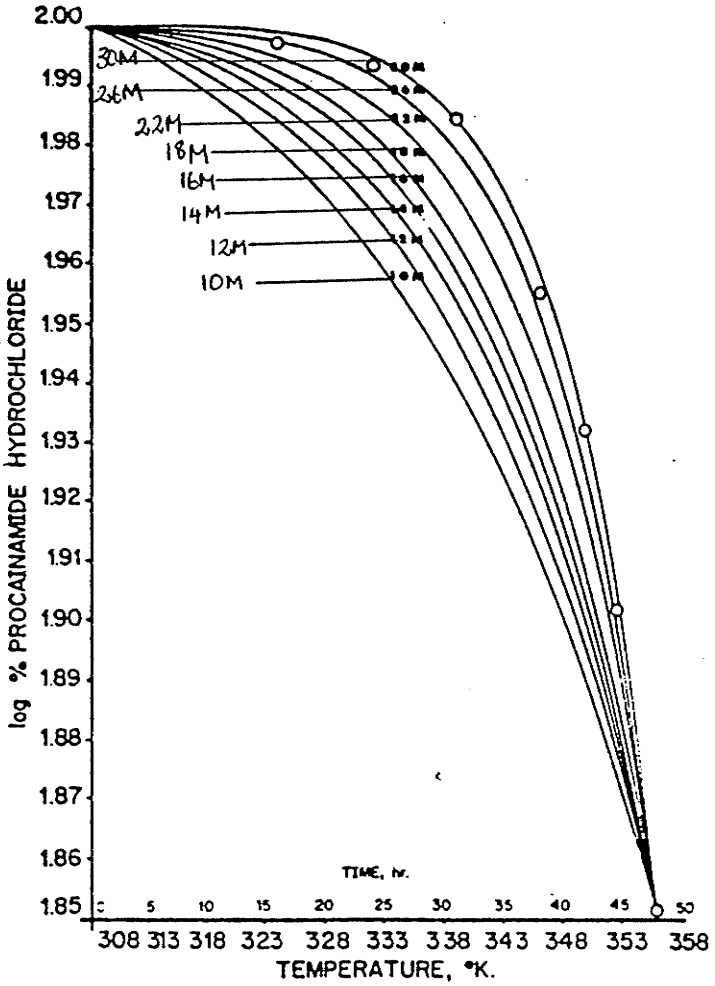


Fig.8 Nonisothermal hydrolysis of procainamide hydrochloride in 0.934M perchloric acid. [13]

The activation energy, the actual experimental degradation and the generated computer data were used to calculate the specific reaction rate, or stability prediction at any desired temperature. The Arrhenius equation may be used with these rates

to calculate the rate constants outside of the temperature range studied. Therefore it is possible to calculate the shelf-life using this temperature programme.

The equipment used was a temperature programmed convection oven, Thelco model 17 equipped with a thermocouple and a saturable core reactor.The temperature was continuously recorded, and the temperature range was arbitrarily set at 35 to 85 degrees Celsius, with a linear rise of 1 degree per hour. The linear nonisothermal temperature programme method has been used to a greater extent than other nonisothermal programmes. With the linear method, temperature rises at a slower rate than in a logarithmic temperature programme, allowing easier sampling.

A disadvantage of this method is that it places unjustified weight on the first and last points, which alone are used to estimate the value of A. The generated curves all pass through the first and the last data points, so that the curves lie close together at the extremities. Researchers who developed this method eliminated this extremity data from their calculations. As a result, on some occasions values of the activation energy were estimated from data covering very narrow ranges of temperatures which could lead unreliable results. Also the order of reaction cannot be determined from the nonisothermal data.

2.2.3 STEPPED TEMPERATURE PROGRAMME

For this temperature programme, the temperature is increased in a stepwise fashion with consecutive equal steps [23]. The number and duration of the steps are predetermined. Each temperature stage can be regarded as a miniature isothermal kinetic model.
A plot of temperature versus time result in the figure 9.

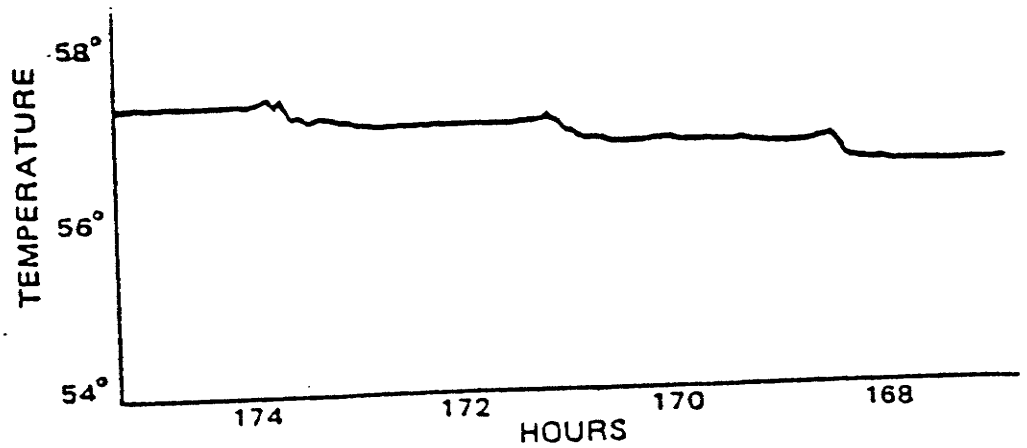


Fig.9 Experimental temperature profile of stepped temperature programme. [23]

This method was designed by Edel et al. , who claim that the error due to the time required to raise the temperature between each stage can be regarded as negligible. The equation for degradation is

$$\text{Ln } C - \text{Ln } C_o = t/n \ (k_1 + k_2 + k_3 + \ldots\ldots\ldots + k_n) \qquad (25)$$

Like the linear temperature programme, to solve the above equation for any rate constant, the constant must be expressed in

terms of all the other constants in the equation. In the final analysis the equation is solved for each rate constant, k_i

When k_i = k_1, the equation was found to be

$$\text{Ln } C - \text{Ln } C_0 = k_1\, t/n\{1 + \Sigma e^{[illegible]}\} \qquad (26)$$

Equations similar to this are then solved for rate constants k_1 to k_n. Calculations involved in solving this equations are tedious and very long, and are best computerised.

For a first order reaction and a particular activation energy, this programme calculates the k value corresponding to the first temperature stage. It is then possible to determine the theoretical concentration at the end of this stage, which is also the concentration at the beginning of the next stage. Repeating this process, the theoretical concentrations of all the stages can be calculated.

The concentrations calculated theoretically for a particular activation energy are plotted for each time interval given by the computer as shown in figure 10.

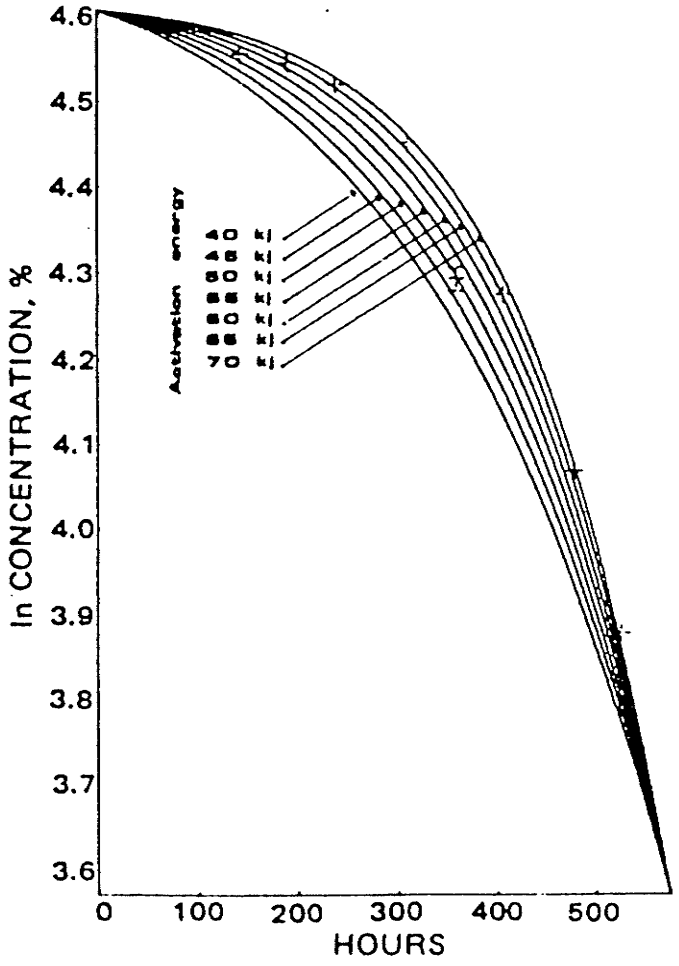


Fig.10 A plot of theoretical concentration for the stepped temperature programme. [23]

The experimental data points are also plotted on the graph. This enables one to determine the activation energy for the reaction. The specific rate constant, or any rate constant outside the studied temperature range can be calculated using the Arrhenius equation.

The investigation of this temperature programme was carried out on a substituted benzazepine with a 0.2% solution. The experiment was carried out in a thermostatically controlled bath of glycerol-water. Samples were removed from the bath at predetermined times coinciding with the end of a temperature stage.

A programmer was used to control the rise of temperature in the bath. The system enables the bath temperature to be altered automatically stepwise from a preset starting temperature over a range of up to 115°C at 10 rates. The kinetic experiments were carried out between 40°C and 97.7°C in consecutive equal steps which in this case were temperature changes of 0.3 degrees every three hours.

2.3 ANALYTICAL METHODOLOGY

Ibuprofen was synthesised in the early 1960,s [59]. The assay method then was a simple volumetric analysis. Ibuprofen was dissolved in ethanol and titrated with sodium hydroxide and phenolphthalein solution as indicator. This assay lacks sensitivity for routine quality control. The assay is not as specific as GC/HPLC methods.

In 1965 some researchers utilised quantitative paper chromatography to estimate the amount of ibuprofen in serum [60]. Their method involved an extraction process followed by paper chromatography. They used Whatman no.1 and a volume of 5 μL of

both standard and test was used. The papers were sprayed with a solution of 0.1% w/v bromocresol purple and Ibuprofen appeared as a yellow spot on a blue background. The amount of Ibuprofen was estimated by comparing the area and intensity of unknown with the standard. This method requires 48 hours to complete. Although this method is more specific than the titration method, it is too slow for routine quality control.

A GLC assay method was reported [61] requiring time consuming extraction of ibuprofen into benzene, followed by evaporation of the extract to dryness and subsequent derivatization of ibuprofen to the methyl ester.

Equipment for extraction was a two-speed reciprocating shaker for shaking the samples in a horizontal position. GLC measurements were made with a gas chromatograph equipped with a hydrogen flame-ionization detector and a -0.2 to 1.0 mv recorder. All cylinders of gases used for chromatography, helium, hydrogen and oxygen, were fitted with filters containing molecular sieve 4A.

GLC conditions were conducted on a U-shaped glass column (1.5 m * 3 mm) of 3% (w/w) OV-17 on 60 mesh Gas Chrom Q. The column was preconditioned at 250° for 1 hr without carrier gas flow and for 16 hr with a carrier gas flow of 10 mL/min. This method involves a lot of manipulation and is very slow therefore it is not an ideal method.

A GC method for ibuprofen was included in the USP XX supplement 3 [62]. The gas chromatograph was equipped with a flame ionization detector and contained a 120 cm * 3 mm glass column packed with 10 percent G3 liquid phase support. The column and detector block were maintained isothermally at about 185° and 200° respectively. Dry helium was used as the carrier gas at a flow rate of about 60 mL per minute.

The assay involved preparation of the internal standard solution, the standard and test solutions.

Internal standard solution: A solution of n-nonadecane in chloroform containing about 2.2 mg per mL.

Standard: 100 mg of USP standard dissolved in 50 mL of the internal standard solution. 5 mL of hexamethyl disilazane was added and the solution warmed to about 45°, shaken vigorously and allowed to stand for 30 minutes. The test solution was prepared as above like the standard. 2 μL of both standard and test solutions was injected into the gas chromatograph and ibuprofen peak located.

For analysis of tablets the above GC method is used. The procedure involves an initial extraction phase of ibuprofen from the tablet formulation into chloroform. For routine quality control analysis this method is log and could incorporate a lot of errors.

A sensitive and simple rapid method for the quantitation of ibuprofen have been developed which have superseded the GC method. The methods involve the use of HPLC.

Savage et al. [63] performed an HPLC assay of ibuprofen in bulk drug and tablets and for dosage uniformity testing. HPLC was carried out on a stainless steel octadecylsilane column using 25 % 0.25 M glacial acetic acid in acetonitrile as the mobile phase, with UV detection at 254 nm. Their results were comparable with those of USP 20" revision supplement 3, 1982. USP adopted an HPLC method this method is described in detail in later chapters.

A rapid and sensitive HPLC method for ibuprofen in plasma was reported by Ali et al. [64]. The method is based on reversed phase HPLC with a mobile phase containing acetonitrile and 0.1 M acetic acid (55:45 v/v). The chromatographic elution time was 8.5 minutes, and ibuprofen quantities as low as 0.1 μg/mL can be assayed.

Samples were chromatographed on HPLC equipped with a universal liquid chromatography injector, a UV absorbance detector and a strip-chart recorder. The deprotenated plasma samples were injected at room temperature on a microparticulate reversed phase HPLC column 4 mm * 30 cm. The flow rate was adjusted to 1 mL/min with an inlet pressure of 1500 psi. The ratio of peak height of ibuprofen to that of internal standard was used to calculate the ibuprofen concentration. This method was designed for assay of ibuprofen at low concentrations in plasma and was not considered ideal for assay of tablets.

CHAPTER 3

3. EXPERIMENTAL AND METHODOLOGY-IBUPROFEN ASSAY

3.0 Materials used

MATERIAL	LOT #	MANUFACTURER
Ibuprofen BP, USP	8911295	Shasun Drugs
Lactose monohydrate USP	JA180	Spectrum Chemicals
Microcrystalline cellulose	000693	Spectrum Chemicals
Corn Starch	78F-0895	Sigma
l-Leucine	L800	Sigma
Sodium lauryl sulphate	L-0105	Sigma
Silicone Dioxide	GA291	Spectrum Chemicals
Magnesium Stearate USP	IF 187	Spectrum Chemicals
Polyvinyl pyrrolidone (PVP)	HI203	Spectrum Chemicals
Sodium carbonate monohydrate		Sigma Chemicals

3.1 Other chemicals

CHEMICAL	LOT #	MANUFACTURER
Ibuprofen USP reference standard	Cat # 33550	
Chloro acetic acid	2793	Fisher Scientific
Acetonitrile (HPLC grade)	A998B4	Fisher Scientific
Phosphoric acid 85%	863537	Fisher Scientific
Valerophenone	42H3666	Sigma
Ammonium chloride	06067	Fisher Scientific

3.2 EQUIPMENT

3.2.0 Equipment for assay

HPLC Method 1 (USP method)

Equipment: HPLC pump, Shimadzu LC 16A
Injector, Hewlett Packard model number 1050
UV Detector, Hewlett Packard model number 1050
Integrator, Hewlett Packard HP 3396A
IEC Centrifuge, Micro MB Centrifuge
Balance, Mettler AE50

Column: Octadecyl Silane, (Supelcosil LC-18)
25 cm * 4.6 mm
Particle size 5μm (Supelco Inc)
Lot number 071212AB
Catalog number 5-8231

HPLC Method 2 (improved assay)

Equipment: HPLC pump, Shimadzu LC 16A
Injector, Hewlett Packard model number 1050
UV Detector, Hewlett Packard model number 1050
Integrator, Hewlett Packard HP 3396A
IEC Centrifuge, Micro MB Centrifuge
Balance, Mettler AE50

Column: CSC-S Nitrile

15 * 0.46cm

Particle size 3μm

Lot number 079032

3.2.1 Equipment for tablet manufacturing

Granulation Equipment

Granulator: Basic food processor, model by Cuisinart with capacity of 2 litres.

Balance: Mettler AE50

Sieve # 10

Plastic trays for drying the granules

3.2.2 Equipment for tablet compression

Tablet press: A single punch tablet press.

Manufacturer: Stokes, Merrill-Alfa and Laval group.

Model: 511-7

Serial # 089047

3.2.3 Equipment for isothermal method

Ovens: Blue M Oven, a single wall transite oven by Blue M Electric

Can Lab oven, By Labline Inc.

Freezer : Up-Right freezer by Viking, temp -18°C

For incubation: 20ml brown glass bottles

3.2.4 Equipment for non-isothermal method

Oven: Gas chromatography oven, model 3700 Varian

Freezer: Up-right freezer by Viking, temp -18°C

For incubation: 20ml brown glass bottles

3.3 ANALYSIS AND ASSAY

Drug assay refers to determination of drug concentration in mixtures such as dosage forms and biological fluids. Assays as well as other tests and standards are developed by industrial quality control groups to assure quality during manufacturing [44,45,46]. Also enable stability of the active ingredient to be assayed. Other factors considered during dosage form assay development include,

. Sensitivity
. Accuracy
. Precision
. Convenience
. Cost
. Ease of Automation

3.4 IBUPROFEN ASSAY

3.4.0 Method 1 USP XXII 1990

Assay method: HPLC
Mobile phase: 4.0 g of chloroacetic acid was dissolved in 400 mL of water. pH was adjusted to 3.0 with ammonium hydroxide. 600 mL of acetonitrile was then added to the solution.

Column: Octadecyl Silane, (Supelcosil LC-18)

25 cm * 4.6 mm

Particle size 5 μm (Supelco Inc)

SAMPLE PREPARATION

Internal standard solution

A solution of valerophenone in mobile phase at a concentration of 0.35 mg/mL.

Standard solution

A standard solution of ibuprofen, at a concentration of 12 mg/mL was prepared using Ibuprofen USP reference standard, cat. 33550, and the internal standard solution was used as solvent.

Test solution

About 1200 mg of ibuprofen manufacturing grade was weighed accurately and transferred to a container, 100 mL of internal standard solution was added, and the solution was mixed.

Procedure

Equal volumes (5 μL) of the standard solution and the test solution were auto-injected separately into the HPLC. The peak area for the major peak was recorded. The relative retention times were found to be 1.4 for the internal standard and 1.0 for

ibuprofen. The quantity, in mg, of ibuprofen was calculated using the following formula:

100 mL C(Ru/Rs)

where C is the concentration in mg/ml of standard

Rs is the peak area of the standard

Ru is the peak area of the test sample

This method showed many of inconsistencies and variability during analysis, and had to be abandoned since the results were found to be unreliable. A second method was developed that proved to be reliable and consistent.

3.4.1 Method 2

Assay : HPLC method (Improved procedure) Details are given below.

Mobile phase: Acetonitrile 500 mL

0.0004 M Phosphoric acid

SAMPLE PREPARATION

Standard solution

A Standard solution of ibuprofen, at a concentration of 10 mg/mL was prepared using Ibuprofen USP reference standard, Cat. number 33550 , and the mobile phase was used as solvent.

Test solution

Sample solutions of ibuprofen manufacturing grade with a concentration of 10 mg/mL were prepared, using the mobile phase as solvent.

HPLC Parameters:

Detection, uv absorption at 264 nm

Injection volume: 10 μL

Chart speed: 0.2 cm/min

Flow rate: 0.1 mL/min

Pressure: 200 Kgf/cm^2

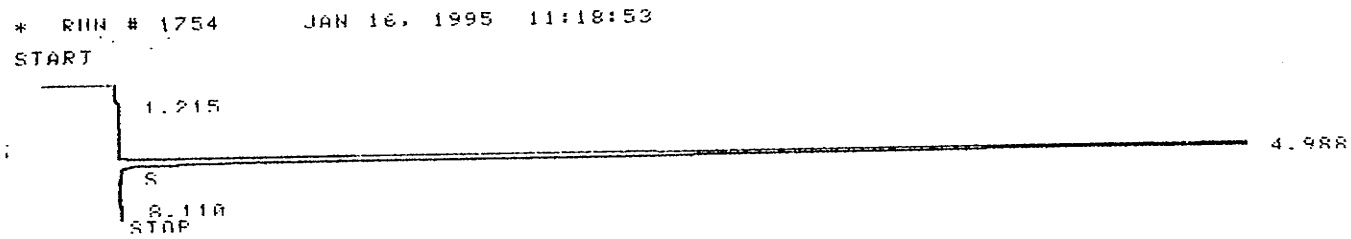
Running time: 10 min

Procedure

Solutions of unknown and standard were placed in glass vials for HPLC automatic injection of 10 μL was performed. For each analysis, two injections of the standard solution, followed by three injections of the test solution were performed. Amount of ibuprofen in milligrams was then calculated using the peak areas of both standard and test.

3.5 HPLC Chromatogram of ibuprofen

HPLC chromatogram of ibuprofen manufacturing grade. Retention time of ibuprofen with method 2 was around 4.5 to 5 minutes.



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AREA%

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4.988	26429888	SBB	.185	100.00000

3.6 ASSAY VALIDATION OF METHOD 2

According to USP 1995, to validate the assay, several parameters have to be checked, including sensitivity, accuracy, precision and linearity.

3.6.0 SENSITIVITY

Sensitivity pertains to the smallest amount of drug that can be reliably measured in an assay procedure [45,46]. One way of demonstrating this is by calculating the relative standard deviation of test parameters, in this case, peak area of several chromatograms of the least quantifiable concentration. Usually if the relative standard deviation is below 4% then the concentration can be reliably quantified. Minimum quantifiable

concentration was found to be 0.5 mg/mL equivalent to 0.13 μg

Procedure:

A solution containing about 0.5 mg/mL of ibuprofen raw material was prepared using mobile phase as solvent. Six aliquots of 10 μL of the solution were injected onto the column.

Table 1: A table of number of injections and peak area in the determination of sensitivity of the assay method

INJECTION NUMBER	PEAK AREA	REF #
1	1170445	1863
2	1163106	1864
3	1170671	1865
4	1161967	1866
5	1257069	1867
6	1160552	1868

Mean peak area 1180635

Relative standard deviation 3.19 %

Concentrations below 0.5 mg/mL could not be detected. These determinations of sensitivity and other parameters used in validation conform with the system suitability guidelines of the USP XXII 1990. Results obtained are consistent with those reported subsequently by other researchers using the assay.

3.6.1 PRECISION

Precision is a measure of the scatter or dispersion about the mean and among a set of replicated measurements [45-46]. It is usually expressed in terms of a standard deviation or relative standard deviation derived from statistical analysis of multiple determinations. Precision determinations permit an estimate of the reliability of single determinations and are commonly in the range of ± 0.3 % to 3 % of standard deviation. The mean in this case is based the assay of tablets containing a normal 50 mg of ibuprofen per tablet.

Procedure:

A standard solution of ibuprofen approximately 10 mg/mL was prepared using the USP reference standard, with the mobile phase as solvent. Three test solutions were prepared by weighing about 0.3 g of a powdered mixture of 50 mg ibuprofen tablets left at room temperature. The mixtures were transferred to three 10 mL volumetric flasks and diluted to volume with the mobile phase. The flasks were shaken for about 5 minutes. The solutions were then centrifuged for about 3 minutes and the clear solution transferred into HPLC glass vials. Injections of both standard and test were automatically injected onto the column.

Table 2: A table of peak area and amount of drug in the determination of precision of the assay method

SAMPLE #	PEAK AREA STD	PEAK AREA TEST	# mg of DRUG
1	21389552	20582832	50.7
2	25067936	15402928	50.2
3	22595280	20933456	51.6
4	24038432	16022256	52.8
5	22427456	21261472	52.4
6	21663304	13159296	51.5
7	24435680	14807608	48.7
8	20256919	12657496	49.3

Mean amount of drug = 50.9 mg

Standard deviation = 1.4 mg

50 mg was selected for this determination, since that is the quantity in a standard ibuprofen tablet.

Number of milligrams of drug in each sample was calculated. The eight determinations of the mean fall within the acceptable range of the standard deviation as defined in the USP.

3.6.2 LINEARITY

Linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of an analyte in samples within a given range [45-46]. One parameter that can be used to determine linearity is the regression coefficient that is obtained from the regression line.

Procedure:

Four standard solutions of ibuprofen of concentrations 15, 10, 5 and 2.5 mg/mL were prepared using the mobile phase as solvent. Four replicate aliquots of each standard were injected onto the column. The average peak area of each standard was plotted against concentration. A least squares regression analysis was performed on the data to check linearity.

Table 3: A table depicting peak area and concentration in determining linearity of the assay method

STANDARD	CONCENTRATION	PEAK AREA	AVERAGE PEAK AREA
A	15 mg/mL	37174688	37077608
		37975744	
		36678144	
		36481856	
B	10	24249072	24242560
		24346592	
		24202608	
		24171968	
C	5	12351480	12144582
		12125936	
		11955776	
		12145136	
D	2.5	5926874	5996956
		5988989	
		6093725	
		5978237	

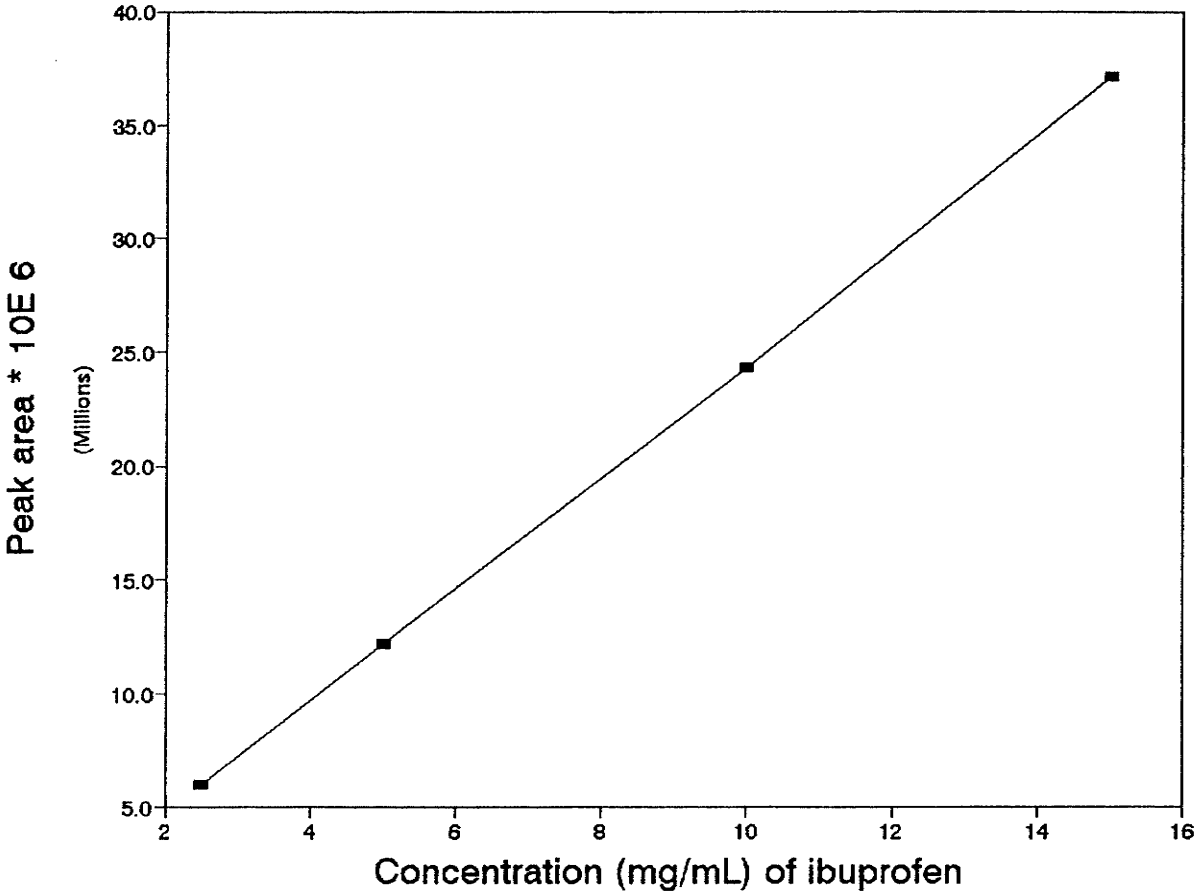

Fig. 11 A graph of peak area versus concentration depicting linearity of assay method.

Slope = 2404213

Intercept = 631720

Correlation coefficient = 0.99920

Results shown in this representative calibration curve are typical of those obtained throughout this study and related analyses performed in the same laboratory with the same compound. This involved separate studies over the course of two years.

The range of concentration selected for linearity study was based on concentrations which would be obtained in a quality control assay of standard 50 mg ibuprofen tablets.

3.6.3 ACCURACY

It is the degree to which an experimental mean agrees with the accepted or true value for the drug in the sample [45-46]. Accuracy of an assay can be determined through a series of experimental assays, the determined amount of drug from the assay is compared to the amount of drug added in the dosage form and the difference between the means is used to asses the accuracy. Dosage form assays commonly provide accuracy within 3 to 5 % of true values [46].

Procedure

A standard solution of ibuprofen approximately 10 mg/mL was prepared with the USP reference standard, using the mobile phase as the solvent.

Six test solutions were prepared by weighing about 0.2 g to 4 g of a powdered mixture of 50 mg ibuprofen tablets left at room temperature. The mixture was transferred into a 10ml volumetric flask and diluted to volume with mobile phase. The flasks were shaken for about 5 minutes. The solutions were then centrifuged for about 3 minutes and the clear solution transferred to HPLC glass vials.

Three aliquots of both the standard and test were automatically injected into the column. Only the mean peak area of standard were recorded. The amount of ibuprofen was then calculated in each sample, and then compared to the quantity added.

Table 4: A table depicting peak area and amount of drug in determining the accuracy of the assay method (50mg tablets)

sample #	Peak area STD	Peak area Test	# mg Ibuprofen	Mean (mg)
1	24460000	13980256	48.1	49.3
		14541160	50.0	± 1.4 %
		14511592	49.9	
2	25067936	15496408	50.2	49.9
		15744200	51.0	± 0.2 %
		14968176	48.5	
3	22127429	20582832	50.7	51.6
		2093345	51.6	± 3.2 %
		21261472	52.4	
4	20057224	12538656	51.4	50.1
		12031464	49.3	± 0.2 %
		12093192	49.6	
5	24435680	14807608	48.7	48.2
		14667504	48.2	± 3.6 %
		14582296	47.9	
6	23323304	16696952	50.7	51.1
		17170176	52.1	± 2.2 %
		16578048	50.3	

The variation of the means is indicated as a percentage of the true mean. Therefore determination of the accuracy of the analytical method indicates that the means are within the acceptable range of 50 mg ± 5% as defined in the United States Pharmacopoeia.

CHAPTER 4

4. STUDIES ON IBUPROFEN TABLETS

4.0 PREFORMULATION STUDY

Preformulation study or testing is usually defined as an investigation of physical and chemical properties of a drug substance alone and when combined with excipients [44,47,48]. Excipient compatibility with a drug substance is essential in order to formulate a stable and effective dosage form. Excipients facilitate administration, promote the consistent release and bioavailability of a drug. Excipients also protect a drug substance from degradation.

Tablet formulations contain disintegrants which facilitate complete break up of a tablet into smaller particles and facilitate dissolution of the active ingredients. Diluents, sometimes called fillers, are present in a formulation unless the active ingredient exceeds 250 mg. A binder is included to promote bonding and cohesion in the formation of granules. Other excipients include colourings, flavours, sweeteners and coating agents [44,45,48].

The technique commonly employed in drug-excipient compatibility screening is an accelerated approach utilizing differential thermal analysis, usually referred to as DTA, to screen the compatible or the incompatible drug-excipient

combinations [47]. Another technique involves chromatography, whereby the mixture of drug-excipient is stressed by incubating it at elevated temperatures for a certain amount of time.

The samples are examined periodically for appearances and any signs of decomposition are detected by the use of thin layer chromatography, TLC, or high pressure liquid chromatography, HPLC. Unstressed samples are used as control. If significant interaction is noticed at elevated temperatures, corroborative evidence must be obtained by examining mixtures stored at lower temperatures for longer durations [47].

4.0.0 Compatibility of Ibuprofen with excipients

Procedure

Equal amounts of excipient and Ibuprofen were mixed thoroughly using a pestle and mortar to produce a homogeneous mixture, which was incubated at 65°C for seven days. Control samples were left at room temperature.

An HPLC assay was performed on all the mixtures to check whether ibuprofen had been altered or had reacted with the excipients at elevated temperature or whether decomposition of ibuprofen had occurred.

Assay : HPLC 2 method

Mobile phase: Acetonitrile 500 mL

0.0004 M Phosphoric acid

Table 5: Results of compatibility study, expressed as a percentage of Ibuprofen present in the mixture

NAME OF MIXTURE	% IBUPROFEN IN HEATED MIXTURE	% IBUPROFEN IN UNHEATED MIXTURE
PVP + Ibuprofen	50	49
Mg Stearate + Ibuprofen	51	48
MCC+ Ibuprofen	55	46
Lactose (USP) + Ibuprofen	48	47
Corn Starch + Ibuprofen	42	44
Na lauryl sulphate + Ibuprofen	50	54
l-Leucine + Ibuprofen	46	54
Sodium carbonate + Ibuprofen	0.6	17

The mixture of ibuprofen and sodium carbonate was eliminated from further study because ibuprofen reacted with this excipient within the first week after mixing.

4.1 FORMULATION STUDY

Tablets may be defined as solid pharmaceutical dosage forms, usually prepared with the aid of suitable pharmaceutical excipients [44,45,48]. Different tablets may vary in size, shape, weight, hardness, thickness, disintegration characteristics. In other aspects, tablets vary depending upon the intended use and their method of manufacture.

In general most pharmaceutical excipients are thought of as inert ingredients, but in some studies it has been shown that excipients can significantly affect the bioavailability of a drug. A classic example is of calcium salts that interfere with the absorption of tetracyclines from the gastro-intestinal tract [44]. Another classic case of a chemical incompatibility is the interaction of certain amine drugs with lactose in the presence of a metal stearate lubricant like the commonly used magnesium stearate, which produce discolouration of the final tablet [44].

Excipients may be classified into two groups, according to the part they play in the finished tablet. The first group includes diluents, binders, glidants and lubricants. These excipients help to impart satisfactory processing and compression characteristics to the formulation. The second group of excipients includes disintegrants, colourants, flavours and sweetening agents.
This second group of excipients help to give additional desirable physical characteristics to the finished tablet.

4.1.0 DILUENTS

Diluents, sometimes referred to as fillers, are used mainly to add the necessary bulk to a formulation when the active ingredient is inadequate to produce the required bulk. when the active ingredient forms the bulk of the tablet, little or no diluent is required, and other excipients are kept at a minimum to avoid producing a tablet that is very large. Diluents include lactose, microcrystalline cellulose, hydrolysed starches and other cellulose derivatives. The physical properties of the diluent can have a marked effect on the disintegration properties of the tablet and release of the active ingredient.

4.1.1 DISINTEGRATING AGENTS

Disintegrants include corn and potato starch, starch derivatives like sodium starch glyconate, and cellulose derivatives such as sodium carboxymethyl cellulose. Disintegrants promote the break up of tablets after administration making the drug more available to go into solution and be readily absorbed. Disintegrating action is due to capillary action, with the disintegrant making the tablet matrix more porous. This affects both the rate of disintegration and the effectiveness of dispersion of the resulting particles.

4.1.2 LUBRICATING AGENTS

Lubricants enhance the flow of the granules from the hopper into the die cavity and also prevent the adhesion of granulate material to the punches and dies. Lubricants usually produce tablets that have a desirable sheen. Commonly used lubricants include magnesium stearate, talc, calcium stearate, stearic acid, hydrogenated vegetable oils and polyethylene glycol. Lubricants are mostly hydrophobic, therefore excessive amounts can result in poor tablet disintegration and hence delayed dissolution of the drug substance.

4.1.3 BINDING AGENTS

The binder is fundamental to promoting the adhesion of the formulation to give the granulation particle size uniformity, producing the free flowing granules with adequate hardness, influencing ease of compression and also the general quality of the tablet. Materials commonly used as binders include starch paste, gelatine, sugars e.g. sucrose and glucose, natural and synthetic gums such as acacia, sodium alginate, polyvinyl pyrrolidone (PVP) and tragacanth.

4.1.4 GLIDANTS

Glidants generally improve the flow characteristics of a powder mixture. Poor glidant action can result in soft tablets and can also create "rat-holing". The most commonly used glidant is colloidal silicon dioxide. It is used at concentrations of 1% or less. Talc frequently serves the function of lubricant/glidant.

4.1.5 COLOURING AGENTS

Colourants have no other functions other than making the tablet more aesthetic in appearance. Colourings are used by the manufacturer to control the product during processing as well as serving as a means of identification to the patient. Another use of colours is to disguise the off-colour drugs and produce a more elegant finished product.

4.1.6 FLAVOURING AGENTS

Flavours and sweeteners are added primarily to chewable tablets and these include certain dyes and lakes, natural and artificial sweeteners.

4.2 TABLET MANUFACTURE

A key step in tablet manufacturing involves forming granules from the tablet mixture for tablet compression. There are three established methods of granulation. The most widely used method is wet granulation. Its popularity is due to the greater probability that granulation will meet all the physical requirements for the compression of good tablets. The other two methods are dry granulation and direct compression. Which ever method of manufacture is used, the resulting tablet must meet a number of physical and biological standards.

4.2.0 PROPERTIES OF TABLETS.

1. A tablet must be hard and should resist shock and abrasions during all the manufacturing processes including shipping and handling.
2. Uniformity of tablet weight and drug content per tablet is important and this is usually tested by the weight variation test and content uniformity test.
3. Tablets should remain stable throughout their shelf-life
4. The active ingredient of the tablet must be available. This can be assessed by the disintegration test and the dissolution test.
5. Tablets must be elegant in appearance and must have a characteristic shape, colour and other markings necessary to

identify the final product. [48]

4.3 DESIGN OF FOUR IBUPROFEN TABLET FORMULATIONS

The wet granulation method was used in all four formulations in this study. One of the disadvantage of this method is that it is the most labour-intensive and expensive although it is used because of its versatility. The four formulations were named formulation A, B, C and D.

Formulation A

Name: Ibuprofen tablets

Strength: 50 milligrams

Average weight per tablet : 200 milligrams

Batch size: 1000 tablets.

MANUFACTURING PROCEDURES

Step 1: Weigh separately into labelled containers

Ibuprofen BP,USP.....50.0 g

Lactose monohydrate.....44.7 g

PVP 10 % w/v in water.....30.0 g

Microcrystalline cellulose.....63.3 g

l-Leucine.....10.0 g

Silicone dioxide.....2.0 g

Theoretical weight 200.0 g

Step 2: Sieve ibuprofen, lactose monohydrate, microcrystalline cellulose through a 40 mesh sieve.

Step 3: Mix the sieved raw materials in a 2 litre food processor for 5 minutes.

Step 4: Add granulating solution in small increments, in the food processor, and mix for 10 minutes.

Step 5: Sieve the wet mass through 12 mesh sieve.

Step 6: Transfer the wet mass to a plastic tray and dry over night.

Step 7: Screen the dry granules through a 12 mesh sieve into a plastic jar.

Step 8: Add l-leucine and silicone dioxide to the dry granules. Mix the ingredients by gently shaking the plastic jar for 5 minutes.

Step 9: Weight of granulate = 173.06 grams.

Step 10: Compress the granulate into tablets using 7 mm punch.

FORMULATION B

Name: Ibuprofen tablets

Strength: 50 milligrams

Average weight per tablet: 200 milligrams

Batch size: 1000 tablets

MANUFACTURING PROCEDURE

Step 1: Weigh separately into labelled containers,

Ibuprofen.....50 g

Microcrystalline cellulose.....44.7 g

PVP 10 % w/v in water.....30.0 g

Corn starch.....63.3 g

l-Leucine.....10.0 g

Silicone dioxide.....2.0 g

Theoretical weight 200 g

Step 2: Sieve ibuprofen, Microcrystalline cellulose and starch through a 40 sieve.

Step 3: Mix the sieved raw materials in a 2 litre food processor for 5 minutes.

step 4: Add granulating solution in small increments, in the food processor, and mix for 10 minutes.

Step 5: Sieve the wet mass through a 12 mesh sieve.

Step 6: Transfer the wet mass to a plastic try and dry over night.

Step 7: Screen the dry granules through a 12 mesh sieve into a

plastic jar.

Step 8: Add l-Leucine and silicone dioxide to the dry granules and mix the ingredients by gently shaking the plastic jar for 5 minutes.

Step 9: Weight of granulate = 172.50 grams

Step 10: Compress the granulate into tablets using 7 mm punch.

FORMULATION C

Name: Ibuprofen tablets

Strength: 50 milligrams

Average weight per tablet 200 milligrams

Batch size: 1000 tablets

MANUFACTURING PROCEDURES

Step 1: Weigh separately into labelled plastic containers,

Ibuprofen.....50.0 g

Lactose.....42.7 g

PVP 10 % w/v in water.....30.0 g

Corn starch.....63.3 g

Sodium lauryl sulphate.....12.0 g

Silicone dioxide.....2.0 g

Theoretical weight 200 g

Step 2: Sieve ibuprofen, lactose and starch through a 40 mesh sieve.

Step 3: Mix the sieved raw materials in a 2 litre food processor for 5 minutes.

Step 4: Add granulating solution in small increments in the food processor, and mix for 10 minutes.

Step 5: Sieve the wet mass through a 12 mesh sieve

step 6: Transfer the wet mass to a plastic tray and dry over night.

Step 7: Screen the dry granules through a 12 mesh sieve into a plastic jar

Step 8: Add sodium lauryl sulphate and silicone dioxide to the dry granules and mix the ingredients by gently shaking the plastic jar for 5 minutes.

Step 9: Weight of granulate = 170.1 grams

Step 10: compress the granulate into tablets using a 7 mm punch

FORMULATION D

Name: Ibuprofen tablets

Strength: 50 milligrams

Average weight per tablet: 200 milligrams

Batch size: 1000 tablets

MANUFACTURING PROCEDURES

Step 1: Weigh separately into labelled plastic containers

Ibuprofen.....50.0 g

Microcrystalline cellulose.....42.7 g

PVP 10 % w/v in water.....30.0 g

Corn starch.....63.3 g

Sodium lauryl sulphate.....12.0 g

Silicone dioxide.....2.0 g

Step 2: Sieve ibuprofen, microcrystalline cellulose and corn starch through a 40 mesh sieve

Step 3: Mix the sieved raw materials in a 2 litre food processor for 5 minutes.

Step 4: Add granulating solution in small increments, in the food processor and mix for 10 minutes.

Step 5: Sieve the wet mass through a 12 mesh sieve

Step 6: Transfer the wet mass to a plastic tray and let the granules dry over night.

Step 7: Screen the dry granules through a 12 mesh sieve into a plastic jar.

Step 8: Add sodium lauryl sulphate and silicone dioxide to the dry granules and mix the ingredients by gently shaking the plastic jar for 5 minutes.

Step 9: Weight of granules = 173.4 grams

Step 10: Compress the granules into tablets using a 7 mm punch.

4.4 DETERMINATION OF SHELF-LIFE OF IBUPROFEN TABLETS

4.4.0 ISOTHERMAL METHOD

PROCEDURE:

Formulations A,B,C,and D of ibuprofen were incubated at four different temperatures, 40° 50° 60° and 70°C. Five tablets were placed into 20 mL brown glass bottles, and for each formulation and temperature, there were twelve samples (12), giving a total of 48 samples in each incubator.

A sampling schedule was written as shown on Table. 6 below. At the appropriate times, samples were then rapidly transferred to a freezer at -18° C and stored until analyzed. This precaution ensured that degradation of the drug was terminated following removal from the incubator.

The average weight of five tablets for each sample was determined, then the tablets were powdered and analyzed using HPLC method 2.

Table 6: A sampling schedule for temperatures 40° 50° 60° 70°C

ACCELERATED STABILITY TESTING PROGRAMME FOR IBUPROFEN TABLETS

INCUBATION PERIOD (Days)

sample #	40°	50°	60°	70°
1	16	5	3	0.25
2	33	8	5	0.75
3	50	12	10	1.0
4	67	16	12	1.75
5	84	20	14	2.0
6	102	23	18	2.75
7	120	30	19	3.0
8	138	35	24	3.75
9	156	40	26	4.0
10	174	97	31	6.75
11	-	104	35	7.0

4.4.1 ANALYSIS OF SAMPLES

Assay : HPLC method 2

Mobile phase: Acetonitrile 500 mL

0.0004 M Phosphoric acid

SAMPLE PREPARATION

Standard solution

A USP reference standard, Cat number 33550 was used to prepare a solution of 10 mg/mL in the mobile phase.

Sample solution

A test solution was prepared by weighing about 0.3 g of powdered tablets. The mixture was transferred into a 10 mL volumetric flask and diluted to volume with the mobile phase. The flask was shaken for about 5 minutes. The solution was then centrifuged for about 3 minutes and the clear solution transferred to an HPLC glass vial.

HPLC Parameters:

Detection: UV absorption at 264 nm

Injection volume: 10 μL

Chart speed: 0.2 cm/min

Flow rate: 0.1 mL/min

Pressure: 200 Kgf/cm'

Running time: 10min

Procedure

The solutions were transferred to glass vials for HPLC for auto-injections of 10 μL. In each analysis, two injections of the standard solution, followed by three injections of the test solution were performed.

4.4.2 NON-ISOTHERMAL METHOD

The focus of this research was to determine if non-isothermal stability studies could be used on solid dosage forms, such as ibuprofen tablets.

The temperature programme adopted for the study was the linear temperature programme. This has been used to a greater extent than other non-isothermal temperature programmes, probably because with this programme, temperature rises at a slower rate than with other systems.

4.4.3 LINEAR TEMPERATURE PROGRAMME

Initial temperature: 50°C

Temperature Programme: An increase of 1°C every 48 hours

Sampling time: A sample was removed from the oven every 5th day.

Final temperature: 70°C

Procedure:

Five tablets of each of the four formulations were placed into the glass bottles, and incubated under linear programmed conditions, starting at 50°C.

Table.7 below shows the sampling date as well as the incubation period, and also it shows the temperature of the oven at the time of sampling.

Samples were rapidly transferred to the freezer and were later analyzed using HPLC method 2.

Table 7: A sampling schedule for the linear temperature programme

NONISOTHERMAL STABILITY PROGRAMME FOR IBUPROFEN TABLETS

TEMPERATURE RANGE : 50°- 70°C

Sample #	Temperature	Incubation period (d)
1	51°C	4
2	53°	9
3	55°	14
4	57°	18
5	59°	23
6	61°	28
7	64°	32
8	65°	37
9	67°	42
10	69°	46
11	70°	51

4.4.4 ANALYSIS OF SAMPLES

As described for the isothermal method

CHAPTER 5

5. STABILITY STUDY RESULTS AND TREATMENT OF DATA

5.0 ISOTHERMAL METHOD

Drug remaining undecomposed, of formulations A, B, C and D described under section 4.3, was assayed in triplicate the mean content and the relative standard deviation were recorded in a tabular form as shown on Tables 8 to 19. The tables show how much drug remained after a certain period of time at a particular temperature. The USP pharmacopoeia stipulates a relative standard deviation of not more than 6% to show reliability of assay results, hence the %RSD was included in the tables.

Initially samples were incubated at four different temperatures, 40° 50° 60° and 70°C. The lowest temperature, 40°C was eliminated from the study because ibuprofen showed insufficient breakdown at this temperature. In order for 40°C data to be included in the study, storage would have had to have exceeded twelve months.

In treatment of data, the first step involves determination of rate constants at respective temperatures, i.e. 50°, 60° and 70°C, by plotting the best fit data points, log concentration against time as shown on Figures 12, 14, 16 and 18. Rate constants are then calculated from the slope of line. The second step involves plotting a graph of log K, where K is rate constant from the first step, against the reciprocal of the absolute

temperature as illustrated by Figures 13, 15, 17 and 19. From the graph, Log K_{rt}, the rate constant at storage conditions, is obtained by extrapolation. Energy of activation is obtained from the slope of this graph.

Figures 12, 14, 16 and 18 clearly show that the rate of degradation was fast at 70°C which is demonstrated by the steep slope of the curve. The rate of degradation was very slow at 50°C as expected. The slope of this curve in all cases was almost zero showing how stable ibuprofen was at this temperature. Rate of degradation at 60°C was slightly faster than the rate at 50°C.

Figures 13, 15, 17 and 19 are the extrapolation curves for the rate constant at 25°C. As expected the rate constants obtained at this lower temperature are much smaller than those at higher temperatures.

From the results, the calculated shelf-life of formulations A, B, C and D was found to be comparable with shelf-life of ibuprofen tablets available in the market.

Table 8: Results in milligrams of drug remaining undecomposed of Formulation A in 50°C incubator

TEMPERATURE : 50°C

FORMULATION A

Sample #	Incubation Period(d)	Drug remaining(mg)	% RSD
0	0	51.6	1.6
1	5	47.4	2.0
2	8	48.2	1.3
4	16	48.2	3.9
5	20	47.2	3.4
6	23	46.8	0.25
8	35	46.5	0.33
10	97	45.1	3.2
11	104	43.9	4.0

BEST FIT DATA

Calculated from linear regression for drug remaining(mg).

Days	0	5	10	15	20	25	30	35	40	120
# mg Drug	48.7	48.5	48.3	48.1	47.8	47.6	47.4	47.1	46.9	43.4

Table 9: Results in milligrams of drug remaining undecomposed of Formulation A in 60°C incubator

TEMPERATURE : 60°C

FORMULATION A

sample #	Incubation period(d)	Drug remaining(mg)	% RSD
0	0	51.6	1.6
1	3	47.6	1.8
2	5	47.9	2.7
3	10	47.5	2.8
4	12	48.4	1.3
5	14	48.7	0.12
6	18	47.9	0.12
7	19	49.5	0.43
8	24	47.8	6.8
9	26	47.5	4.8
10	31	45.5	6.3
11	35	42.3	1.4

BEST FIT DATA

Calculated from linear regression for drug remaining(mg).

Days	0	5	10	15	20	25	30	35	40	50
# mg Drug	50.1	49.4	48.5	47.8	47.1	46.4	45.7	44.9	44.3	42.9

Table 10: Results in milligrams of drug remaining undecomposed of Formulation A in 70°C incubator

TEMPERATURE : 70°C

FORMULATION A

Sample #	Incubation period(d)	Drug remaining(mg)	% RSD
0	0	51.6	1.6
1	0.25	49.9	2.6
2	0.75	48.3	0.83
3	1.0	49.2	0.2
4	1.75	48.9	3.0
5	2.75	46.7	0.49
6	3.75	45.5	0.79
7	4.0	43.9	0.0
8	6.75	45.1	0.34
9	7.0	44.4	0.52
10	7.75	45.2	0.71
11	8.0	43.3	0.5
12	13.0	39.5	1.1
13	26.0	39.8	1.3

BEST FIT DATA

Calculated from linear regression for drug remaining(mg).

Days	**0**	**4**	**6**	**10**	**14**	**18**	**20**	**24**	**28**	**30**
# mg Drug	**48.4**	**46.4**	**45.6**	**43.9**	**42.2**	**40.5**	**39.7**	**38.1**	**36.7**	**36**

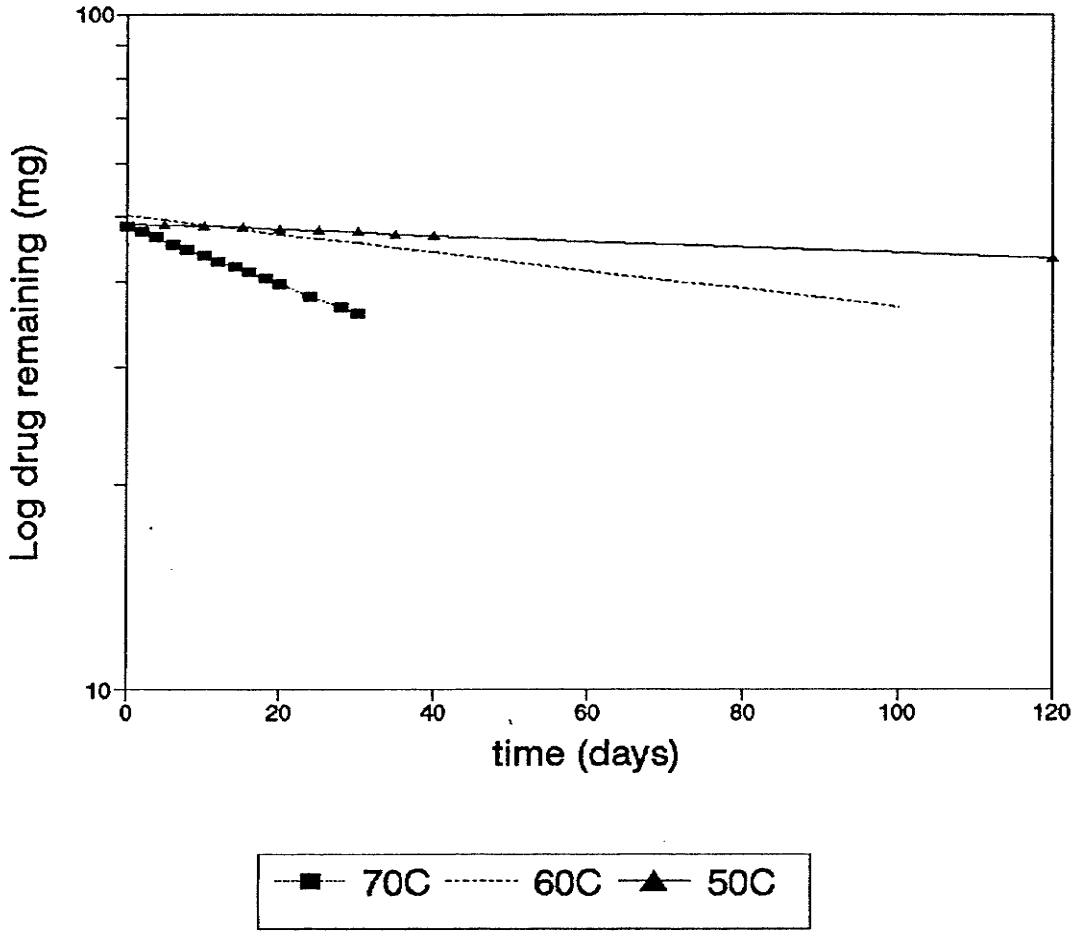

Fig.12 A graph of log concentration versus time for formulation A

	50°	60°	70°
-K/2.303	-4.254805*10⁻⁴	-1.313130*10⁻³	-4.30414*10⁻³
K	9.7654*10⁻⁴	3.09982*10⁻³	9.91243*10⁻³
R	-0.84	-0.73	-0.86

where

K/2.303 is the slope

K is the degradation rate constant

R is the correlation coefficient

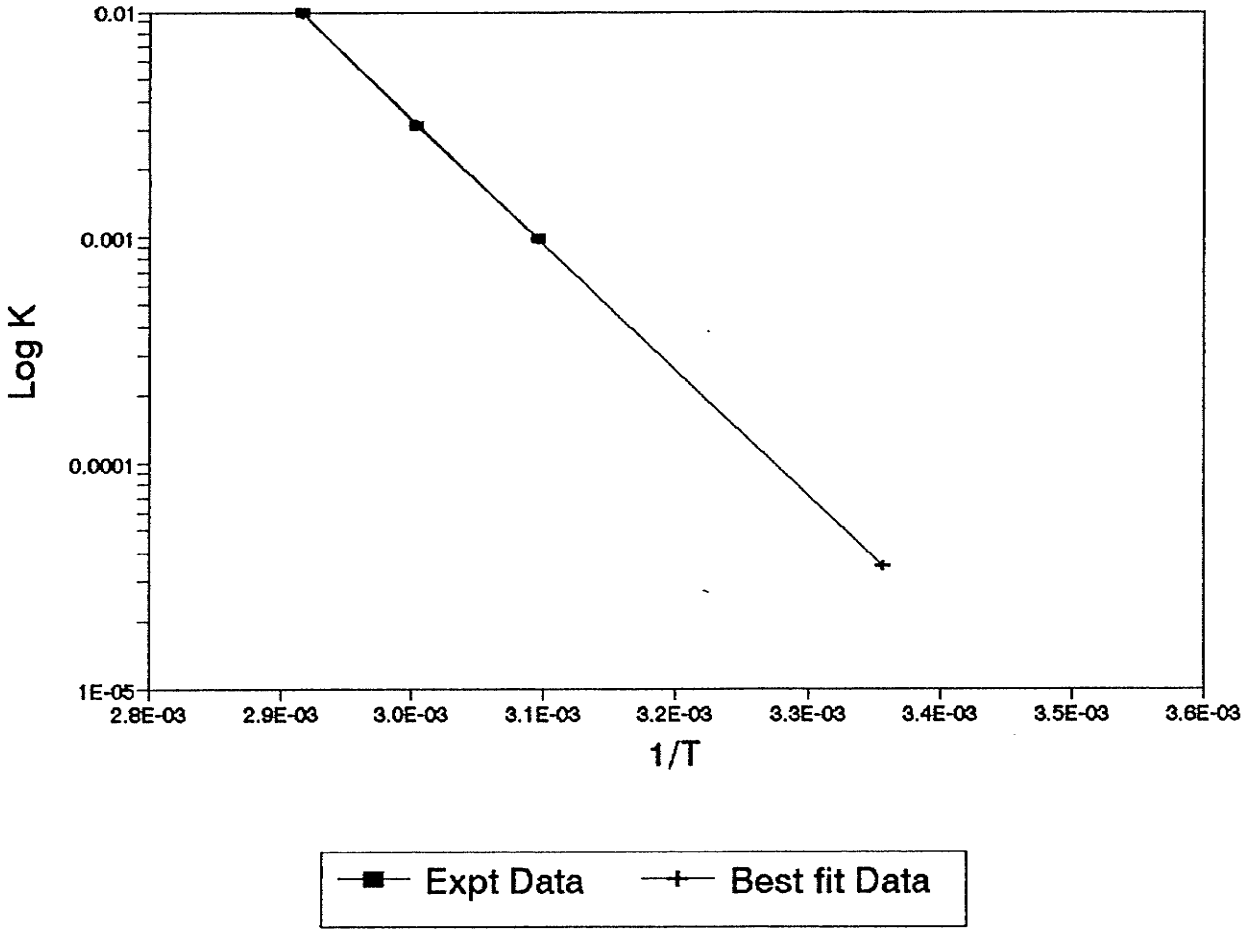

Fig.13 A graph of log K versus reciprocal temperature for formulation A

From the graph.

Slope = -5567.733

Intercept = 14.21368

Energy of activation (Ea) = 25.47828 Kcal/mol

K_{rt} by extrapolation = $3.46658*10^{-1}$

shelf-life of formulation A = 8.4 years

Table 11: Results in milligrams of drug remaining undecomposed of Formulation B in 50°C incubator

TEMPERATURE : 50°C

FORMULATION B

Sample#	Incubation Period(d)	Drug remaining(mg)	% RSD
0	0	49.9	4.3
1	5	47.6	2.0
2	8	44.7	0.89
4	16	46.0	1.5
5	20	44.3	1.1
6	23	45.4	0.92
7	30	44.5	1.1
8	35	43.2	1.5
10	97	42.9	3.2
11	104	41.0	3.0

BEST FIT DATA

Calculated from linear regression for drug remaining(mg)

Days	0	5	10	15	20	25	30	35	40	120
# mg Drug	46.8	46.5	46.2	45.9	45.6	45.4	45.1	44.8	44.6	40.4

Table 12: Results in milligrams of drug remaining undecomposed of Formulation B in 60°C incubator

TEMPERATURE : 60°C

FORMULATION B

Sample#	Incubation period(d)	Drug remaining(mg)	% RSD
0	0	49.9	4.3
1	3	48.2	8.5
2	5	43.8	5.7
3	10	44.7	2.2
4	12	44.5	1.1
5	14	43.8	1.9
6	18	43.6	3.8
7	19	42.9	2.4
8	24	43.2	0.81
9	26	43.9	4.3
10	31	43.0	3.0
11	35	41.2	2.7

BEST FIT DATA

Calculated from linear regression for drug remaining(mg).

Days	0	5	10	15	20	25	30	35	40	50
# mg Drug	47.2	46.3	45.4	44.6	43.7	42.9	42.1	41.3	40.5	39.0

Table 13: Results in milligrams of drug remaining undecomposed of Formulation B in 70°C incubator

TEMPERATURE 70°C

FORMULATION : B

Sample #	Incubation period(d)	Drug remaining(mg)	% RSD
0	0	49.9	4.3
1	0.25	48.5	1.3
2	0.75	48.3	1.3
3	1.0	49.6	3.1
4	1.75	48.2	2.8
5	2.75	46.7	2.4
6	3.75	45.3	0.0
7	4.0	47.7	1.0
8	6.75	46.2	1.4
9	7.0	45.4	0.25
10	7.75	45.8	0.25
11	8.0	43.5	2.2
12	13.0	42.3	1.5
13	26.0	39.0	1.5

BEST FIT DATA

Calculated from linear regression for drug remaining(mg).

Days	**0**	**4**	**6**	**10**	**14**	**18**	**20**	**24**	**28**	**30**
# mg Drug	**48.6**	**46.9**	**46.0**	**44.4**	**42.7**	**41.2**	**40.4**	**39.0**	**37.5**	**36.8**

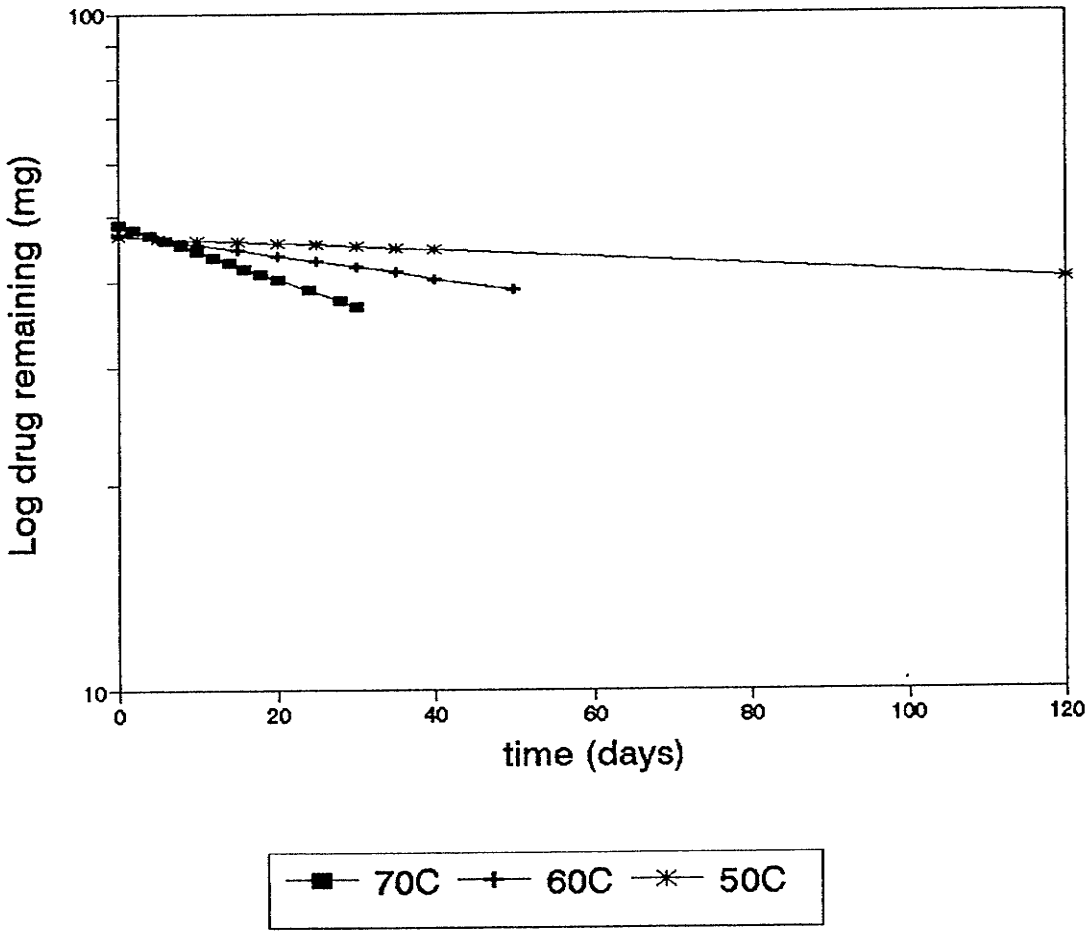

Fig.14 A graph of log concentration versus time for formulation B

	50°	60°	70°
-K/2.303	-5.2583*10⁻⁴	-1.64903*10⁻³	-4.03223*10⁻³
K	1.21424*10⁻³	3.80974*10⁻³	9.29852*10⁻³
R	-0.81	-0.81	-0.95

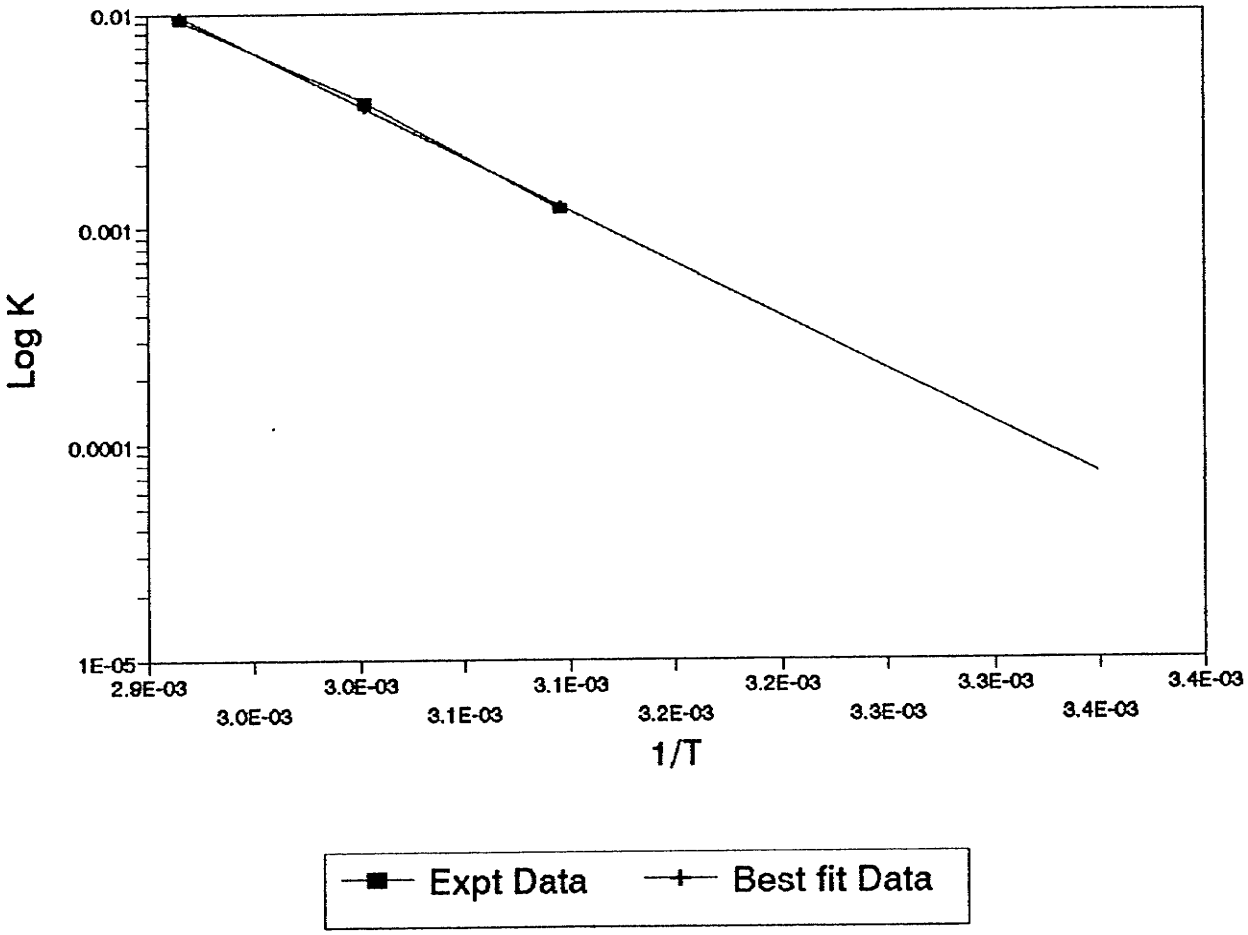

Fig.15 A graph of log K versus reciprocal temperature for formulation B

From the graph.

Slope = -4906.59

Intercept = 12.28082

Energy of activation (Ea) = 22.45286 Kcal/mol

K_{rt} by extrapolation = $6.675*10^{-1}$

Shelf-life of formulation B = 4.4 years

Table 14: Results in milligrams of drug remaining undecomposed of Formulation C in 50°C incubator

TEMPERATURE : 50°C

FORMULATION C

Sample#	Incubation Period(d)	Drug remaining(mg)	% RSD
0	0	52.3	1.1
1	5	50.0	0.7
2	8	48.6	4.8
4	16	47.6	4.5
5	19	47.9	4.0
6	23	46.5	3.0
7	30	45.5	1.5
8	35	47.5	1.7
10	97	42.3	5.4
11	104	39.0	2.7

BEST FIT DATA

Calculated from linear regression for drug remaining(mg).

Days	**0**	**5**	**10**	**15**	**20**	**25**	**30**	**35**	**40**	**120**
# mg Drug	**50.1**	**49.5**	**49.0**	**48.5**	**48.0**	**47.5**	**46.9**	**46.4**	**45.9**	**38.7**

Table 15: Results in milligrams of drug remaining undecomposed of Formulation C in 60°C incubator

TEMPERATURE : 60°C

FORMULATION C

Sample#	Incubation period(d)	Drug remaining(mg)	% RSD
0	0	52.3	1.1
1	3	48.9	1.3
2	5	46.3	3.3
3	10	46.7	1.8
4	12	45.1	3.7
5	14	42.8	1.6
6	18	38.3	3.3
7	19	34.5	2.0
8	24	39.1	2.0
9	26	36.2	4.0
10	31	34.0	1.7

BEST FIT DATA

Calculated from linear regression for drug remaining(mg).

Days	0	5	10	15	20	25	30	35	40	50
# mg Drug	51.2	47.8	44.6	41.6	38.9	36.3	33.9	31.6	29.5	25.7

Table 16: Results in milligrams of drug remaining undecomposed of Formulation C in 70°C incubator

TEMPERATURE: 70°C

FORMULATION C

Sample #	Incubation period(d)	Drug remaining(mg)	% RSD
0	0	52.3	1.1
1	0.25	52.4	4.2
2	0.75	52.5	2.0
3	1.0	43.7	3.3
4	1.75	45.9	5.2
5	2.75	44.6	4.7
6	3.75	37.2	1.2
7	4.0	41.4	0.0
8	6.75	43.5	4.7
9	7.0	39.5	1.1
10	7.75	37.9	0.15
11	8.0	35.0	2.8

BEST FIT DATA

Calculated from linear regression for drug remaining(mg).

Days	**0**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**
# mg Drug	**49.9**	**42.9**	**39.7**	**36.8**	**34.1**	**31.6**	**29.3**	**27.2**	**25.2**	**23.3**

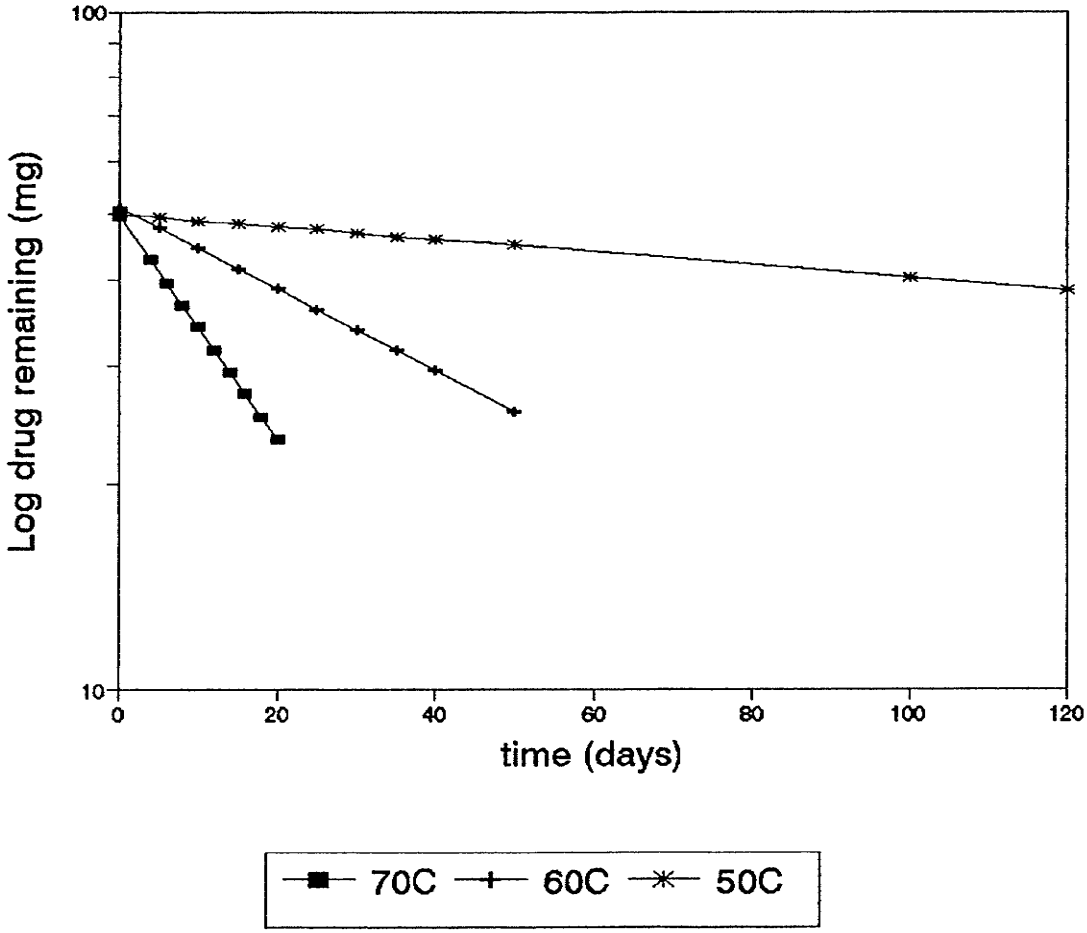

Fig.16 A graph of log concentration versus time for formulation C

	50°	60°	70°
K/2.303	$-9.3331*10^{-4}$	$-5.98519*10^{-3}$	$-1.650270*10^{-2}$
K	$2.14525*10^{-3}$	$1.378881*10^{-2}$	$3.792067*10^{-2}$
R	-0.94	-0.93	-0.84

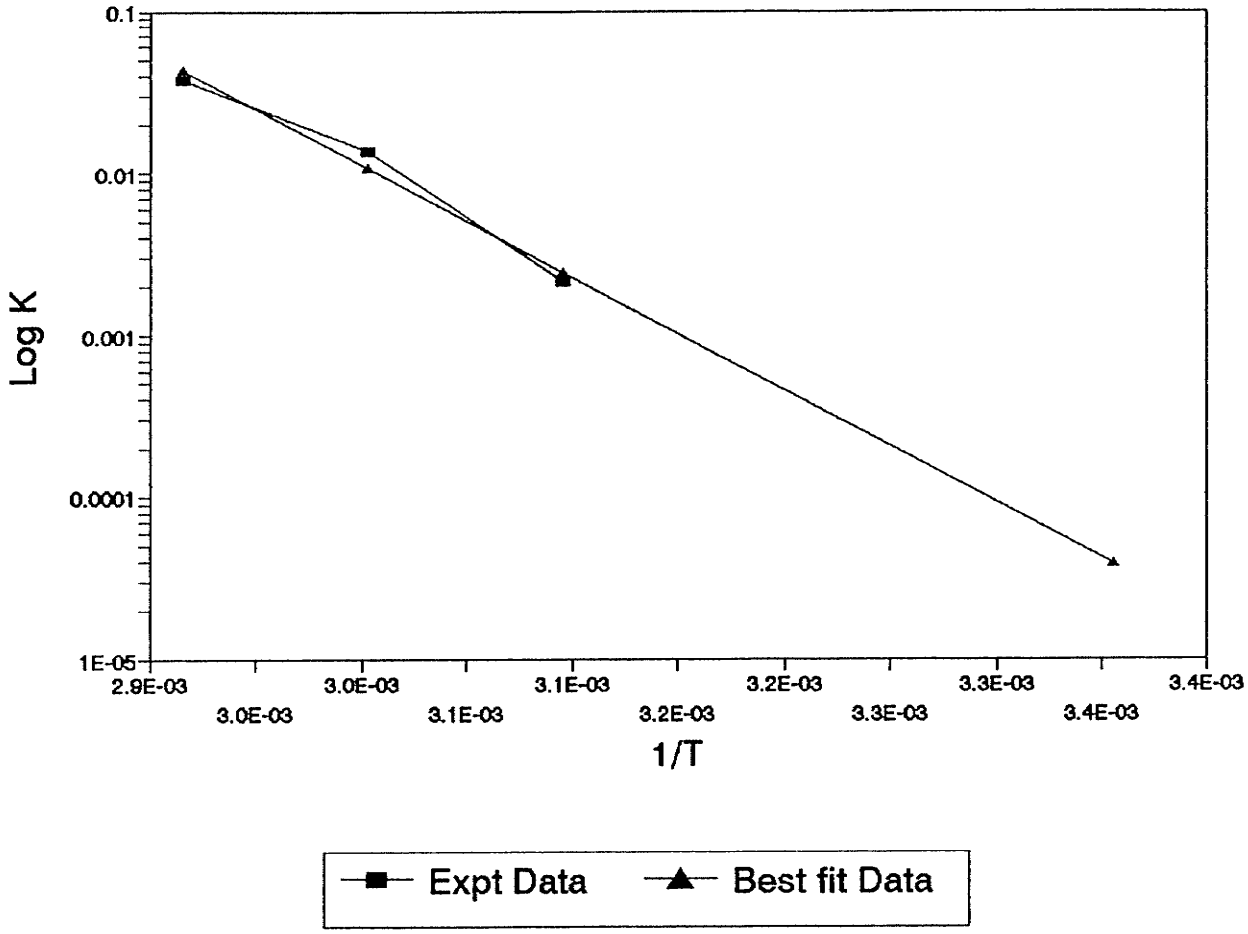

Fig.17 A graph of log K versus reciprocal temperature for formulation C

From the graph.

Slope = -6934.44

Intercept = 18.84328

Energy of activation (Ea) = 31.73242 Kcal/mol

K_{rt} by extrapolation = $3.851*10^{-1}$

Shelf-life of formulation C = 7.6 years

Table 17: Results in milligrams of drug remaining undecomposed of Formulation D in 50°C incubator

TEMPERATURE : 50°C

FORMULATION D

Sample#	Incubation Period(d)	Drug remaining(mg)	% RSD
0	0	48.9	0.83
1	5	47.0	2.7
2	8	47.0	1.3
4	16	47.6	4.1
5	19	47.8	1.5
6	23	47.2	0.6
7	30	45.1	2.9
8	35	46.8	1.1
10	97	39.7	3.8
11	104	38.6	3.1

BEST FIT DATA

Calculated from linear regression for drug remaining(mg).

Days	0	5	10	15	20	25	30	35	40	120
# mg Drug	48.8	47.8	47.3	46.8	45.8	45.3	44.8	39.4	37.8	31.8

Table 18: Results in milligrams of drug remaining undecomposed of Formulation D in 60°C incubator

TEMPERATURE : 60°C

FORMULATION D

Sample#	Incubation period(d)	Drug remaining(mg)	% RSD
0	0	48.9	0.83
1	3	50.1	3.3
2	5	49.5	1.0
3	10	46.7	2.0
4	12	45.9	0.82
5	14	44.8	2.9
6	18	44.5	0.26
7	19	44.0	5.0
8	24	39.6	1.4
9	26	43.6	1.1
10	31	42.0	3.2

BEST FIT DATA

Calculated from linear regression for drug remaining(mg).

Days	**0**	**5**	**10**	**15**	**20**	**25**	**30**	**35**	**40**	**50**
# mg Drug	**49.9**	**48.3**	**46.7**	**45.2**	**43.9**	**42.4**	**41.0**	**39.7**	**38.4**	**36.0**

Table 19: Results in milligrams of drug remaining undecomposed of Formulation D in 70°C incubator

TEMPERATURE :70°C

FORMULATION D

Sample #	Incubation period(d)	Drug remaining(mg)	# RSD
0	0	48.9	0.83
1	0.25	45.2	4.4
2	0.75	41.4	0.98
3	1.0	40.6	0.89
4	1.75	43.9	0.12
5	2.75	43.0	4.0
6	3.75	42.5	3.7
7	4.0	40.5	0.0
8	6.75	37.9	0.70
9	7.0	41.1	0.28
10	7.75	37.9	1.3
11	8.0	37.0	2.8

BEST FIT DATA

Calculated from linear regression for drug remaining(mg).

Days	0	4	6	8	10	12	14	16	18	20
# mg Drug	44.9	41.2	39.5	37.9	36.3	34.8	33.3	32.0	30.6	29.3

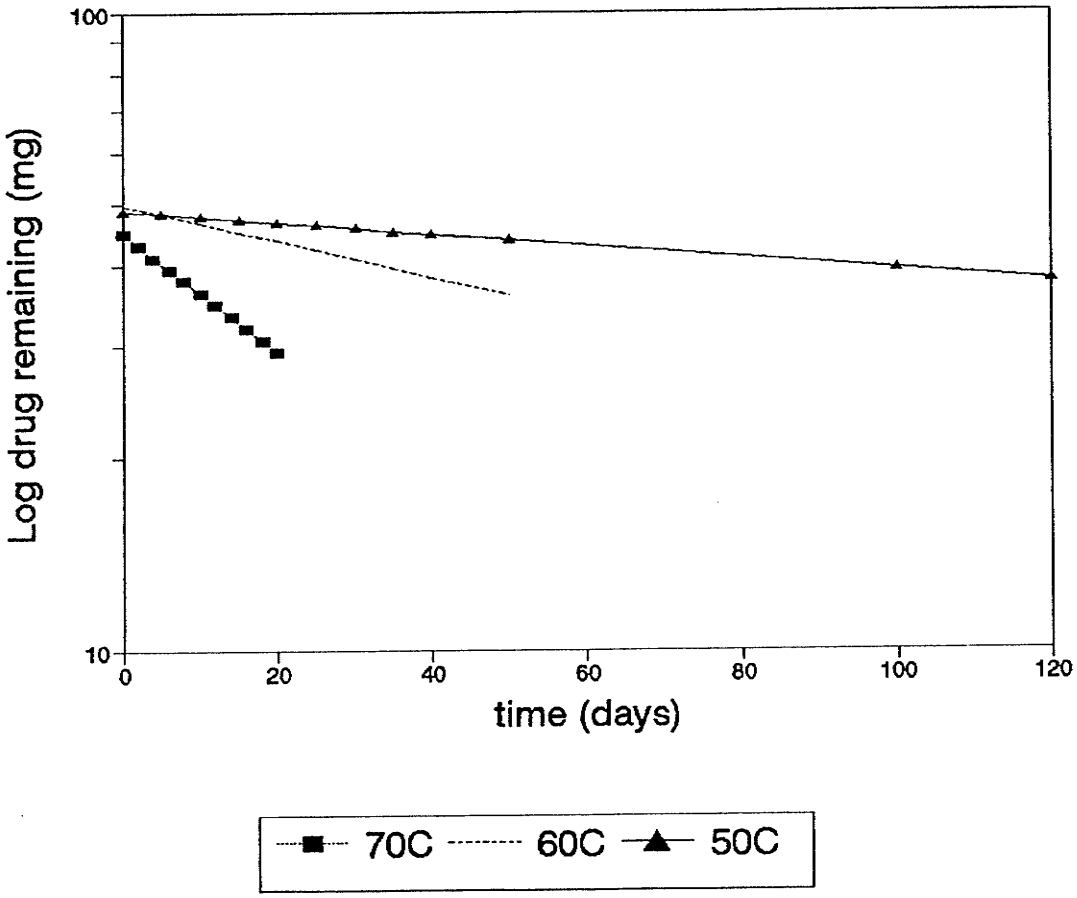

Fig.18 A graph of log concentration versus time for formulation D

	50°	60°	70°
K/2.303	$-9.3109*10^{-3}$	$-2.833*10^{-3}$	$-9.223*10^{-3}$
K	$2.13227*10^{-3}$	$6.53616*10^{-3}$	$2.134636*10^{-3}$
R	-0.97	-0.90	-0.81

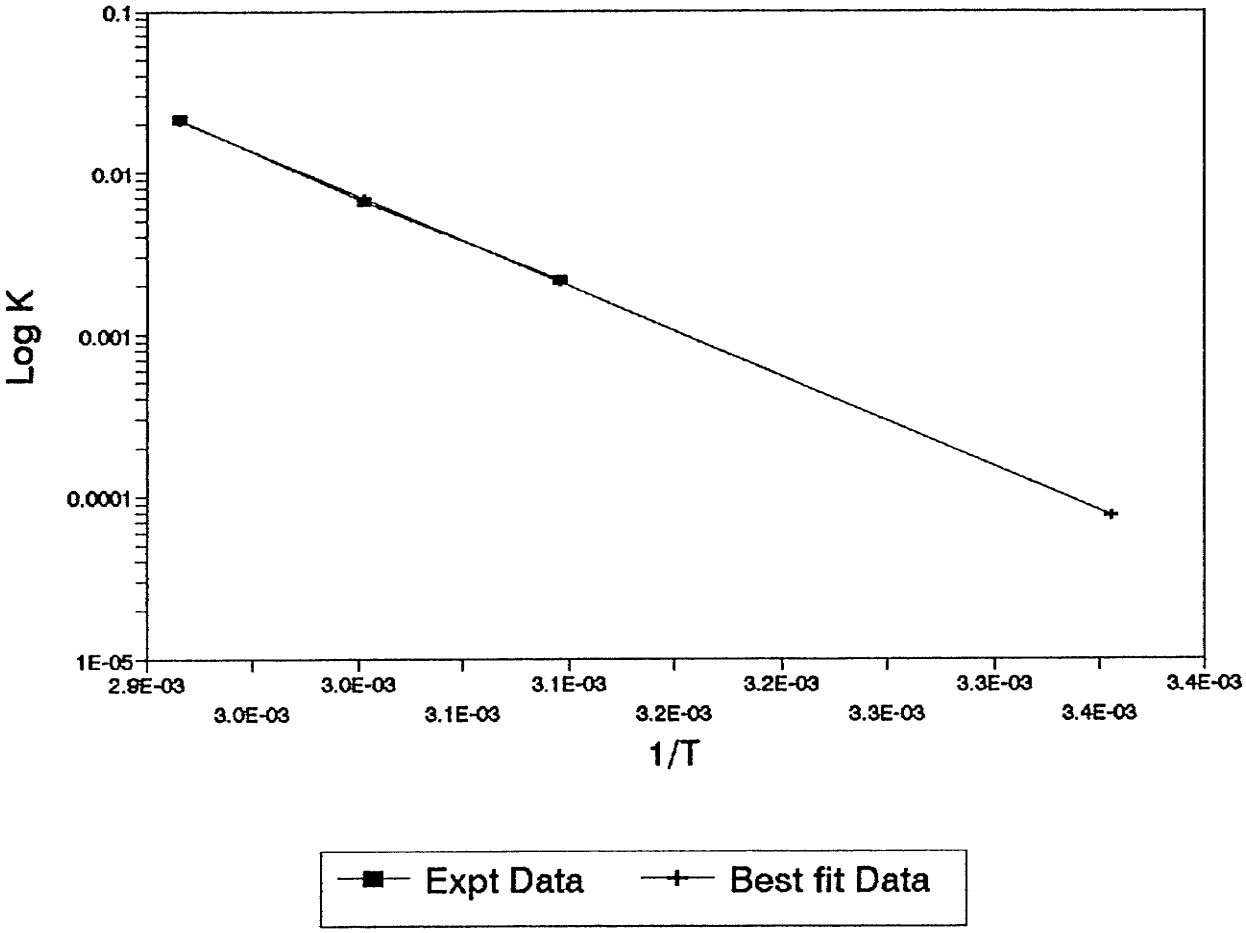

Fig.19 A graph of log K versus reciprocal temperature for formulation D

From the graph.

Slope = -5543.995

Intercept = 14.47514

Energy of activation (Ea) = 25.36966 Kcal/mol

K_{rt} by extrapolation = $7.6018*10^{-1}$

Shelf-life of formulation D = 3.8 years

5.1 NON-ISOTHERMAL RESULTS

In this study, a general method for determination of nonisothermal kinetic profiles was utilised in analysis of data. The method utilised a BASIC computer program NONISO similar to the program used by Hempenstall et al. (1983) in their stability study of penicillin formulations.

Figure 19 is a schematic diagram of the NONISO structure, This BASIC computer program has been written for implementation on a Z80-based microcomputer with 64K RAM and requires approximately 22K for the fully labelled version.

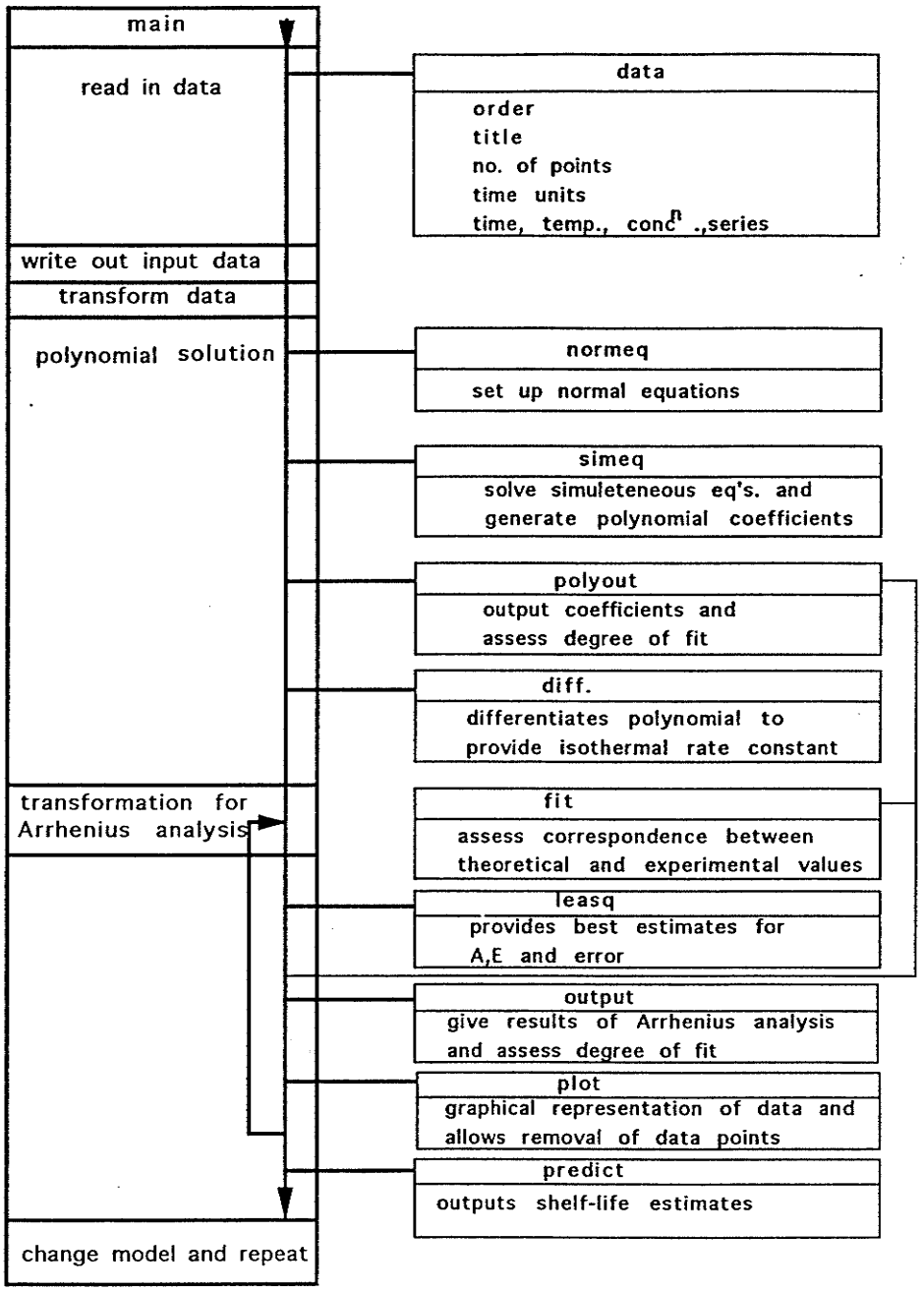

Fig.20 A schematic diagram of the NONISO structure [18]

Mean content, incubation period and the relative standard deviation were recorded as shown on Tables 20 to 23, for each formulation.

Table 20: Non-isothermal results of drug remaining undecomposed of Formulation A

FORMULATION A

Sample #	Incubation period(d)	Drug remaining(mg)	%RSD
1	4	49.0	0.12
2	9	51.0	0.19
3	14	50.2	3.2
4	18	49.7	2.0
5	23	49.3	1.6
6	28	46.4	3.8
7	32	45.2	0
8	37	45.9	0.4
9	42	45.8	1.2
10	46	45.4	0.12
11	51	43.5	0.92

Table 21: Non-isothermal results of drug remaining undecomposed of Formulation B

FORMULATION B

Sample #	Incubation period(d)	Drug remaining(mg)	% RSD
1	4	49.6	1.3
2	9	51.7	1.9
3	14	50.5	0.79
4	18	49.0	1.2
5	23	50.0	2.5
6	28	44.6	1.0
7	32	45.8	0.83
8	37	45.6	0.4
9	42	45.8	0.92
10	46	45.9	1.3
11	51	45.5	0.82

Table 22: Non-isothermal results of drug remaining undecomposed of Formulation C

FORMULATION C

Sample #	Incubation period(d)	Drug remaining(mg)	% RSD
1	4	51.6	0.49
2	9	52.2	0.9
3	14	51.3	2.3
4	18	46.7	0.33
5	23	43.9	1.3
6	28	43.0	5.1
7	32	43.3	2.4
8	37	42.9	2.0
9	42	40.1	2.6
10	46	40.6	1.3
11	51	42.1	1.2

Table 23: Non-isothermal results of drug remaining undecomposed of Formulation D

FORMULATION D

Sample #	Incubation period(d)	Drug remaining(mg)	% RSD
1	4	49.5	0.4
2	9	48.9	0.51
3	14	52.9	0.80
4	18	50.2	1.4
5	23	46.0	2.6
6	28	46.4	3.7
7	32	40.0	4.9
8	37	42.6	2.5
9	42	41.4	3.0
10	46	38.4	2.0
11	51	39.3	1.1

Analysis of data utilised the NONISO computer programme which generates several rate constants from the data points. These are then plotted to produce a polynomial curve. From this curve, the rate constant for normal storage conditions, as well as other parameters are obtained.

It was not possible in this case to generate a polynomial curve because of the following:

* Only eleven data points were obtained after 51 days of the study. And these points were inadequate to generate a valid polynomial curve. The time required to obtain an adequate set of data points would be too long for the method to be a practical alternative to isothermal study for this drug.
* Ibuprofen showed insufficient decomposition to be a good model compound for the study
* All the graphs of amount of ibuprofen remaining vs time showed some abnormal behaviour at approximately the 30th day.

Only one graph from formulation A, Fig.21 is included to show this abnormal behaviour of ibuprofen.

The difference between the nonisothermal and isothermal results is that with isothermal method the temperature range is limited. 40° degradation takes too long for realistic study and upper temperature is limited by the low melting point. Isothermal studies would require about 12 months to obtain valid data. Non-isothermal method is shorter but still too long for a practical alternative to isothermal method.

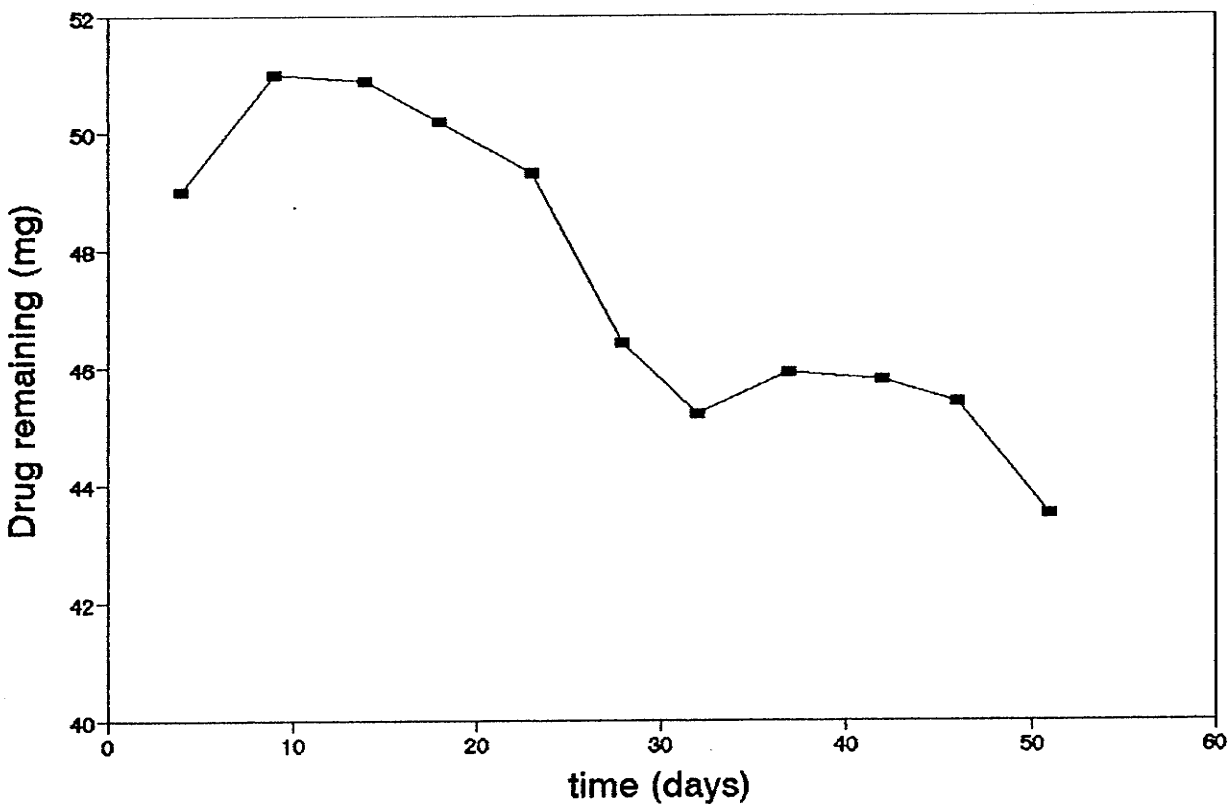

Fig. 21 A graph of concentration versus time for formulation A (nonisothermal)

CHAPTER 6

6. DISCUSSION AND CONCLUSION

6.0 SHORTCOMINGS OF THE USP ASSAY METHOD

Ibuprofen USP assay method

The HPLC method described in USP XXII 1990 showed a lot of variability amongst assay results and there were other problems involving the corrosive nature of the mobile phase.

6.0.0 Assay results

The assay was performed on preformulation samples as well as the first 10 samples from the 50°C incubator. Three determinations of the assay were performed on each sample. On 8 of these samples, the relative standard deviation on the triplicate analysis was found to be more than 6 %. The acceptable limit of the relative standard deviation is not more than 4 %. Consequently, it was necessary to develop an improved alternative procedure.

6.0.1 HPLC Equipment

The corrosive nature of the mobile phase used in the USP assay affected the pumping system, such that it pumped very slowly with a lot of resistance. This was corrected by thoroughly washing the system with water every day after analysis.

Before starting analysis the system had to be equilibrated with the mobile phase and left pumping for about 45 minutes to stabilize. Not only did the mobile phase damage the equipment but time was wasted each day while stabilizing the system.

6.1 SHORTCOMINGS OF NON-ISOTHERMAL METHOD FOR IBUPROFEN

One major problem encountered in this study was that ibuprofen proved to be too stable to be a good model compound. This was reflected also in the isothermal method where the 40°C data had to be eliminated early in the study because of inadequate decomposition.

Another factor limiting the use of the non-isothermal method with ibuprofen is that ibuprofen has a low melting point, 75-78°C. The temperature could not be increased beyond the melting range.

Because of the good stability of the drug, coupled with a low melting point, not enough data points were generated to run the NONISO computer programme. The non-isothermal study took 51 days and a total of eleven data points were generated. Ideally a non-isothermal method should take at the most three weeks.

The ibuprofen study utilised a BASIC computer programme similar to the programme used by Hempenstall et al. [18]. In their study a total of 28 data points were obtained in 112 minutes.

For a study to run for almost three months, it defeats a primary purpose of the non-isothermal method, a major advantage of which is decreased study time.

Ibuprofen showed some abnormal behaviour at approximately the 30th day. At this point, decomposition slowed markedly until the end of the study. This abnormality resulted in many errors in the NONISO analysis such that no meaningful results were obtained.

This abnormal behaviour of ibuprofen might be explained by changes in the hydrolytic degradation pathway as well as the mechanism involved. Storage at elevated temperature could reduce the moisture content of the tablets to a level below that required for degradation. The details of the decomposition profile of this drug in storage were beyond the scope and objectives of this research.

6.1.0 ADVANTAGES OF NONISOTHERMAL METHOD

Advantages of this method over the isothermal methods are numerous. Perhaps most important is that all the parameters can be calculated from a single experiment in order to predict the shelf-life of a drug. Thus activation energy, reaction rate constant and the order of the reaction can be calculated from a single experiment.

There is a reduction in the number of samples used during the experiment, since one sample is used for one full experiment from which all the parameters can be calculated.

Another advantage is that the experiment can be terminated at any time, provided sufficient experimental points have been obtained, and the amount of degradation is large enough to determine the order of the reaction with sufficient accuracy [17]. According to Cole et al. [8], the order of the reaction may be determined provided more than 20 % degradation has occurred. Also the small volume of controlled temperature space required for this experiment increases its potential further.

With a typical drug for which the method would be ideal, results can be obtained in about one week, using a sampling schedule which causes inconvenience.

There are still many problems to overcome before nonisothermal methods can replace the existing isothermal methods for stability determination. Nonisothermal methods have yet to be introduced in industrial setting.

6.1.1 DISADVANTAGES OF NONISOTHERMAL METHODS

A major disadvantage of all nonisothermal programmes currently in use is that the drug's half-life must be known before the nonisothermal experiment can be conducted. This knowledge enables the researcher to select a programme that will allow a reasonable percentage of decomposition to occur before

the maximum temperature is reached [11].

Two major limiting factors in the development of the nonisothermal methods are the necessity for computers to reduce the overwhelming and complex data treatment [14-22], and the lack of a commercially available instrument. The lack of hardware, to provide the desired temperature-time programme is a factor in limiting the wider use of the nonisothermal accelerated method.

Manually adjusted thermoregulators in the water bath and variable transformer-controlled heater in the oil bath have been used. Manual adjustment of the temperature is tedious and can be a source of errors.

6.2 GENERAL DISCUSSION AND CONCLUSIONS

The HPLC method developed for this study represents a significant improvement over that described in the USP XXII 1990. It was used effectively to perform the stability studies on Ibuprofen formulations and evaluated accelerated stability study methods.

The improved HPLC method for ibuprofen is better than the USP method in many aspects. Variability among the assay results was minimal and the triplicate assay determination of each sample resulted in a relative standard deviation of less than 4 %. Results from assay validation of this method reflected the superior qualities of this method.

The mobile phase of the improved method was "user friendly" to the HPLC pumping system as opposed to the corrosive nature of the mobile phase of the USP method.

Most work on non-isothermal stability studies in the pharmaceutical field has been confined to solutions. This is surprising given the predominance of the solid dosage forms.

Problems associated with non-isothermal studied on solid dosage forms include uneven heat transfer through a solid, and the difficulties associated with automatically assaying a solid and measuring its temperature as opposed to doing the same thing with a liquid. More research is required to determine the applicability of the nonisothermal techniques to the testing of solid dosage forms. Results from this study show that they are not applicable in all situations. Ibuprofen was shown to be an unsatisfactory candidate for application of the method to solid dosage forms.

The nonisothermal methods cannot be expected to replace the conventional stability testing programmes, but would rather be employed in cutting down the number of poor formulations during the preliminary formulation screening. The isothermal method is still very useful to researchers and there are many advantages in using this conventional method.

One major advantage of shelf-life prediction from isothermal studies is that the shelf-life of a drug can be known in a few months. With ibuprofen the shelf-life was determined in about 9 months. This compares with storing the drug at normal conditions

and determining the shelf-life in two to three years, which is time consuming and uneconomical. This accelerated shelf-life prediction method seemed very appropriate and in some cases the results obtained from the predictions have been shown to be in close agreement with the results obtained when the product was stored at normal storage conditions.

Problems arise with multi-component products such as multi-vitamins. Consider a situation where an accelerated stability test is performed on a product with several active ingredients, stored at four different temperatures, and having thirteen sampling periods.

There would be numerous assays performed on each active ingredient for each temperature. Also considerable controlled temperature storage space is required, and there is difficulty in maintaining assay precision constant over a prolonged period of time.

The isothermal method can be tedious, time consuming and uneconomical, especially if it is to be incorporated in the early stages of product development. It is necessary to note that the Arrhenius equation holds only if the reaction mechanism does not change as a function of temperature. This implies that the activation energy should be independent of temperature.

Another important feature to note is that accelerated stability testing is most useful when the reaction at ambient temperature is too slow to be monitored conveniently. Ibuprofen was a good model to demonstrate this. The data for 40°C had to be

eliminated from the study because of insufficient degradation. Also when the activation energy is relatively high. For example consider an activation energy Ea of 25 Kcal/mol, an increase from 25 degrees to 45 degrees brings about a 14-fold increase in the reaction rate constant. When comparing with an Ea of 10 Kcal/mol, a rate increase of just three fold is obtained for the same elevation in temperature [5].

Non Arrhenius behaviour does occur in pharmaceutical systems. This may be due to multiple reaction pathways, evaporation of the solvent that may lead to an unstable product, or change in the physical form of the preparation when the temperature of the reaction is changed.

One interesting example of non-Arrhenius behaviour is the increased rate of decomposition of ampicillin upon freezing. Savello et al. [50] showed that for a 1% ampicillin sodium solution in 5% dextrose, the percentage of degradation at 4 hours storage time is approximately 14% at -20°C, compared to 6% at zero degrees and 10% at 5°C.

In conclusion, it is important to note that the procedures which can be used to predict stability of pharmaceutical products have reached a reasonable degree of sophistication. Even though the use of nonisothermal methods have not yet received official recognition, there is no doubt that the use of these methods will increase in the future. This increase will probably occur primarily with liquid dosage forms. Applications to solids will require further evaluation on an individual basis.

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