STABILITY OF PROCHLORPERAZINE IN SOLUTION AND IN THE SOLID-STATE

THESIS

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Edith Martins Antunes
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ABSTRACT

Prochlorperazine, a member of the piperazine subclass of phenothiazines, widely used as an anti-emetic, is susceptible to oxidation to sulfoxides. These are main metabolites and degradants of all phenothiazines which are found to be inactive at the dopamine receptors. Prochlorperazine causes photosensitivity effects in patients attributed to dechlorination at C_2 with the release of HCl (Huang and Sands, 1967; Nejmeh and Pilpel, 1978; Moore and Tamat, 1980).

The aim of this study is to investigate the thermal and photostability of prochlorperazine edisylate and mesylate salts in the solid state and in solution. Prochlorperazine is available as a fine chemical and in a variety of dosage forms, including injectables and tablets. According to ICH guidelines, any degradants greater than 0.1% are required to be isolated and identified.

In order to assess the photostability of the two salts, an HPLC method was developed and validated for linearity, accuracy and precision, selectivity, limit of detection, quantitation and ruggedness.

Sulfoxides were synthesised for use as standards in the rate studies according to the well-known hydrogen peroxide method (Owens *et al.*, 1989). The rate of prochlorperazine degradation in solution under various light sources (254 nm UV light, diffuse light and sunlight) was studied. The light sources used above were quantified using potassium ferrioxalate as a chemical actinometer). The photodegradation rate was found to be greater in ampoules sealed under nitrogen than air, but the thermal degradation was faster in ampoules sealed with air than those purged with nitrogen. Amber ampoules retarded the rate of degradation under all photolytic conditions. This is a vital consideration for the packaging and storage of prochlorperazine in injectables. Degradation was found to occur mainly by first-order kinetics and the degradation rate decreased in the following order: sunlight >> UV light 254 nm > fluorescent / diffuse light. Solid state samples, however, were found to be relatively stable to the various light / heat conditions over a 6 month period when compared to prochlorperazine solutions, but still considerably unstable. Thus both storage and packaging is a vital consideration for prochlorperazine injectables.

The thermal behaviour of mixtures of prochlorperazine with standard excipients, was assessed for potential interactions, using differential scanning calorimetry. For most of the excipients (magnesium stearate, stearic acid, Explotab®, Ac-Di-Sol®, Encompress® and Ludipress®, lactose and Starch 1500®) disappearance or broadening of the melting endotherm of the drug indicated interactions. Lubritab®, however, was the only 'inert' excipient tested.

Liquid chromatography - mass spectrometry (LC-MS) was used to determine the nature of the degradation products. The major degradation pathways included dechlorination and demethylation of the parent drug, as well as sulfoxidation and *N*-oxidation. Prochlorperazine underwent dechlorination and sulfoxidation with subsequent photosubstitution to yield the 2-hydroxy derivative. The solid state photostudies showed the formation of dealkylated, oxidised and hydroxylated products, sulfoxides and dimers. Since *N*-demethylation, *N*-oxidation, sulfoxidation and aromatic hydroxylation are reported to occur in the *in vitro* metabolism of perazine derivatives, it does appear that there is some relationship between metabolites and photoproducts (Breyer, 1974).

This study has been successful in providing understanding of the photolytic and thermal degradation pathways of prochlorperazine.

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dedicated to the memory of my

grandfather

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

BP	-	British Pharmacopoeia
CN HP	-	Cyano hydroxy propyl
DSC	-	Differential Scanning Calorimetry
DRS	-	Diffuse Reflectance Spectroscopy
E_a	-	Activation Energy
ESR	-	Electron Spin Resonance
FDA	-	Food and Drug Administration
GC	-	Gas Chromatography
HPLC	-	High Performance Liquid Chromatography
ICH	-	International Committee on Harmonisation
IR	-	Infra-red Spectroscopy
k	-	Rate Constant
LC-MS	-	Liquid Chromatography-Mass Spectrometry
LOD	-	Limit Of Detection
LOQ	-	Limit Of Quantitation
MCC	-	Medicines Control Council
mp	-	Melting Point
MS	-	Mass Spectrometry
m/z	-	Molecular ion
NMR	-	Nuclear Magnetic Resonance
R	-	Gas Constant
RH	-	Relative Humidity
RO	-	Reaction Order
RSD	-	Relative Standard Deviation
TIC	-	Total Ion Chromatogram
TLC	-	Thin Layer Chromatography
USP	-	United States Pharmacopoeia
UV	-	Ultra Violet
UV/Vis	-	Ultra Violet/Visible
Z	-	Pre-Exponential Factor

CHAPTER 1. INTRODUCTION

This chapter is a general survey of factors that determine the stability or lack of stability of drugs.

1.1 Drug stability

Drugs in the pure or dispensed dosage form are required to be stable. Stability refers to the capacity of the drug formulation to remain within the specifications stated to ensure its identity, strength, quality and purity (Carstensen, 1995).

The purpose of stability testing is to provide evidence on how the quality of a drug or a drug formulation varies with time under the influence of a variety of environmental factors such as temperature, humidity and light. Such testing enables recommended storage conditions, re-test periods and shelf lives to be established.

Regulatory bodies such as the Food and Drug Administration (FDA), and the Regulatory Authorities of the European Union and Japan, which are represented on the International Committee on Harmonisation (ICH), have put forward protocols for the stability testing of pharmaceuticals. Important documents on the stability testing of drugs are the FDA and ICH Guidelines which seek to define the core stability data package required for new drug substances and products. It is, however, not always necessary to follow these guidelines exactly when there are scientifically justifiable reasons for using alternative approaches.

1.2 Photostability

Photosensitivity in humans and animals is a relatively common problem that can be the cause of great discomfort and can severely restrict the lifestyle of the sufferer. Reactions may include skin rashes, itching, reddening and brown patches of skin discolouration. There are two main mechanisms for photosensitivity: a) a phototoxic reaction which occurs if the light energy absorbed by a molecule, such as a drug, results in tissue damage via a reactive chemical species such as free radicals, and b) a photoallergic reaction, which occurs if the light energy absorbed

by the molecule, causes a chemical or structural change that then renders that substance allergenic, causing an immune reaction.

Photosensitivity reactions may be caused by some systemic (oral) drugs and some topical therapeutic or cosmetic creams and perfumes. A systemic drug can induce a rapid photosensitivity reaction in susceptible individuals because the entire blood volume of a resting adult can circulate throughout the skin approximately once every eleven minutes. This allows exposure of the drug in the small blood vessels and capillaries present in the dermis to penetrating UV-A and visible light and to small amounts of UV-B rays. As these reactions may cause distress to the patient, it is important that the patient is aware of such drugs, so that an effective sunscreen can be used as required. Drugs which can cause photosensitivity include: chlordiazepoxide, naproxen, piroxicam, ibuprofen, trimeprazine, chlorpromazine, promethazine, sulphonamides and prochlorperazine.

A significant number of drugs containing one or more chlorine (Cl) atoms as substituents have been known to cause adverse photosensitivity effects. Photosensitivity refers to an abnormal reaction to a normal amount of light. Many chloroaromatic compounds have demonstrated the photolabile nature of chlorine. The free radicals formed on dissociation of the carbon (C)-Cl bond are thought to be able to combine readily with proteins and to remain localised to prolong photobiological effects in the skin (Moore and Tamat, 1980). These compounds are capable of undergoing both Type I (free radical) and Type II (singlet molecular oxygen) reactions. The photolability of the aromatic Cl substituent and the accompanying generation of free radicals *in situ* are thus thought to be important precursors of these photosensitivity reactions, although the mechanism by which the phenothiazine derivatives cause photosensitivity has not been entirely resolved. Moore and Tamat (1980) have reported that the polarity of the environment appears to determine whether the photoreaction occurs *via* a free radical formation or molecular oxygenation of a suitable oxidisable species, particularly when the drug is located in a region where the oxygen concentration is significant. The degradation of the compounds with Cl and trifluoro- (CF_3) substituents is thus thought to occur via photooxidation.

Huang and Sands (1967) reported that, at the end of a period of irradiation of a patient dosed with chlorpromazine, a shift in the pH from 4.5 to 1.9 was observed.

The liberation of hydrogen chloride (HCl) and the subsequent formation of a polymer of chlorpromazine in the tissues was investigated as the possible cause of the photosensitivity and of the skin discolouration observed in patients receiving chlorpromazine medication over a prolonged period. The chloride substituent is cleaved easily under acidic conditions (Huang and Sands, 1967).

The ICH Harmonised Tripartite Guidelines recommend that light testing, in addition to studying the effect of temperature, should be an integral part of stress testing. Stress testing is normally conducted to provide data on forced decomposition products and decomposition mechanisms for the drug and are normally carried out under more severe conditions than those used for accelerated tests. New drug substances and formulations should demonstrate on evaluation that they do not undergo unacceptable changes when exposed to light.

A systematic approach to photostability testing covers studies such as a) tests on the drug substance, b) tests on the exposed drug formulation outside the immediate pack, c) tests on the drug formulation in the immediate pack, and d) tests on the drug formulation in the marketing pack.

1.3 Packaging

Packaging plays an important part in determining the stability of a drug in the dosage form. Improper packaging and / or viscicitudal storage may cause losses in strength of the drug and for this reason the FDA requires that accelerated stress tests be done with exposure to both moisture and temperature (Carstensen, 1995).

The three main dosage forms in which drugs are marketed are: a) liquids, b) solids and c) aerosols.

The liquid dosage forms can be broadly classified as: aqueous solutions (e.g. solutions for injections), suspensions and emulsions; and non-aqueous solutions and suspensions such as ointments in petrolatum. Packing materials include: glass or plastics such as Aclar, PVA, PVDA (blisters).

Glass is non-permeable to moisture and oxygen so that any mass exchange with environment must take place through the closure system. Glass is, however, not

the reaction rate is then given by:

$$-d[A] / dt = -k[A]^n[B]^m \qquad [4]$$

where the sum n+m denotes the overall order of the reaction.

Very common behaviour found for degradation reactions is that the value of n in equation [2] is 1, i.e. the reaction is first-order, with the rate being directly proportional to the concentration of unreacted drug.

The integrated form of the first-order rate equation is:

$$\ln ([A] / [A]_o) = -kt \qquad [5]$$

where $[A]_0$ is the initial (t=0) concentration. Proof of such behaviour is obtained if a plot of $\ln ([A] / [A]_o)$, or of $\ln [A]$, against time is linear.

In photochemical reactions, it is not uncommon to find that:

$$-d[A] / dt = k \quad (\approx k[A]^o) \qquad [6]$$

i.e. n = 0 or the reaction is zero-order, and the rate of degradation is independent of the reactant concentration (over a large portion of, but not the complete reaction). The rate of reaction may, however, be affected by factors other than concentration (and temperature) such as the intensity of irradiation.

The integrated rate equation for a zero-order reaction is then:

$$[A] = -kt + [A]_o \qquad [7]$$

and such behaviour is easily recognised by the linearity of plots of [A] against time, t.

For reaction type [3], if reactant B is present in large excess, the rate equation may be of the form:

$$-d[A] / dt = \{k[B]_o\}[A] = k'[A] \quad [8]$$

which is described as pseudo first-order behaviour.

e.g. aspirin which decomposes by a pseudo first-order reaction to salicylic acid and acetic acid (Mroso *et al.*, 1982; Ager *et al.*, 1986; Carstensen *et al.*, 1995). The decomposition is best monitored by the appearance rate of the decomposition product which follows the rate equation:

$$[B] = A_o [1 - \exp(-k_1 t)] \quad [9]$$

More complex reactions

Where the decomposition product itself is not stable, consecutive reactions may occur:

$$A \xrightarrow[k_1]{} B \xrightarrow[k_2]{} C \quad [10]$$

The simplest kinetics (Carstensen *et al.*, 1995) result when each of the steps is a first-order reaction.

Parallel reactions may take place if, for example, A can decompose into two different products, B and C, the reactions are then represented by:

$$A \xrightarrow[k_1]{} B \quad [11]$$

and

$$A \xrightarrow[k_2]{} C \quad [12]$$

If both steps are first-order reactions, the overall rate equation is then

$$-d[A] / dt = +k_1[A] + k_2[A] = (k_1 + k_2)[A] \quad [13]$$

A third type of complication may occur when the equilibrium constant, K_{eq} is not so large that significant amounts of reactant(s) remain(s) at equilibrium in reaction [1a + 1b] and [3], such that K_{eq} does not greatly exceed unity.

$$A \rightleftarrows B \qquad [14]$$

The equilibrium constant, K_{eq}, is given by

$$[B]_{eq} / [A]_{eq} = K_{eq} \qquad [15]$$

If the forward reaction (f) and the reverse (r) reactions both follow first-order kinetics:

$$-d[A] / dt = k_f[A] - k_r[B] \qquad [16]$$

$$\text{At equilibrium, } -d[A] / dt = 0 \qquad [17]$$

$$\text{and } k_f[A]_{eq} = k_r[B]_{eq}, \qquad [18]$$

$$\text{so } K_{eq} = k_f / k_r \qquad [19]$$

Instead of using the actual concentrations, the fraction decomposed may be calculated as:

$$\alpha = 1 - [A] / [A]_o \qquad [20]$$

The first-order equation is then:

$$d\alpha / dt = k(1 - \alpha) \qquad [21]$$

for small α, $(1 - \alpha) \approx 1$ so the reaction appears to be zero-order. Since it is actually a first-order reaction, such a situation is referred to as a pseudo zero-order reaction.

Note: α is commonly used in the chemical literature, while the "x" notation is used by Carstensen (1995).

The order of a reaction is determined from experimental measurements of concentrations of reactant (the drug) or products of degradation at various times at constant temperature. The goodness of fit of data to the expected rate equations is then determined by statistical methods, and the order of the reaction is established if possible. If no simple order of reaction is indicated, the reaction mechanism is probably more complex than suspected.

Effect of Temperature

Rate constants are a function of temperature. Accelerated testing methods involve storing the reactant (drug or dosage form) at elevated temperatures which then causes larger degrees of decomposition in a short time so that the extent of reaction may be assessed with accuracy. The temperature dependence of a chemical reaction is given by the Arrhenius equation:

$$k = Z\exp[-(E_a / RT)] \quad \text{or} \qquad [22]$$

$$\ln k = -(E_a / RT) + \ln Z \qquad [23]$$

where E_a is the activation energy, Z is the pre-exponential factor, R is the gas constant and T the kelvin temperature (K).

Measurements of k values at, at least, two temperatures are required to estimate values for the important Arrhenius parameters, E_a and Z. The more temperatures used to obtain data, the more reliable these parameters will be.

Once the rate equation (e.g. first-order) has been identified (and shown not to change with temperature), and the Arrhenius parameters have been estimated, it is possible to extrapolate beyond the conditions for which experimental data are available. Such extrapolations are required, for example, to calculate the extent of degradation of a drug, or dosage form, during storage at a temperature far below that used for the accelerated testing. For example, storage may be at room temperature or in a refrigerator.

Extrapolations have to be carried out with great caution and are based on the assumption that the mechanism of the reaction under consideration does not

change with temperature or with extent of reaction. If a change of phase, for example freezing of a liquid or solution, occurs in the temperature interval considered, any kinetic extrapolation will be invalid.

1.5 Solid state reactions

The stability of pharmaceuticals in the solid state has not been extensively covered in the literature (Byrn, 1976; Carstensen, 1974, 1995). Little of the substantial knowledge of physical and chemical factors has been applied to the study of the decomposition of drugs and related compounds. Through knowledge of the mechanisms of solid state reactions and the effects of crystal structure on the solid state reactions of drugs, rational approaches to drug stabilisation may become possible (Byrn, 1976).

Solid state reactions differ from homogenous reactions in that: a) pronounced differences may be found in the reactivities of closely related compounds, b) different reaction products may be obtained from those formed when the reaction is carried out in solution or in the liquid state, or c) different crystalline modifications of the compound may have different reactivities or form different products (Byrn, 1976).

Many photochemical reactions in the solid state can be explained in terms of the "topochemical postulate" which states that solid state reactions proceed with a minimum of atomic and molecular movement. The crystal structure of the product may thus depend on the structure of the reactant crystal (Byrn, 1976).

Colour changes may be induced in a solid by light (photochroism) or by heat (thermochroism). Because both photochroism and thermochroism are usually reversible occurrences, these colour changes may cause concern even though the drug has not degraded (Byrn, 1976). For example, some sydnones are photochromic and at least one derivative of morphine has thermochromic properties. *N*-(3-pyridyl)sydnone gives colourless crystals which turn blue on exposure to sunlight. The infrared (IR) spectra of the blue and colourless crystals were identical. Similar results were obtained with salicylideneanilines where the change in the crystals were due to a small concentration of highly coloured species (Byrn, 1976).

In the solid state, reactions that occur include a) free radical reactions initiated by heat or light, b) polymerisation and c) thermal rearrangement reactions. Reaction with the surrounding atmosphere can provide oxidation *via* d) singlet-oxygen addition and e) reaction of an excited state drug molecule with ground state oxygen. An example of a) above is ergocalciferol, while b) and c) are examples of the pathways involved in the degradation of solid polyenes (Byrn, 1976).

There are two main purposes for conducting studies of decompositions of solids in pharmaceuticals: a) to elucidate the mechanism of a reaction, and b) to predict stability.

1.6 Solid-solid interactions

Solid-solid interactions in pharmaceutical dosage forms are of great importance. For example, Troup and Mitchner (1964) showed that in solid dosage forms containing aspirin and phenylephrine, there was a direct correlation between the loss rates of both compounds (Carstensen, 1974). Chromatography proved the existence of acetylated phenylephrine in the dosage form, so that the acetic acid liberated in the aspirin decomposition resulted in the acetylation of phenylephrine. Aspirin in an alkaline environment was also shown by Eppich *et al.* to degrade by a topochemical reaction (Carstensen, 1974).

The detection of solid-solid interactions in drug dosage form development programmes is thus also of great importance. By detecting incompatibilities in the pre-formulation stage, it is possible to formulate dosage forms with ingredients which are least likely to interact. This type of screening programme is referred to as a "compatibility programme" or a "pre-formulation screening programme". Tests normally consist of mixing the drug and the excipient thoroughly in the stated amounts in a sealed vial, which is then stored. The occurrence of interactions may be gauged visually or, more quantitatively, by differential scanning calorimetry (DSC).

Blaung and Huang demonstrated the interaction between dextroamphetamine sulfate and spray-dried lactose in a quantitative fashion by use of an (ethanol mediated) diffuse reflectance spectroscopy technique. Conclusions regarding the therapeutic availability cannot be drawn from such tests.

1.7 Solid state kinetics

The decompositions of pure solids gives curves of fractional decomposition alpha (α) against time at constant temperature that are usually i) either initially steep and then deceleratory as they approach 100% decomposition, or ii) are characterised by an induction period, followed by an acceleratory region and then a deceleratory region. Several models have been developed to account for the types of α-time curves observed (Brown *et. al.*, 1980). Type (i) curves are normally accounted for by considerations of contracting geometry models (Fig. 1.1 (b)), reaction order models (RO) (Fig. 1.1 (c)) or by diffusion models (Fig. 1.1 (d)), while shape (ii) may be accounted for by either nucleation theories or liquid layer theories (Fig. 1.1 (a)) (Carstensen, 1995).

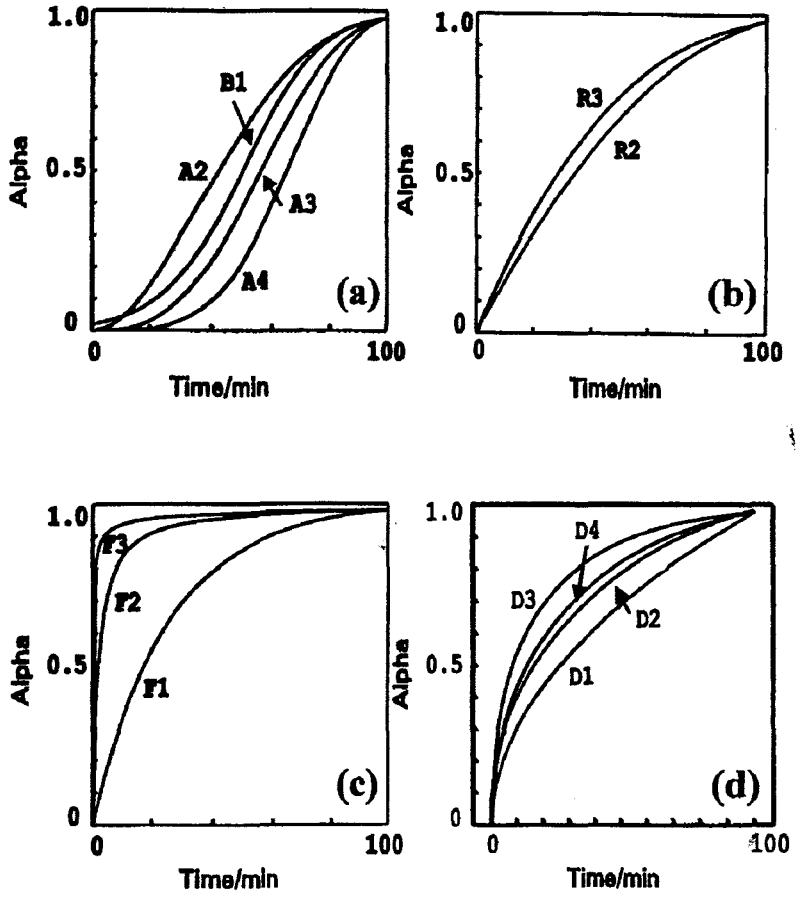


Fig. 1.1 α-time plots for the most important rate equations used in kinetic descriptions of reactions involving solids: a) sigmoid models (S-shaped), b) geometrical models, c) reaction order (RO) models, d) diffusion models

Deceleratory curves are often assumed, by analogy with homogenous kinetics (see earlier) to be described by simple order-of-reaction (RO) models (Fig. 1.1 (c) and especially first-order rate equations.

At low extents of decomposition, α, as discussed earlier, the order-of-reaction may appear to be zero, i.e. a significant segment of the α-time curve is approximately linear.

In the pharmaceutical literature, few attempts have been made to test the applicability of the full range of solid state kinetic models (Brown *et al.*, 1980).

S-shaped curves (e.g. Figure 1.1 (a)) are usually assumed to be described by Prout-Tompkins or Bawn models. When moisture is present, decomposition is often accounted for by solution kinetics of a saturated, sorbed solution, in which case the decomposition is zero-order. Apparent zero-order reactions also occur where the decomposition is diffusion controlled, but the solubility is controlled by a smaller constant concentration. In the case of interacting substances, the three-dimensional diffusion equation $[1 - (1 - x)^{1/3}]^2 = kt$ often holds (Carstensen, 1995).

Where a second reactant, such as moisture, is involved, reaction may lead to some sort of equilibrium and the temperature dependence of the equilibrium constant in terms of the van't Hoff equation may be of more importance than contributions from the Arrhenius-type temperature dependence of the rate coefficients (Carstensen, 1995).

Equilibria are much more commonly found in solid dosage forms than in pure solids and solutions. This may be due to an apparent equilibrium but it offers a procedure for extrapolation in predictive work. Reasons for equilibria may be chemical, for example excipient interactions, catalysed isomerisation, de-esterification and even the presence of impurities (Carstensen, 1995).

Interactions of solids with oxygen have been reported by Tingstad and Garrett (1960) and Garrett (1954), who established that fumagillin (A) oxidised *via* a steady state intermediate (AO_2) which reacted with A to form AO (Carstensen, 1974):

$$A + O_2 \underset{k_r}{\overset{k_f}{\rightleftarrows}} AO_2 \xrightarrow[k_2]{+A} 2AO \qquad [24]$$

If $k_2[A] \gg k_r \gg k_f[A]$, then:

$$d[AO]/dt = -2d[A]/dt = (k_2[O_2]/k_r)\cdot[A]^2 \qquad [25]$$

so that the reaction would be second-order and follow:

$$1/[A] = 1/[A]_o + kt \qquad [26]$$

1.8 Drug stability testing

There are several differences between chemical kinetic studies and pharmaceutical stability studies. a) Chemical kinetics are often studied through several half-lives, while stability is usually only studied down to 85% of the initial drug strength. b) Chemical kinetics are mostly carried out in as a pure a system as possible, while in stability studies, the systems contain many components and the purity is controlled to a practical range and not to the highest degree of purity. c) One of the goals of chemical kinetics is to determine the reaction mechanisms, while that of stability studies is to estimate an expiration date. This last aspect involves a fair amount of sophisticated statistics which may not be necessary in the chemical kinetics studies (Carstensen, 1995).

The objective of stability testing is thus to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light. Such tests enable recommended storage conditions, re-test periods and shelf lives to be established. A program for stability assessment might include storage at ambient temperature and under stressed conditions. Stress testing is part of the development strategy and is normally carried out under more severe conditions than those used for accelerated tests. Stress testing includes the effect of temperature, humidity and exposure to various wavelengths (both ultraviolet (UV) and visible regions) of electromagnetic radiation (photolysis) in open containers. Where applicable, the effects of oxidation (high pressures of oxygen), susceptibility to hydrolysis across a

wide range of pH values (acidic and alkaline), and the presence of added substances under consideration for product formulation when in solution or suspension, are also examined. Such stress testing helps to determine the intrinsic stability of the drug and also provides data on forced decomposition products. Results from these studies form an integral part of the information provided to the regulatory authorities. It is recognised that some degradation pathways are complex and that, under forcing conditions, decomposition products may be observed which are unlikely to be formed under accelerated or long-term testing. This information is useful in the development and validation of suitable analytical methods, but it is not always necessary to examine specifically for all degradation products (resulting from accelerated stress tests) if it has been demonstrated that, in practice, these are not formed. Analytical instruments or techniques used in the quantitation of the active ingredient and related substances include: High Pressure Liquid Chromatography (HPLC), Ultraviolet (UV) spectrophotometry, Nuclear Magnetic Resonance (NMR), Mass Spectrometry (MS), Thin Layer Chromatography (TLC) and non-aqueous titrations.

Stability information on the drug substance before formulation is valuable in identifying characteristics of the intact molecule that can change under defined storage conditions. When such labile conditions are found, those storage conditions are then included in the stability study protocol designed for all drug products of the drug substance. These studies are then also useful in establishing packaging requirements, storage conditions, and an expiration date period when required, e.g. antibiotics (Carstensen, 1995).

1.9 International Committee on Harmonisation (ICH) guidelines for stability testing

All stability equipment and procedures should be operating and validated in a satisfactory manner, with all standard operating procedures written.

1.9.1 The sample

The ICH guidelines for stability testing recommend that care should be taken to ensure that the physical characteristics of the samples are taken into account and care exercised so that effects on the physical state such as cooling and / or placing

the samples in sealed containers, sublimation, evaporation and melting are minimised. Possible interactions between the samples and any materials used for containers and for general protection of the sample should be considered and eliminated whenever a test is being carried out.

1.9.2 Packaging

The ICH guidelines state that stability testing should be carried out in the final packaging proposed for marketing. Additional testing of the unprotected drug product can form a useful part of the stress testing and pack evaluation, as can studies carried out in related packaging materials, in support of the packaging chosen.

1.9.3 Photostability testing

The ICH stability guidelines call for light cabinet testing and some of the light sources used include: 1) fluorescent lights, 2) xenon light, and 3) UV light.

Problems with light cabinets include ventilation and monitoring of light bulbs. Even with good ventilation, temperatures in the light cabinets are usually higher than the environment (e.g. 27°C) thus necessitating the routine monitoring of temperature. Monitoring of light intensity using actinometry (as specified by the recent ICH guidelines) rather than light meters is absolutely necessary and a bulb replacement schedule should be in effect. Where quantitation in light stability testing is necessary, quinine may be used as a chemical actinometer (as specified by the recent ICH guidelines).

When exposing solid drug substances to light sources for stability testing, they should be spread (with a thickness of not more than 3 mm) across a suitable glass or plastic dish with a transparent cover, if deemed necessary. Liquid drug substances should be exposed in chemically inert and transparent containers. These samples should always be positioned so as to provide a maximum area of exposure to the light source. When testing the drug product in the immediate container (marketed packaging), the samples are placed either transversely or horizontally with respect to the light source, whichever provides for the most uniform exposure of the samples (Carstensen, 1995).

At the end of the exposure period, samples are examined for any changes in physical properties (e.g. appearance, clarity or colour of solution, dissolution / disintegration of dosage forms such as capsules, tablets, etc.) and are assayed for degradants by a method suitably validated for the products likely to arise from the photochemical degradation processes.

When testing powder samples, representative portions should be ensured for individual tests. For solid forms of oral dosage products, testing is conducted on an appropriately sized composite of, for example, 20 tablets or capsules. Similar sampling considerations, such as homogenisation or solubilisation of the entire sample, apply to other materials that may not be homogenous after exposure (e.g. creams, ointments, suspensions, etc.). This analysis should be performed simultaneously with that of any protected samples used as dark controls.

1.9.4 Thermal stability testing

1.9.4.1 Temperature and humidity

Tests of the effect of temperature on the stability of a drug or dosage form are usually combined with the testing of any effect of controlled humidity (Carstensen *et al.*, 1995).

Storage under conditions of high relative humidities (RH) is of particular importance for solid dosage forms. For products such as solutions, suspensions etc., contained in packs designed to provide a permanent barrier to water loss, specific storage under conditions of high relative humidity is not necessary, but the same range of temperatures should be applied. Low relative humidity (e.g. 10-20% RH) can adversely affect products packed in semi-permeable containers (e.g. solutions in plastic bags, nose drops in small plastic containers etc.) and consideration should be given to appropriate testing under such conditions.

1.9.4.2 Oxidation studies

The ICH guidelines state that conditions involving a high oxygen content must be evaluated in stability studies of solutions and suspensions. Oxidation reactions are, however, relatively rare as main reactions in pharmaceutical dosage forms. Some

oxidation probably takes place in many drugs and results in small amounts of unidentified degradation products.

Oxidation reactions are usually the sum of a series of reactions (sometimes chain reactions) and these start with a particular reaction (initiation reaction) which usually does not involve molecular oxygen. If oxygen is abundant, then all the drug can decompose and the kinetics may be approximately zero-order, because $[O_2]$ becomes (virtually) constant. For example, captopril undergoes autoxidation. Oxidations in aqueous solution are a function of the dissolved oxygen. Nitrogen may be bubbled through the aqueous solution to remove oxygen, or antioxidants, such as ascorbic acid, may be included, where possible, in oxygen-sensitive drugs.

1.9.4.3 Standard conditions

The standard conditions for thermal stability testing are:

	Conditions	*Minimum Time*
Long term testing	25°C ± 2°C / 60% RH ± 5%	12 months
Accelerated testing	40°C ± 2°C / 75% RH ± 5%	6 months

Where significant change occurs during accelerated testing, additional testing at an intermediate condition, e.g. 30°C ± 2°C / 60% RH ± 5%, should be conducted. 'Significant change' under accelerated conditions is defined as:

1) A 5% potency loss from the initial assay value of a batch;
2) Any specified degradant exceeding its specification limit;
3) The product exceeding its pH limits;
4) Dissolution exceeding the specification limits for 12 capsules or tablets.
5) Failure to meet specifications for appearance and physical properties, e.g. colour, phase separation, resuspendability, delivery per actuation, caking, hardness, etc.

The length of the studies and the storage conditions are normally sufficient to approximate for actual storage, shipment and subsequent use (e.g. reconstitution or dilution as recommended in the labelling). Assurance is provided by long term testing which covers the expected shelf life (Carstensen, 1995).

The ICH guidelines recommend that other testing conditions should be allowed for heat-sensitive drug products that are normally stored at lower temperatures (which will eventually become the designated long term storage temperature). Special consideration is normally given to products which change physically at lower temperatures, e.g. suspensions or emulsions which may sediment or cream and oil and semi-solid preparations which may show an increased viscosity. Where a lower temperature condition is used, the six months accelerated testing is normally carried out at a temperature at least 15°C above the designated long-term storage temperature (together with appropriate relative humidity conditions for that temperature). For example, for a product to be stored long-term under refrigerated conditions, accelerated testing should be conducted at 25°C ± 2°C at 60% RH ± 5% RH. The designated long-term testing conditions will be reflected in the labelling and expiration date.

Should significant change occur under the standard test conditions at 40°C / 75% RH, then the initial Registration Application will also include a minimum of 6 months data from an ongoing one-year study at 30°C / 60% RH, where the same criteria for significant change apply. The long-term test then continues for a sufficient time beyond 12 months to cover the proposed shelf life. The further accumulated data is then submitted to the authorities during the assessment period of the Registration Application.

Frequency of testing should be sufficient to establish the stability characteristics of the drug formulation. Testing will normally be every three months over the first year, every six months over the second year, and then annually where the testing covers those features which are susceptible to change during storage and likely to influence the quality, safety and / or efficacy. Stability information also covers the physical, chemical and microbiological test characteristics. Statistical tests are usually applied to validate the stability results. The need for replication of several tests is dependent on the results of the validation studies.

1.9.4.4 Degradation products

When degradation products are detected at 0.1% level or more, they must be isolated and identified. Information on the procedures for the isolation and purification of degradation products must be provided along with their physical and

chemical properties. Indications of pharmacological action or inaction, as well as any cross references to available information on biological effects and the significance of these effects at the concentrations most likely to be encountered, should be supplied.

The results of kinetic studies may provide information on the mechanism of formation of the degradation products.

1.9.4.5 Statistical methods

A stability study protocol must describe, in addition to how the stability study is designed and carried out, the statistical methods used in analysing the data.

Sampling times are chosen so that the degradation curve can be adequately characterised. Degradation curves are constructed in terms of width confidence intervals around the average of the sampling times included in the study.

The statistical methods and formulas used in the analysis, as well as the evaluation of the data (including calculations and graphs), should normally be documented.

1.10 Project proposal

1.10.1 Introduction

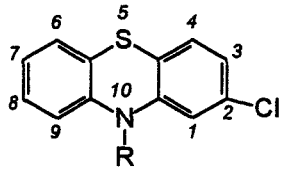
This study was initiated in response to a request from a South African generic pharmaceutical manufacturer to undertake a photostability programme, in accordance with the new ICH photostability guidelines. The request was to investigate prochlorperazine edisylate in solution, despite the fact that prochlorperazine mesylate was originally used to formulate the injectable. Prochlorperazine is light sensitive and a fine chemical, thus if degradants are found to be 0.1% or greater, they must be isolated and identified according to the ICH regulations (Ortiz *et al.*, 1983), especially since phenothiazines are known to be significantly phototoxic. It was proposed to investigate the stability of both of the prochlorperazine salts in different packaging materials, under various light and heat conditions, in the solid and solution states. Dosage forms for prochlorperazine include injectables. Because market forces are dictating that the industry package

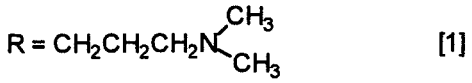
these injectables in clear glass ampoules, the extent of degradation in clear glass needed to be considered. The objective of this investigation was thus to determine the kinetics of degradation, in solution and in the solid state, of two prochlorperazine salts, edisylate and mesylate, under various light and heat conditions. The identities and structure of the major degradation products will also be determined using liquid chromatography-mass spectrometry (LC-MS).

1.10.2 The phenothiazines and prochlorperazine

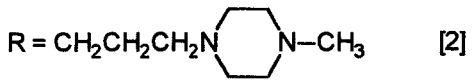
The phenothiazines are a class of structurally related, widely used neuroleptic drugs which may be divided into the piperazine, piperidine and aliphatic subclasses, depending on their chemical structure, pharmacological action and potency (Foye *et al.*, 1995). Phenothiazines are well known phototoxic drugs which also cause photoinduced genetic defects. Chlorpromazine [1], on irradiation with light, forms toxic photoproducts which have the ability to react with cell constituents and cause adverse effects. Chlorpromazine, because it has been widely studied in the literature available, is also commonly taken as the prototype for the many neuroleptic drugs in clinical use today. The chlorine in position 2 has been proposed to cause the photosensitivity effects (Moore and Tamat, 1980) and prochlorperazine [2], a chloro-derivative, is thus expected to behave similarly.

In the *British Pharmacopoeia* (*BP*) and the *United States Pharmacopoeia* (*USP*), the 10-substituted phenothiazine derivatives constituted one of the largest drug classes in the official monographs between 1980 and 1985, rendering the phenothiazines an important class of drugs (Chagonda and Millerson, 1989). Although these statistics may not apply in 1998, there are increasing requirements of the regulatory authorities for more detailed information on the stability profiles and the nature of degradants formed. These drugs may also play an important role in the attempts of the International Conference on Harmonisation forum (ICH) towards the standardisation of light stability studies.





[1]



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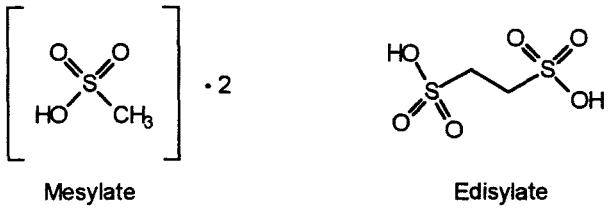


Mesylate Edisylate

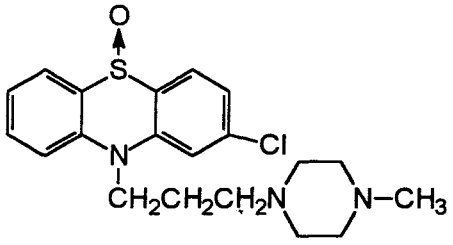
Fig. 1.2 Structures of two phenothazine chloro-derivatives: chlorpromazine [1] and prochlorperazine [2]. The structures of the mesylate and edisylate salts are also shown.

As a result of reports in the literature of the phototoxicity of chlorpromazine, its photostability has been investigated and decomposition products including the N-oxide, the sulfoxide, promazine, 2-hydroxypromazine and a dimer and polymer of chlorpromazine have been isolated and identified (Sharples,1981). Because phenothiazine and its derivatives are sensitive to oxidation, it has been proposed that the cation radical formed on oxidation is an intermediate of the metabolism of the drug and is thus the active pharmacological entity. The stability of the 10-alkylphenothiazine free-radicals has been shown to depend on the nature of the 2-substituent on the phenothiazine nucleus and the nature of the 10-alkyl substituent (Ortiz *et al.*, 1983).

The poor stability of the phenothiazine series results in the *in vitro* degradation of these compounds being similar to that *in vivo*. The major degradant or metabolite identified in both cases is the 5-sulfoxide. The sulfoxides were thus synthesised by a reported method to provide standards for comparison in the stability studies (Owens *et al.*,1989). A complete profile of prochlorperazine and its main metabolite and degradant, the 5-sulfoxide, together with the synthetic method and structural

elucidation is given in Chapter 2. To investigate the stability of both prochlorperazine salts, a high performance liquid chromatographic (HPLC) method has been developed and validated. This method is required to be precise and accurate enough to allow for day-to-day variability, and to reflect the system's ability to resolve the degradation products from the drug. This method is described in Chapter 3.

The availability of the prochlorperazine salts in a variety of liquid dosage forms prompted an investigation into the thermal and photo-stability in the liquid and the solid states under normal and accelerated conditions. The results are discussed in Chapters 4 and 5. Because the two prochlorperazine salts were found to be very stable under the various heat and light conditions in the solid state, a preformulation screening study, using DSC, was carried out to evaluate any chemical interactions which may take place between prochlorperazine and various excipients. Once the selection process was complete, various prochlorperazine - excipient tablets were prepared. The various interactions are discussed in Chapter 5. Chapter 6 contains information on some of the major degradants found in the various stability studies and some conclusions concerning the study are drawn in Chapter 7.



[3]

Fig. 1.3 Structure of prochlorperazine 5-sulfoxide [3] (Foye *et al.*, 1995)

CHAPTER 2. PHARMACEUTICAL CHEMISTRY OF PROCHLORPERAZINE

This chapter introduces prochlorperazine, discusses its mechanism of action and highlights the presence of the chloro-group which not only contributes to the enhanced activity, but also causes adverse photosensitivity effects. Clinical uses of prochlorperazine are discussed. Its metabolism is regarded as important because there may be similarities between degradants and metabolites (West *et al.*, 1974; Breyer *et al.*, 1974; Aravagiri *et al.*, 1985; Jaworski *et al.*, 1993).

2.1 Introduction

Prochlorperazine is a member of the piperazine subclass of phenothiazines. Because of the presence of the sulfoxides *in vivo,* the conditions under which they are formed have been studied and this has led to the influences of both light and oxygen stability being extensively studied on. There are numerous references to the synthesis of various phenothiazine sulfoxides (Whelpton and Curry, 1976; Huang and Bhansali, 1968; Abdel-Moety *et al.*, 1996; Roseboom and Perrin, 1977; West *et al.*, 1974).

2.2 Clinical uses

Since the 1950's, the phenothiazines have been the most frequently used anti-emetic and anti-psychotic agents (the latter are used for the treatment of schizophrenia and manic-depressive illness). Prochlorperazine has been widely studied in the management of antineoplastic-induced vomiting. Anti-emetic agents are thought to act by inhibiting dopamine transmission in the chemoreceptor trigger zone (Merrifield and Chaffee, 1989).

Phenothiazines are currently formulated in a variety of dosage forms, either as sole medicaments or in combination with other drugs (Davidson, 1976).

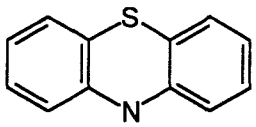
A number of adverse effects are associated with phenothiazines, particularly with chloro-substituted phenothiazines. The chloro-substituent, while enhancing neuroleptic activity, is thought to be responsible for the phototoxic side products. (Refer to chapter 4.1.3.1). Phototoxicity is a major side-effect in chlorpromazine therapy and its photochemical breakdown has been studied extensively (Sharples,

1980). A study of the *in vivo* photosensitivity of the phenothiazines showed that both photoallergy and phototoxicity reactions are mainly due to the decay products formed (Abdel-Moety *et al.*, 1996). A number of chlorpromazine decomposition products, which include the *N*-oxide, the sulfoxide, promazine dimers and polymers, have been isolated and identified (Huang and Sands, 1967). The *N*-oxides and sulfoxides have been found to be pharmacologically inactive. The sulfoxides tested were found to have little or no dopamine receptor binding. Sulfoxides of some phenothiazine derivatives have been found to retain some α-adrenergic binding activity (e.g. thioridazine sulfoxide) and antipsychotic activity, but still possess cardiotoxic side effects. Phenothiazines that contain one or more chiral centres exist as stereoisomeric mixtures and the diastereomeric pairs of thioridazine have been identified in fatal thioridazine intoxications and in *in vivo* metabolic studies in rats (Owens *et al.*, 1989).

Ljunggren and Möller (1977) investigated the relative phototoxicities of phenothiazines and found that the phenothiazine ring system was essential for phototoxic activity.

2.3 Mechanism of action

Phenothiazine (Figure 2.1), first synthesised in 1883, was used as an anthelmintic which had no anti-psychotic activity. Prochlorperazine was produced by the molecular modification of promethazine, an antihistamine with a sedative side effect, and this resulted in the neuroleptic phenothiazines. Thus, what initially involved minor molecular modifications of an antihistamine with sedative side effects, resulted in the development of a major class of drugs that initiated a new era in the drug therapy of the mentally ill (Foye *et al.*, 1995).



[4]

Fig. 2.1 Structure of phenothiazine [4]

Gordon and co-workers (Foye *et al.*, 1995) postulated that an R (N_{10}) group consisting of a three- carbon side-chain attached to the endocyclic nitrogen is optimal for neuroleptic potency. Phenothiazines with a two-carbon R side chain are useful in Parkinson's syndrome because of their anti-cholinergic effect, while promethazine, with its two carbon chain, is used as an antihistamine (Refer to Figure 2.4). The nature of the R group is important. If R is a hydrogen, good neuroleptic potency is attained, whereas introduction of a methyl group would have an inconsistent effect on the neuroleptic activity, although it increases the antihistaminic and antipruritic properties of some compounds. Bulky groups, such as phenyl or dimethylamino groups, around a three-carbon chain completely restrict the free rotation of the R group, resulting in a decrease of neuroleptic potency (Foye *et al.*, 1995).

The activity of a 2-substituted phenothiazines, such as prochlorperazine, varies according to the nature of the electron-withdrawing substituent, provided that it has no ionic character, e.g. Cl, $COCH_3$, CF_3 and $SO_2N(CH_3)_2$ which have increased neuroleptic activity. Substitution of these groups with methoxy or hydroxy groups resulted in a decrease in neuroleptic activity. Compounds containing SCH_3, SCF_3 and SO_2CH_3 substituents were found to be active (Foye *et al.*, 1995).

By virtue of their ability to mimic the trans-α conformation of dopamine (Figure 2.2 B), the phenothiazines are classified as dopamine antagonists. X-ray structures show the preferred conformations of dopamine and chlorpromazine to be partly superimposed, thus providing clues to the molecular mechanism and site of action of phenothiazines. In its preferred conformation, the side chain of chlorpromazine tilts away from the midline toward the chlorine-substituted ring (Figure 2.2 A). Structure D shows the non-superimposability of dopamine with chlorpromazine that is predicted to be inactive (Foye *et al.*, 1995).

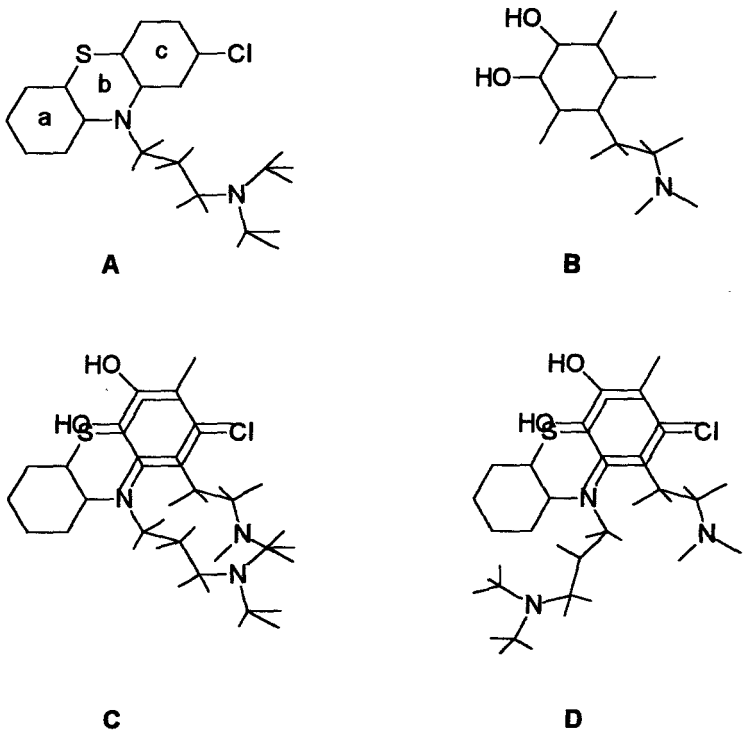
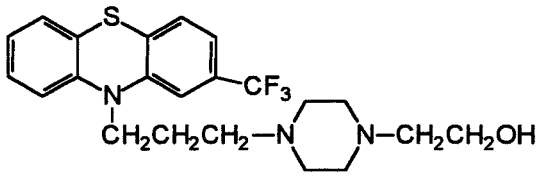


Fig. 2.2 Conformations of chlorpromazine (A), dopamine (B), and their superposition (C). The a, b and c in (A) designate rings. D shows another conformation in which the alkyl side-chain of chlorpromazine is in the *trans* conformation (ring **a** and amino side-chain), which is not superimposable on dopamine. Adapted from Horn and Snyder (Foye *et al.*, 1995).

The chlorine on the ring is responsible for imparting asymmetry to the molecule. The side-chain amine of phenothiazines that contain three carbons separating the two nitrogen atoms, is a major requirement for therapeutic efficacy. For example, promethazine, which is primarily antihistaminic, has two carbons separating the two nitrogen atoms and thus lacks anti-psychotic activity. Incorporation of the dimethylamino function of chlorpromazine, for example, into a piperazine ring (such as prochlorperazine) further increases the neuroleptic activity. Dopamine has unrestricted rotation about the β-carbon phenyl bond and unlimited flexibility and thus much information about dopamine and its receptor is obtained from such drugs (Foye *et al.*, 1995).

The duration of action of neuroleptics can be prolonged by the preparation of long-chain fatty esters. Fluphenazine-decanoate and -enanthate were the first of these esters to appear in clinical use and are longer acting, with fewer side-effects, than

their unesterified precursors (Foye *et al.*, 1995). Fluphenazine [5] was found to be the most potent of the four structurally-related piperazine phenothiazines, i.e. prochlorperazine, perphenazine, trifluoperazine and fluphenazine. The presence of the piperazine ring seems to provide increased neuroleptic activity because it increases the van der Waals interactions between the piperazine ring (as opposed to the dimethylamino function of the aliphatic class) and the substituent on position 2. Similarly, the β-hydroxy ethyl group, that replaces the methyl group on the piperazine ring, provides more sites of interaction between the substituent on position 2 and the side chain, thus further enhancing the van der Waals interactions and increasing the neuroleptic potency.



[5]

Fig. 2.3 Structure of fluphenazine [5]

2.4 Metabolism

Metabolism is important in stability considerations because there is a similarity between the *in vitro* degradants and the *in vivo* metabolites and the metabolism of antipsychotic drugs is of major importance in the action of phenothiazines. Considerable information about the metabolism of chlorpromazine is available in the literature, but information on the other phenothiazine neuroleptics is scant (Foye *et al.*, 1995). The phenothiazine antipsychotic agents are extensively metabolised by various routes which include: aromatic hydroxylation at the 7-position of the phenothiazine ring, ring S-oxidation, *N*-dealkylation, and *N*-oxidation of chlorpromazine (Aravagiri *et al.*, 1985). Breyer *et al.* (1974) studied the four structurally-related phenothiazines (prochlorperazine, fluphenazine, trifluoperazine and perphenazine) and found that a series of ethylenediamine metabolites were formed including: *N'*-methylethylenediamine, ethylenediamine and β-hydroxyethyl-ethylenediamine. The piperazine ring cleavages were thought to proceed via two consecutive *N*-dealkylation reactions.

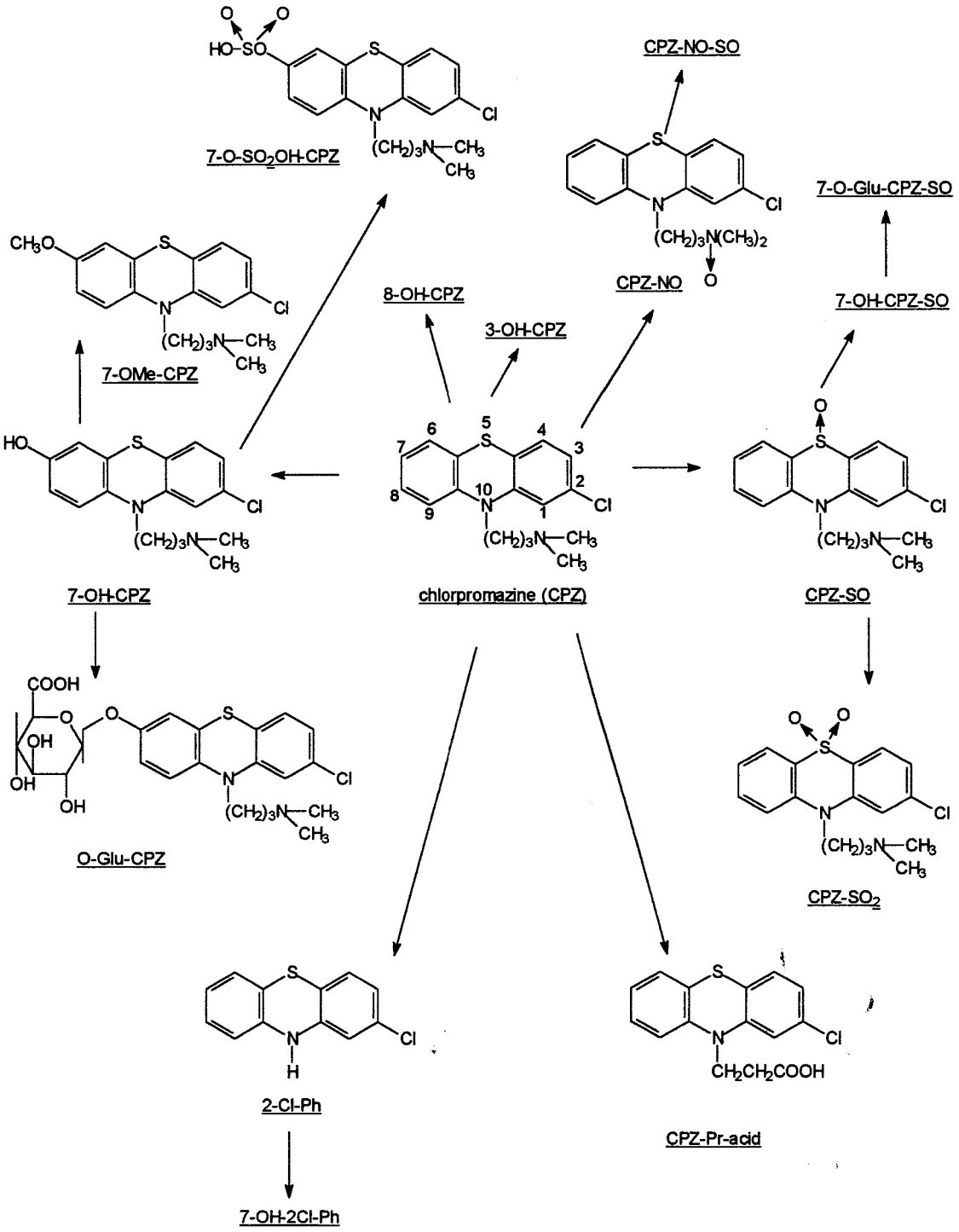
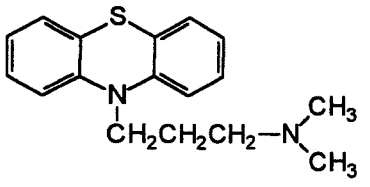


Fig. 2.4 Some metabolic pathways for chlorpromazine. Abbreviations: CPZ, chlorpromazine; NO, N-oxide; SO, sulfoxide; SO_2, sulfone; Ph, phenothiazine; Pr-acid, propionic acid; O-SO_3H, sulfate; O-Glu, O-glucuronide.

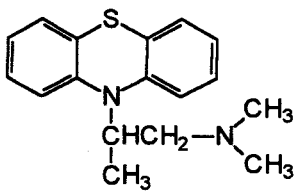
During metabolism, several processes occur. Chlorpromazine can be demethylated, sulfoxidised, hydroxylated and glucuronidated to yield 7-O-glu-nor-CPZ-SO. Species, age, sex, interaction with other drugs, and route of administration can alter the metabolic pathway (Foye *et al.*, 1995).

Radioimmunoassay has provided evidence that the 7- and other hydroxylated derivatives, the mono- and di-demethylated products (nor_1-CPZ, nor_2-CPZ) are active *in vivo* and at D_2 receptors, whereas sulfoxides have been found to be inactive (Foye *et al.*, 1995). The 7-hydroxy metabolite has been found to be at least as effective in antipsychotic treatment as chlorpromazine (Aravagiri *et al.*, 1985).

Formed by oxidation, the sulfoxides are the main metabolites and degradants of all phenothiazines. The three-carbon side-chain of some phenothiazines is implicated in the oxidation processes because promazine [6], which has a three-carbon chain, forms a sulfoxide, while promethazine [7], a structural isomer of promazine [6] with a two-carbon chain, does not. The oxidation process is thus thought to be dependent on the number of carbons between the ring and exocyclic nitrogens.



[6]



[7]

Fig. 2.5 Structures of promazine [6] and promethazine [7]

Sulfoxides have been prepared from phenothiazine itself or from salts using aqueous nitric acid (Owens *et al.*, 1989) and hydrogen peroxide (Juenge *et al.*), whereas *N*-oxides have been prepared using 3-chloroperoxybenzoic acid. The mechanism of sulfoxide formation is described in Chapter 4.

2.5 Synthesis of 5-sulfoxides

Sulfoxidation, resulting in 5-sulfoxide (ring sulfoxide) metabolites, is a common biotransformation pathway for all phenothiazines. Steady-state plasma concentrations of ring sulfoxides several times greater than that of the parent phenothiazines may be attained during therapy. Sulfoxides demonstrate little or no receptor binding and, thus, ring sulfoxides do not contribute to the effects of phenothiazines (Owens *et al.*, 1989).

Several methods for the synthesis of phenothiazine sulfoxides have been reported (Whelpton and Curry, 1976; Huang and Bhansali, 1968; Abdel-Moety, 1996; Roseboom and Perrin, 1977; West *et al.*, 1974). Hydrogen peroxide (H_2O_2) is most frequently used as the oxidising agent. Owens *et al.* (1989) reported a convenient method for the preparation of phenothiazine sulfoxides from their parent drugs by oxidation in aqueous nitric acid. This method was found to be selective for the oxidation of thioridazine, which yielded gram quantities of thioridazine sulfoxide with chromatographically separable pairs of diastereomers. Phenothiazine oxidation by stirring the free base or salt in aqueous nitric acid gave good yields (prochlorperazine sulfoxide at a 74% yield) (Owens *et al.*, 1989). Juenge *et al.* (1983) synthesised sulfoxides using a hydrogen peroxide method that resulted in a complex mixture of oxidation products and intricate procedures were needed to purify these products. Turner *et al.* (1963) used hydrogen peroxide in glacial acetic acid to prepare phenothiazine sulfoxides. Perphenazine, however, which has a sensitive *N*-piperadinylethanol group, gave a sticky dark product with several impurities. Fluphenazine sulfoxide was synthesised by Whelpton and Curry (Huang and Sands, 1967) by shaking a solution of fluphenazine in heptane with a 10% solution of H_2O_2. Potassium metabisulphite was then used to treat the aqueous solution to reduce any *N*-oxides. One of the simpler methods (Huang and Bhansali, 1968) involved dissolving trifluoperazine in 30% H_2O_2 and stirring the solution overnight at room temperature. The resulting sulfoxide was then recrystallised using methanol. Abdel-Moety *et al.* (1996) prepared the sulfoxide by heating the drug (base) with hydrogen peroxide and glacial acetic acid on a water-bath at 60°C for 30 minutes. The solvent was evaporated under reduced pressure and the sulfoxide recrystallised from ethanol (Abdel-Moety, 1996). The aqueous hydrogen peroxide method of oxidation was used in this study (Owens *et al.*, 1989).

CHAPTER 3. PROCHLORPERAZINE CHARACTERISATION

This chapter is concerned with the characterisation of prochlorperazine and the synthesis and characterisation of the 5-sulfoxide by various techniques.

3.1 Reagents

All chemicals were of analytical grade. Methanol (HPLC grade) was obtained from Romil Ltd., Cambridge, hydrogen peroxide from SAARCHEM-HOLPRO Analytic (Pty.) Ltd., Krugersdorp, R.S.A., and redistilled chloroform was used. Water for chromatography was obtained using a Milli-Q Plus® water purification system, filtered with a Q Pak purification pack, suitable for water pretreated by reverse osmosis, consisting of an initial 0.5 µm prefilter, activated carbon, nuclear grade ion-exchange resin, and an Organex-Q organic scavenger mixture.

Prochlorperazine mesylate was kindly donated by Rhone-Poulenc (Port Elizabeth, South Africa) and the edisylate salt by Intramed (Port Elizabeth, South Africa).

Prochlorperazine mesylate: 2-chloro-10-[3-(4-methylpiperazin-1-yl)propyl] phenothiazine di(methanesulphonate), is a white and odourless powder (BP 1993).

Prochlorperazine edisylate: 2-chloro-10-[3-(4-methyl-1-piperazinyl)propyl]-10H-phenothiazine ethanedisulphonate, is a white almost odourless, crystalline powder (BP 1993).

3.2 Hydrogen peroxide oxidation (Owens, *et al.*, 1989)

The general procedure for the synthesis of the sulfoxides of both prochlorperazine salts was: the salt was accurately weighed into a 100 mL Erlenmeyer flask and hydrogen peroxide (5 mL of 100%) added with stirring until the reaction had gone to completion. The reaction was monitored by high performance liquid chromatography (HPLC). The resulting solution was then made alkaline using a 25% sodium hydroxide solution (pH 10) to release the sulfoxide free-base, which was extracted with three 40 mL portions of chloroform. The combined chloroform solutions were washed twice with water (2 x 40 mL), and separated. After removal

of the chloroform by evaporation, the resulting sticky residue was recrystallised twice from ethyl acetate.

3.3 Analytical methods

3.3.1 Spectroscopic studies

The IR spectra of prochlorperazine mesylate and edisylate and their respective sulfoxides were recorded on a Perkin-Elmer FTIR spectrophotometer, Spectrum 2000, using chloroform ($CHCl_3$) on sodium chloride (NaCl) discs. The results obtained were compared with the spectra shown in the BP (1993).

The ultraviolet (UV) spectra for all four compounds were obtained in water on a GBC UV/Vis Model 916. The BP (1993) values were recorded in absolute ethanol with 13.5 *M* ammonia.

The ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectra were obtained at 400.14 MHz using a Bruker NMR spectrometer. The probe temperature was regulated at 303 ± 0.1 K and the prochlorperazine mesylate and edisylate spectra were recorded in D_2O with the corresponding sulfoxides recorded in $CHCl_3$. Spectra were compared with those recorded by Drummond (1997) under very similar conditions.

Mass spectra (MS) were obtained on a Finnigan GCQ low resolution mass spectrometer, using the electron ionisation (EI) mode, while Drummond (1997) reported the mass spectrum of prochlorperazine maleate obtained by direct insertion into a Hewlett Packard 5890 low resolution mass spectrometer.

3.3.2 Thermal behaviour

The DSC curves for prochlorperazine (mesylate and edisylate) and the corresponding sulfoxides were obtained on a Perkin-Elmer DSC 7 differential scanning calorimeter at a heating rate of 10°C min^{-1} from 50°C to 350°C under nitrogen. No comparative literature was available.

The melting points of the four compounds were obtained using a Gallenkamp capillary tube melting point apparatus. The Merck Index quotes melting points of 242°C and 295°C for prochlorperazine mesylate and edisylate, respectively.

3.3.3 High performance liquid chromatography

Equipment

A modular HPLC system consisting of an Iso Chrom LC (SP 8700) constant-flow pump (Spectra-Physics), with a Spectra Physics LC variable wavelength UV detector, a Rikadenki chart recorder, and a Rheodyne fixed loop 20 µL injector (Model 7125) was used.

Chromatographic parameters

Column:	µ-Bondapak, C18 (3.9 x 300 mm i.d.) 10 µm, 125 Å
Mobile phase:	Methanol: water, (60:40 v/v), pH 2.5 (adjusted with orthophosphoric acid)
Flow rate:	1.0 mL per minute
Detector wavelength:	254 nm
Injection volume:	20 µL
Column temperature:	Ambient
Sensitivity:	10 mV
Range:	0.5

Standard preparation

20 mg of each phenothiazine sulfoxide was accurately weighed and transferred to a 250 mL volumetric flask and made up to volume with solvent (mobile phase), to prepare a 8 x 10^{-2} mg mL^{-1} standard solution.

3.3.4 Thin layer chromatography

Thin layer chromatography (TLC) of both prochlorperazine salts and their respective sulfoxides, was done on pre-coated silica gel $60F_{254}$ plates with aluminium support (E. Merck, Darmstadt, Federal Republic Germany). The plates were developed

using 9:1 $CHCl_3$: MeOH as a solvent, air-dried and examined under short-wave ultraviolet light. Prochlorperazine mesylate and edisylate and their respective sulfoxides were dissolved in methanol. Plates were also developed using a 9:1 acetonitrile : 13.5 *M* ammonia solvent system in order to compare the R_f values of the sulfoxides synthesised with those in the literature.

3.4 Results

3.4.1. Physical properties

Some of the physical properties of prochlorperazine mesylate and edisylate are given in Tables 3.1 and 3.2.

Table 3.1

Characterisation of prochlorperazine mesylate and edisylate and their corresponding sulfoxides

			Melting Points	
Compound	**Molecular Mass***	**Molecular Formula***	**Literature[$,#]**	**Experimental**
Prochlorperazine mesylate	556.1	$C_{20}H_{24}ClN_3S,2CH_4SO_3$	242	240 - 243
Prochlorperazine edisylate	564.15	$C_{20}H_{24}ClN_3S,C_2H_6O_6S_2$	295	290 - 292
Mesylate sulfoxide	389.93	$C_{20}H_{24}ClN_3OS$	160 - 162	161 - 162.5
Edisylate sulfoxide	389.93	$C_{20}H_{24}ClN_3OS$	160 - 162	160 - 162

[$] Martindale, The Extra Pharmacopoeia, [#] Pharmaceutical Codex

Table 3.2

Solubilities of prochlorperazine mesylate and edisylate and their respective sulfoxides[$]

Compound	**Water**	**Alcohol**	**Chloroform**	**Ether**
Mesylate	1 in >0.5	1 in 40	Slightly Soluble	Practically Insoluble
Edisylate	1 in 2	1 in 1500	Practically Insoluble	Practically Insoluble
Edisylate sulfoxide*	Slightly Soluble*	-*	Very Soluble*	-*
Mesylate sulfoxide*	Slightly Soluble*	-*	Very Soluble*	-*

-* no literature available

3.4.2 Infrared spectroscopy

The 2,10-di-substituted phenothiazines have a characteristic pattern in their IR spectra (Warren et al., 1966). There are four strong bands in the 1000 - 700 cm^{-1} region including: 915 - 928 cm^{-1}, 840 - 870 cm^{-1}, 785 - 800 cm^{-1} and 730 - 755 cm^{-1}. These bands may be assigned to the phenothiazine ring system and the substituent in the 2-position. A C-Cl band is usually a strong broad signal in the range of 630 - 800 cm^{-1}. Bellamy *et al.* (1958) assigned 770 - 735 cm^{-1} to the out-of-plane bending vibrations of the four adjacent hydrogens in the aromatic ring. Bands found in the 1590 and 1560 cm^{-1} region may also be assigned to the aromatic system. In phenothiazine amine salts, a strong broad band centered between 2300 and 2500 cm^{-1} is present which is characteristic of the R_3NH^+ ion combined with X^-. The relatively small negative ion X^- can approach R_3NH^+ from only one direction and forms the ion pair $R_3NH^+X^-$ with the hydrogen atom strongly bound to the negative ion. Water of hydration tends to lower the intensity and raise the frequency of absorption (Warren *et al.*, 1966).

The spectra of prochlorperazine mesylate and edisylate and their respective sulfoxides are shown in Figures 3.1 and 3.2.

The presence or absence of sulfoxides may be determined from IR spectra by the presence or absence of the S=O bands. The S=O infrared absorption for alkyl and aryl sulfoxides has been reported to fall in the 1070 - 1035 cm^{-1}, range making the sulfoxide easily recognisable (Owens *et al.*, 1989). Table 3.3 contains the infrared data for the two prochlorperazine salts and their respective sulfoxides. The spectra indicate the presence of S=O groups.

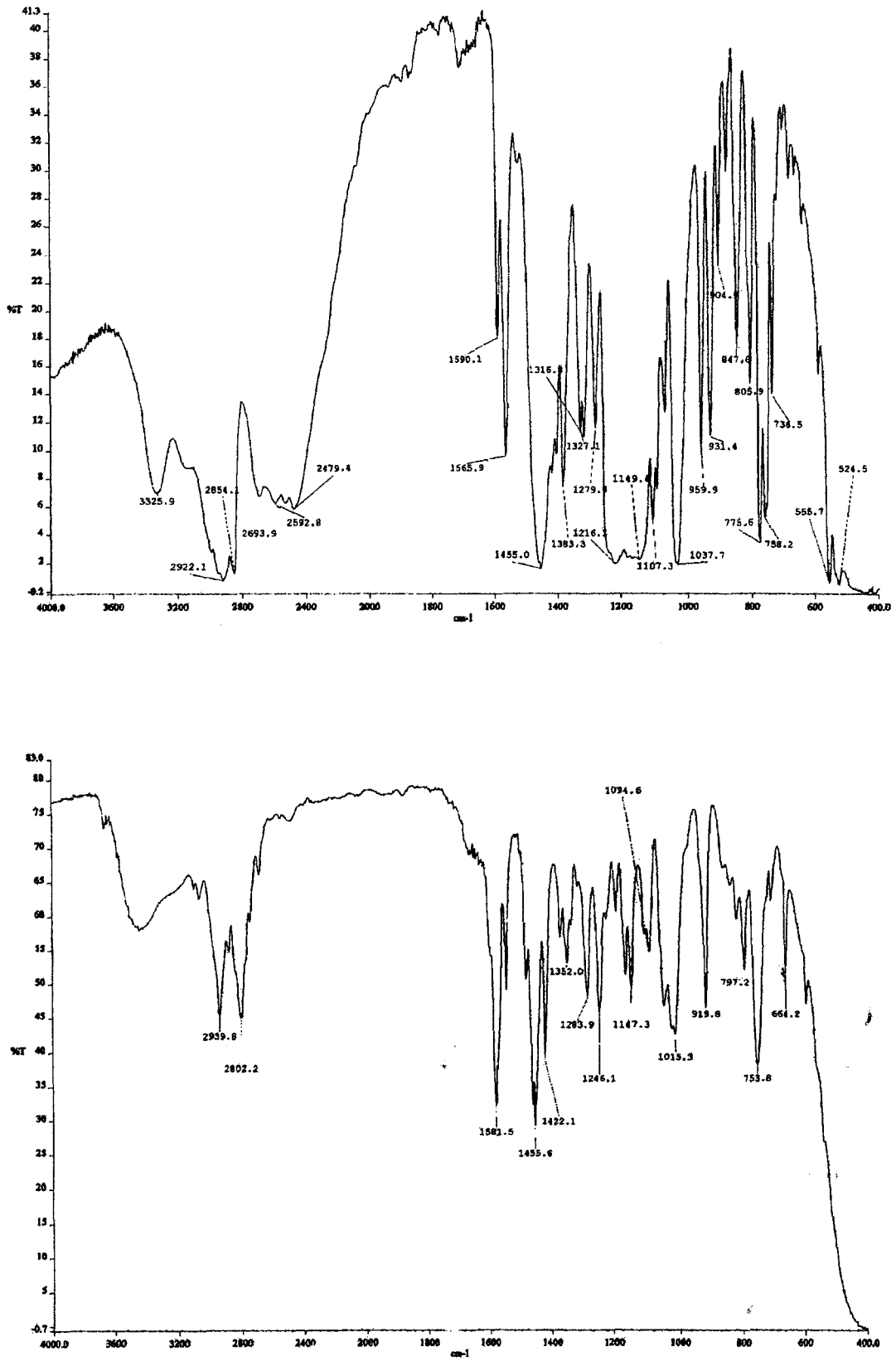


Fig. 3.1 Infrared spectra of prochlorperazine mesylate (top) and its sulfoxide (bottom)

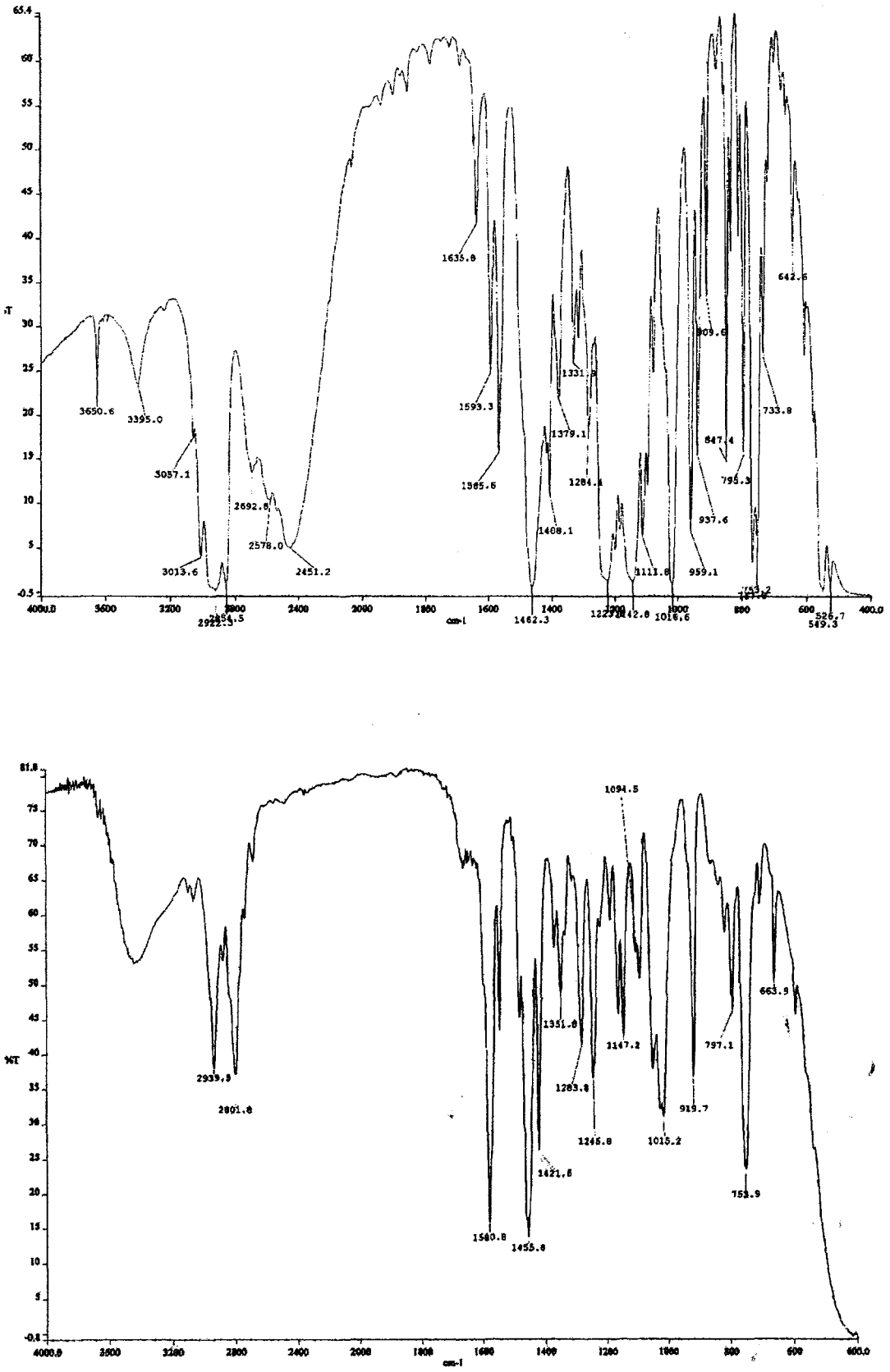


Fig. 3.2 Infrared spectra of prochlorperazine edisylate (top) and its sulfoxide (bottom)

Table 3.3

Infrared spectral assignment of bands for prochlorperazine mesylate and edisylate and their respective sulfoxides

Assignment	Prochlorperazine mesylate cm^{-1}	Prochlorperazine edisylate cm^{-1}	Prochlorperazine mesylate sulfoxide cm^{-1}	Prochlorperazine edisylate sulfoxide cm^{-1}
NH^+	2479	2451	-*	-*
C=C, aromatic	1455, 1565, 1590	1462, 1565, 1593	1461, 1546, 1579	1461.4, 1546, 1579
Cl	758.2	753.8	753.8	753.9
S=O	-*	-*	1029# (1035 - 1070+)	1030# (1035 - 1070+)

*not applicable

#experimental value (+Owens *et al.*, 1989)

3.4.3 UV spectrophotometry

The ultraviolet spectra of phenothiazines are characteristic both in wavelength and intensity (Figures 3.3 - 3.6). The first and most intense absorption is in the range 250 - 265 nm and the second in the range 300 - 325 nm, the exact location being dependent on the nature of the group in position 2. Halogen substituents, such as chloro- or trifluoromethyl, exert a slight influence, in the form of small bathochromic shifts of 2 - 4 nm, on the more intense peak, with the trifluoro methyl substituent exerting a stronger shift than the chloro substituent. An alkyl side-chain containing an amine group causes slight shifts in the peak locations. The amount of the shift is related to the length of the side chain, i.e. the proximity of the amine group to the phenothiazine nucleus. The amine group can exert an influence even when located at the end of a four-carbon chain (Warren *et al.*, 1966). Use of the hydrogen peroxide method to synthesise the sulfoxides may result in continued oxidation with the formation of the sulfones (Wallace, 1971). Although these compounds have structural similarities, their ultraviolet absorption spectra are different and characteristic for the specific functional groups. Table 3.4 shows the differences in the ultraviolet absorption data for a phenothiazine derivative, chlorpromazine hydrochloride, and its sulfoxide as well as prochlorperazine dihydrochloride (Post *et al.*, 1980). Davidson (1976) confirmed the formation of sulfoxides by the presence of a shoulder around 345 nm in the absorption spectra of aqueous solutions of the respective phenothiazines. The UV spectra of the parent drugs, prochlorperazine mesylate and edisylate, and their respective sulfoxides are given in Figures 3.3 - 3.6.

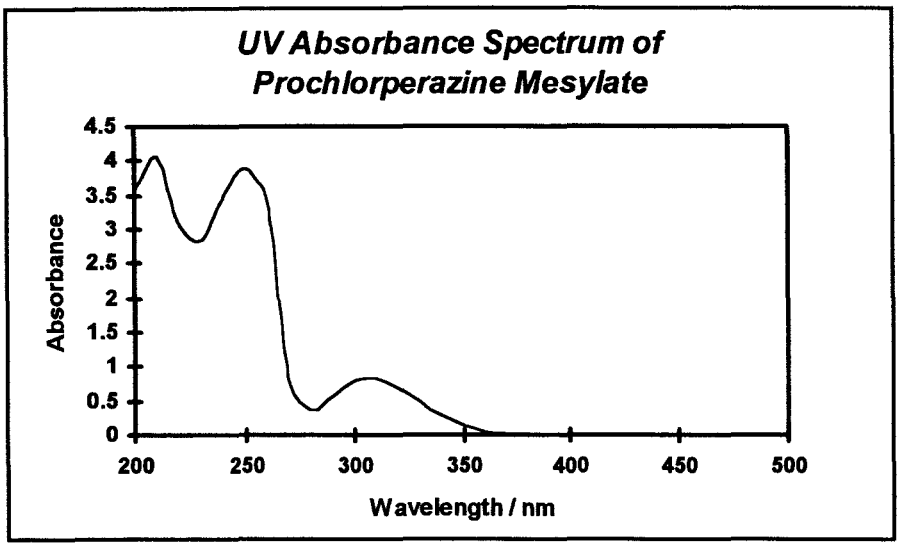


Fig. 3.3 UV spectrum of prochlorperazine mesylate

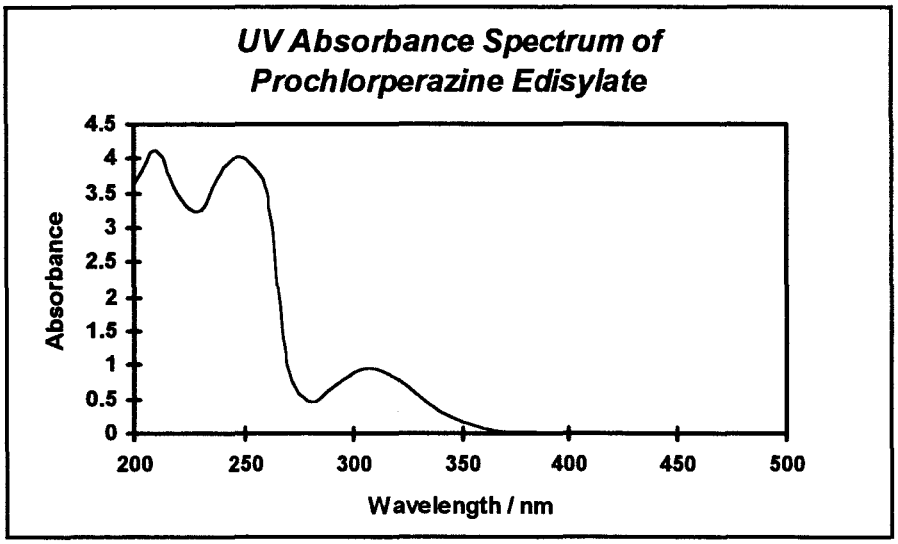


Fig. 3.4 UV spectrum of prochlorperazine edisylate

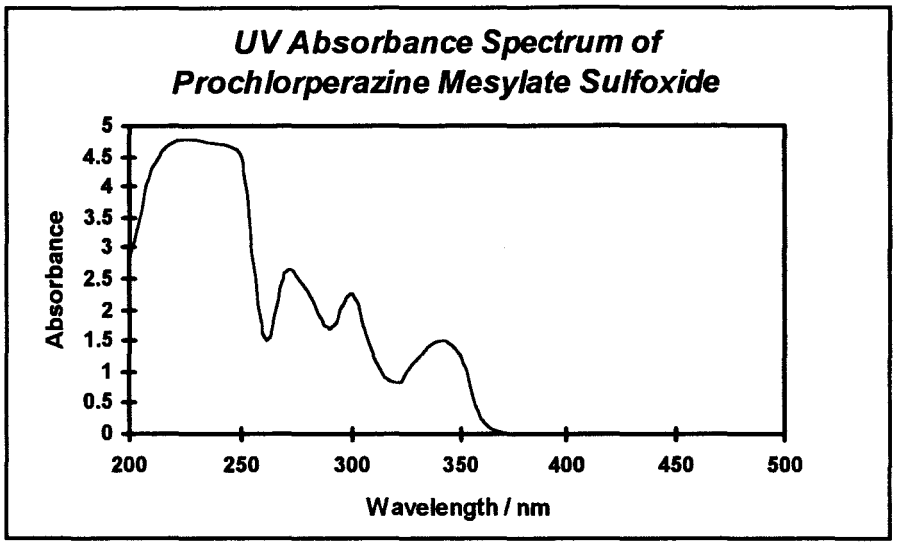


Fig. 3.5 UV spectrum of prochlorperazine mesylate sulfoxide

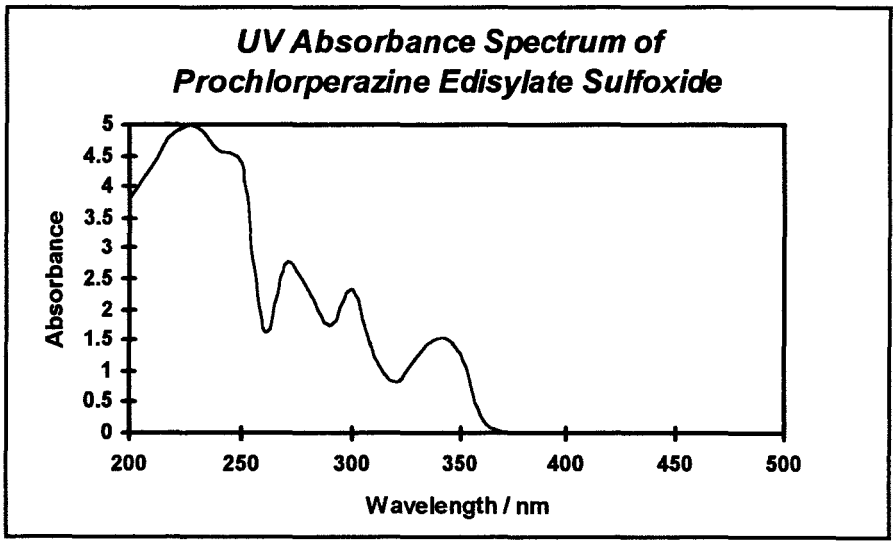


Fig. 3.6 UV spectrum of prochlorperazine edisylate sulfoxide

Table 3.4

Ultraviolet spectral data for prochlorperazine mesylate and edisylate and their respective sulfoxides

Prochlorperazine mesylate / nm (Log ε)	**Prochlorperazine edisylate / nm (Log ε)**	**Prochlorperazine mesylate sulfoxide / nm (Log ε)**	**Prochlorperazine edisylate sulfoxide / nm (Log ε)**	**Prochlorperazine dihydrochloride[Warren *et al.*, 1966] / nm (Log ε)**
307 (3.580)	307 (3.625)	344 (3.699)	343 (3.875)	256 (4.54)
247 (4.247)	246 (4.256)	301 (3.876)	301 (4.052)	309 (3.62)
212 (4.260)	213 (4.265)	275 (4.020)	275 (4.184)	280 (3.11)
208 (4.261)	209 (4.261)	235 (4.225)	237 (4.375)	

3.4.4 Mass spectrometry

Fragmentation patterns obtained from the mass spectrometry data, Table 3.5, for mesylate and edisylate are identical, as are the patterns for the corresponding sulfoxides. The data obtained for the sulfoxides are significantly different to those of the prochlorperazine salts. The MS data for the salts show a loss of 101 daltons which corresponds to the loss of the piperazine ring and the methyl group attached to it. The common product ion *m/z* 141 corresponds to the entire piperazine side chain (i.e. $C_8H_{17}N_2)^+$. Loss of 28 daltons, to give the product ion at 113, corresponds to the partial cleavage of an ethylene group on the piperazine ring to give $(C_6H_{13}N_2)^+$. A loss of a further 43 daltons results in an allyl derivative, $(C_4H_8N)^+$. The MS data for the sulfoxides of the prochlorperazine salts show quite a different fragmentation pattern to that of the parent drugs. Common product ions are found at *m/z* 319, 246, and 139 for both sulfoxides. Partial cleavage of the piperazine ring in the side chain results in the formation of the product ion at *m/z* 319 for the sulfoxides of both salts. Subsequent loss of 73 daltons $(C_3H_7NO)^+$ gives rise to a common product ion at *m/z* 246. The common product ion at *m/z* 139 is a derivative of the side chain $(C_8H_{15}N_2)^+$. The loss of 14 daltons, to form the product ion at *m/z* 232, is due to the cleavage of the methyl group from the ring nitrogen atom.

The mass spectral data obtained for prochlorperazine mesylate and edisylate and their sulfoxides are shown below in Table 3.5.

Table 3.5

Mass spectral data for prochlorperazine mesylate and edisylate and their respective sulfoxides

Prochlorperazine mesylate			Prochlorperazine edisylate		
Ion	***m/z***	**% Relative Abundance**	**Ion**	***m/z***	**% Relative Abundance**
M^+	373	19.99	M^+	373	21.78
$(M-C_5H_{13}N_2)^+$	272	7.36	$(M-C_5H_{13}N_2)^+$	272	6.98
$(C_8H_{17}N_2)^+$	141	34.75	$(C_8H_{17}N_2)^+$	141	35.47
$(C_6H_{13}N_2)^+$	113	73.79	$(C_6H_{13}N_2)^+$	113	74.39
$(C_4H_8N)^+$	70	100.0	$(C_4H_8N)^+$	70	100.0

Prochlorperazine mesylate sulfoxide			Prochlorperazine edisylate sulfoxide		
Ion	***m/z***	**% Relative Abundance**	**Ion**	***m/z***	**% Relative Abundance**
M^+	390	56.78	M^+	390	22.90
$(M-C_3H_4NO)^+$	319	100.0	$(M-C_3H_4NO)^+$	319	100.0
$(M-C_7H_{15}N_2O)^+$	246	72.90	$(M-C_7H_{15}N_2O)^+$	247	39.34
$(M-C_8H_{17}N_2O)^+$	232	42.71	$(M-C_8H_{17}N_2O)^+$	232	36.60
$(C_8H_{15}N_2)^+$	139	7.16	$(C_8H_{15}N_2)^+$	139	6.77

3.4.5 Nuclear magnetic resonance (NMR)

The assignments of protons and carbons for prochlorperazine are shown in Figure 3.7 and the ^{1}H and ^{13}C-NMR data are given in Tables 3.6 and 3.7.

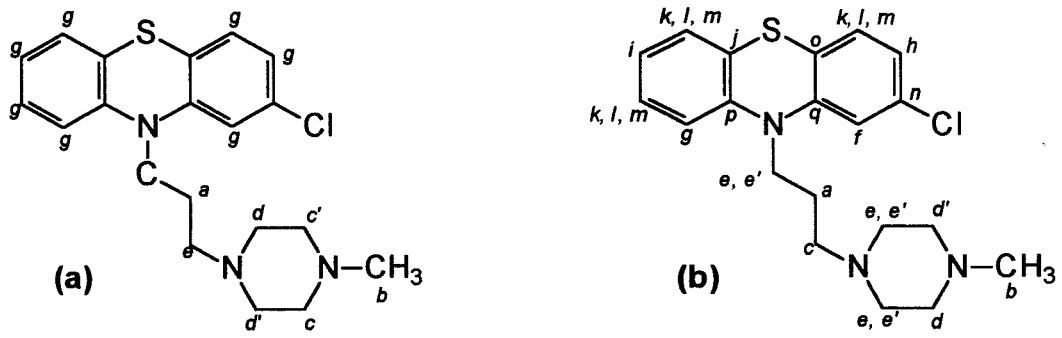


Fig. 3.7 Assignment of protons (a) and carbons (b) for prochlorperazine.

Table 3.6

^{1}H-NMR data for prochlorperazine mesylate and edisylate and their corresponding sulfoxides (* Post *et al.*, 1980)

Protons	Assignment	Mesylate (ppm)	Mesylate sulfoxide (ppm)
a	Multiplet	2.608	2.022
b	Singlet / Triplet	2.8983	2.1459
c, c'			
d, d'	Broad Multiplet	3.3582 - 3.571	2.4575 - 2.4867
e			
f	Triplet	4.0622	4.3143
g	Broad Multiplet	6.786 - 7.550	7.191 - 7.913

Protons	Assignment	Edisylate (ppm)	Edisylate sulfoxide (ppm)	Trifluoperazine hydrochloride* (ppm)
a	Multiplet	2.2424	2.0283	1.94
b	Singlet / Triplet	3.0513	2.1538	2.25
c, c'				
d, d'	Broad multiplet	3.5438 - 3.6282	2.4658 - 2.4994	2.3 - 2.6
e				
f	Triplet	4.0455	4.3245	3.96
g	Broad multiplet	7.0354 - 7.4294	7.2007 - 7.9220	6.75 - 7.30

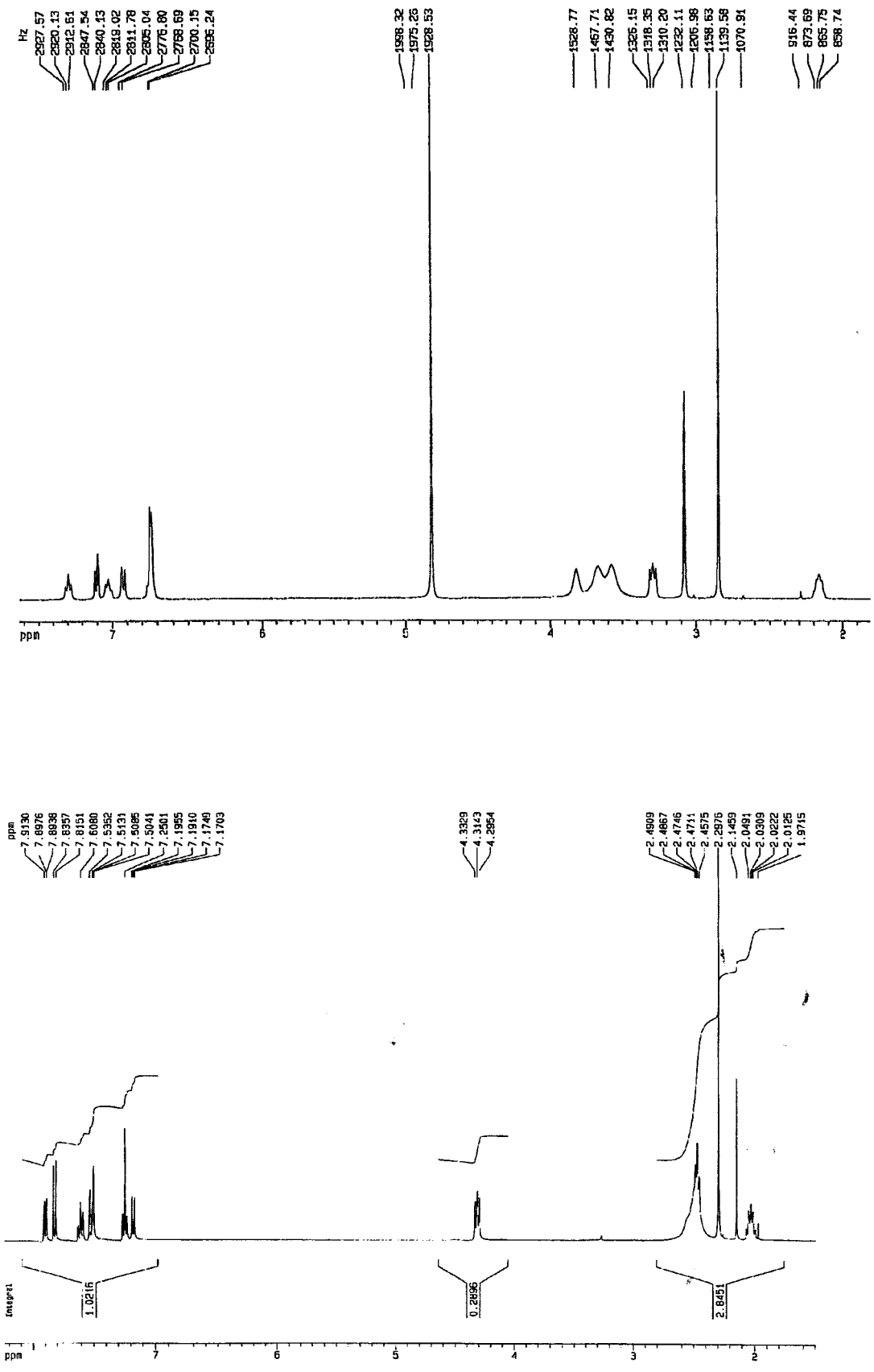


Fig. 3.8 ^{1}H-NMR spectrum of prochlorperazine mesylate (top) and its corresponding sulfoxide (bottom)

The effect of the introduction of oxygen into the system is an increase in the chemical shift of the triplet signal approximately 0.2521 - 0.2790 ppm downfield. The $N(CH_2)_3$ protons shift upfield by about 0.7524 - 0.7632 ppm, while the aromatic protons show additional splitting and shift further downfield. Kreyenbuhl *et al.* (1978) reported the characteristic shift in the triplet signal (f) due to the $N\text{-}CH_2$- protons (nitrogen on position 10) from 3.9 to 4.4 ppm. This is expected because the presence of a sulfoxide would lead to an increased electronegativity on the sulfur, causing the shielding of these protons to be decreased. For comparative purposes, the ^1H NMR data for trifluoperazine hydrochloride are given in Table 3.6, because trifluoperazine has a CF_3 substituent on position 2 in place of the Cl group of prochlorperazine.

Table 3.7

^{13}C-NMR data for prochlorperazine mesylate and its sulfoxide

Carbon	Mesylate Experimental (ppm)	Sulfoxide Experimental (ppm)
a	21.866	24.902
b	39.419	30.270
c	44.175	30.710
d, d'	49.512	46.049
e, e'	51.025	46.252
f	55.268	53.784
g	116.85	55.370
h	117.432	55.965
I	123.525	
j	124.234	
k	124.439	
l	125.513	
m	128.507	
n	128.839	
o	133.768	
p	144.342	
q	146.503	

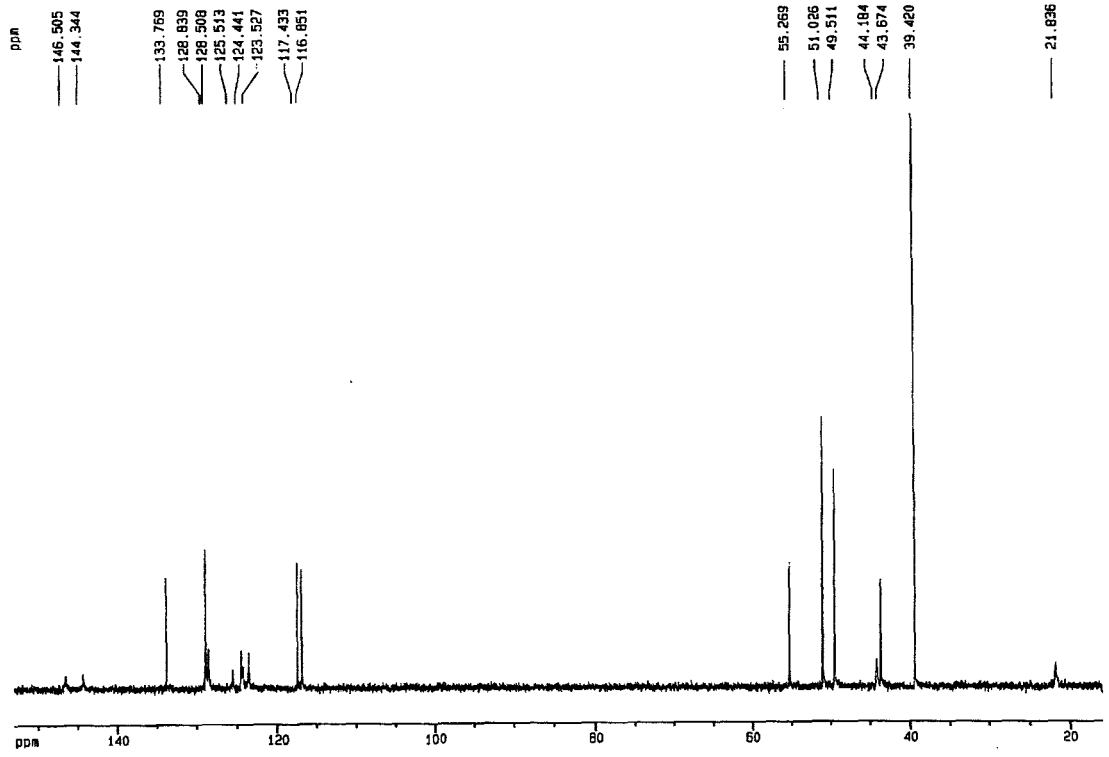


Fig. 3.9 ^{13}C-NMR spectrum of prochlorperazine mesylate

3.4.6 Differential scanning calorimetry

The DSC curves of both prochlorperazine salts and their sulfoxides are given in Figures 3.10 and 3.11. The melting endotherm for the mesylate is strong and sharp at 242°C while the corresponding sulfoxide melting endotherm is slightly broader at 162°C. Melting of the edisylate appears to start at 291°C with rapid decomposition at about 300°C. The endotherm for the edisylate sulfoxide is slightly broader and melting starts at 161°C with no decomposition. These results are in agreement with those given in the literature as shown in Table 3.1.

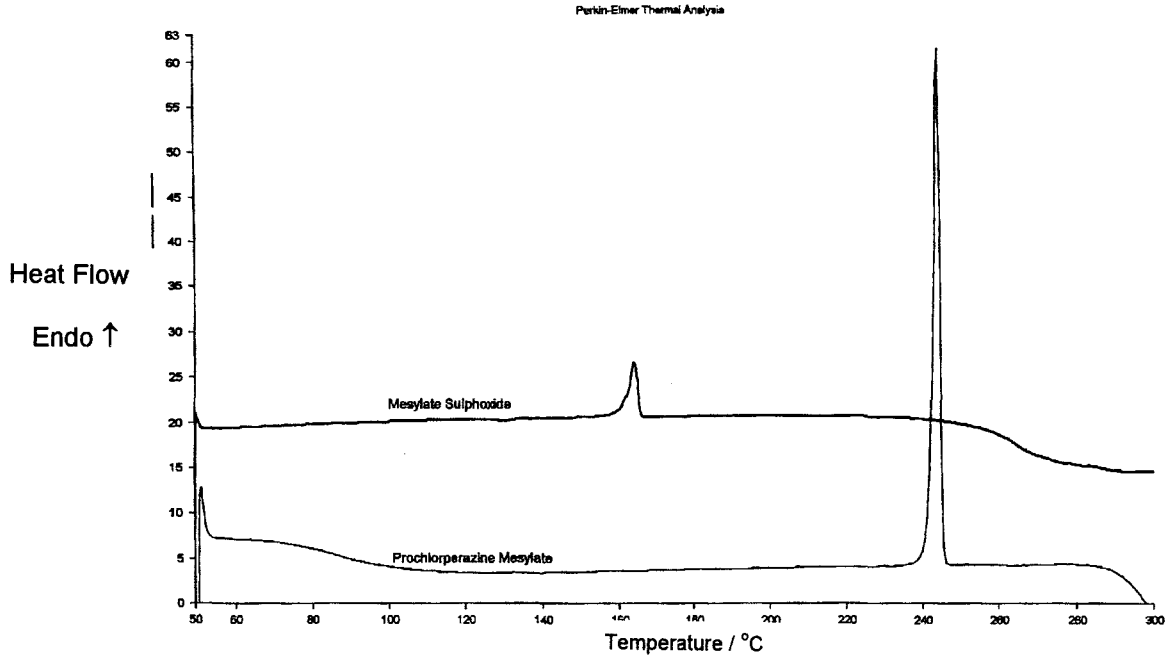


Fig. 3.10 DSC curves for prochlorperazine mesylate and its sulfoxide

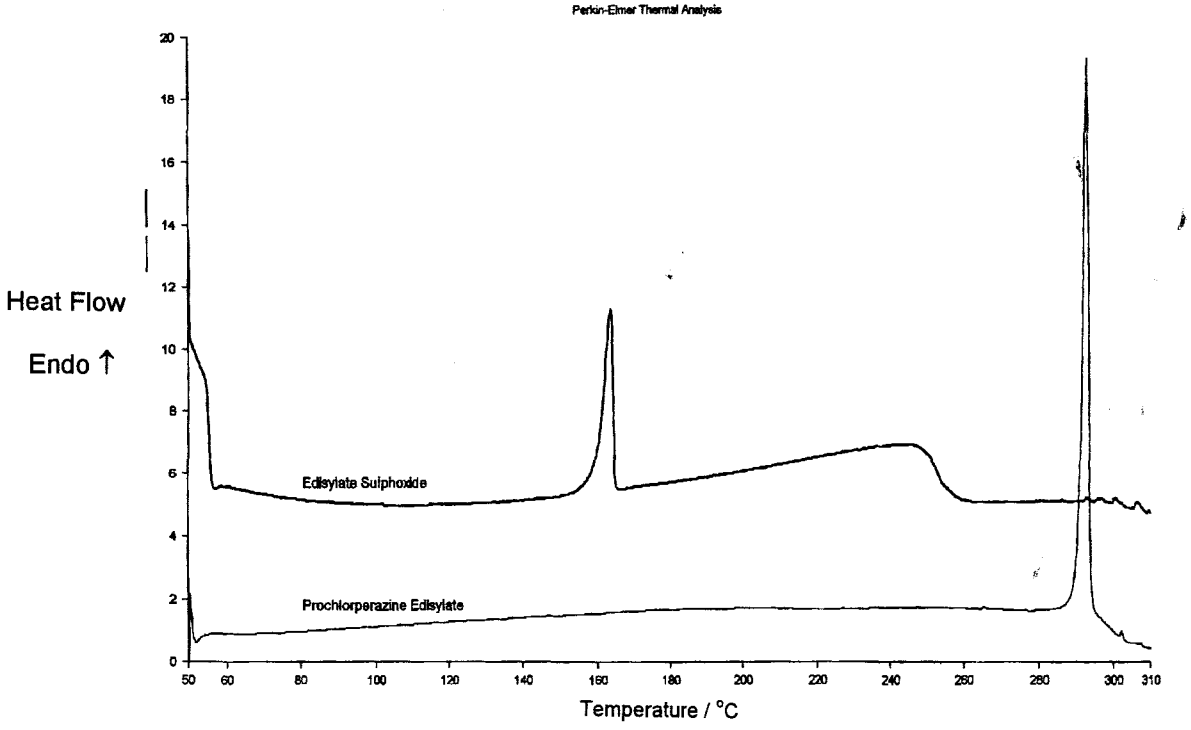


Fig. 3.11 DSC curves for prochlorperazine edisylate and its sulfoxide

3.4.7 High performance liquid chromatography (HPLC)

The presence of a sharp major peak in each of the HPLC chromatograms confirmed the oxidation of the parent drugs to a single compound, the retention times of which are shown in Table 3.8. Due to the polar nature of the sulfoxides, as a result of the addition of the oxygen atom, the retention times were expected to be significantly shorter than those of the prochlorperazine.

Table 3.8

HPLC retention times of the prochlorperazine salts and their sulfoxides

Prochlorperazine salt	**R_t of parent compound / min**	**R_t of sulfoxide / min**
Mesylate	9.9	4.4
Edisylate	9.8	4.3

3.4.8 Thin layer chromatography (TLC)

The R_f values, using the $CHCl_3$: MeOH solvent system for the synthesised sulfoxides are compared with those of the salts in Table 3.9.

Table 3.9

Comparative R_f values for the prochlorperazine salts and their sulfoxides

Prochlorperazine salt	**R_f Value (ave.) 9:1 $CHCl_3$:MeOH**	**R_f Value (ave.) 9:1 CH_3CN:NH_4**	**R_f Value (ave.) (Owens et al. 1989)**
Mesylate	0.43	0.78	-
Edisylate	0.3	0.8	-
Mesylate sulfoxide	0.19	0.55	0.5
Edisylate sulfoxide	0.27	0.55	0.5

In addition to the similarities in R_f values between the synthesised sulfoxides, a single compound on the TLC plates for the two synthesised sulfoxides verified their purity. The TLC plates subjected to the acetonitrile mobile phase indicated that small amounts of a byproduct were formed when the oxidation procedure was carried out. The $CHCl_3$:MeOH solvent system did not resolve the compounds as clearly as the acetonitrile system, where good separation between the sulfoxides and the parent compound (as indicated by the R_f values) was obtained. This mobile

phase could thus be used to identify and determine the purity of the sulfoxides as well as prochlorperazine. The sulfoxides showed an expected lower R_f value (0.55) than prochlorperazine ($R_f \approx 0.8$), indicating the higher polarity of the sulfoxide compared with prochlorperazine.

3.5 Conclusions

The two sulfoxides of edisylate and mesylate salts were synthesised in low percentage yields ranging between 10 - 15 % and their identity and purity confirmed by HPLC, TLC, mp, DSC, MS, UV, IR and NMR, and compared to literature values where available. Chapter 2 deals with the confirmation of the identity, in the form of appearance, solubility characteristics, literature and experimental melting points, NMR, MS and UV of prochlorperazine mesylate and edisylate as well as their sulfoxides.

The results obtained using various techniques described above confirmed the identity and purity of both the prochlorperazine salts and their corresponding sulfoxides.

CHAPTER 4. METHOD DEVELOPMENT AND VALIDATION

4.1 Introduction

The analysis of drug substances and finished products within a pharmaceutical industry is carried out to satisfy both the manufacturing companies and regulatory bodies, equally, about the integrity, quality and stability of the medicine to be administered to the patient. Several reports are available containing guidelines for the validation of analytical methods, (Hokanson, 1994; Boehlert, 1984; ICH Validation Guidelines, 1996) with some reports describing practical minimum requirements for each test in detail (Carr and Wahlich, 1990).

In the past, analytical methods used for monitoring the stability of dosage forms have involved non-specific spectrophotometric or titrimetric procedures for the assay of the active ingredient, together with thin layer chromatography for the estimation of impurities and degradation products. In the last decade, this approach has changed dramatically due to the development of high performance liquid chromatography (HPLC), with HPLC now being the method of choice for the quantitative measurement of the active and degradation products. Advantages of HPLC include the separation and measurement of the desired components, but it is a time-consuming and expensive technique. TLC and gas chromatography (GC), however, offer the advantage of rapid determination of degradation products, provided that the stability of the component of interest does not pose a problem (for GC). Calorimetric, titrimetric, spectrophotometric (UV/Vis), NMR, fluorimetric and polarographic methods may also be used (Boehlert, 1984). The first two procedures are not selective for undegraded compounds and, while UV methods are accurate and convenient for assaying formulations, they are unsuitable for badly degraded formulations due to the presence of other UV absorbing species. If HPLC is to be used, peak purity tests (e.g. photodiode array and mass spectrometry) may be conducted to show that the chromatographic peak of the analyte is not attributable to more than one component.

This chapter discusses the parameters that are considered during the development and validation of the analytical method used, where all the relevant data collected during the validation and the calculations are presented and discussed. The aim of this chapter is to demonstrate that a proposed procedure is suitable for its intended

purpose. The various validation characteristics discussed in separate sections include linearity, range, specificity, accuracy and precision, detection and quantitation limit and ruggedness / robustness. The purity and identity of the reference materials have already been discussed and characterised in Chapter 2. The HPLC method was developed for the separation of both the prochlorperazine salts and their degradants. The separation was performed on a Novapak CN-HP (3.9 x 150 mm i.d.) steel cartridge column with a mobile phase of acetonitrile : 1% aqueous KCl (15 : 85, v/v) with isocratic elution and UV detection. Results indicate that the HPLC method measures the prochlorperazine and its sulfoxide as opposed to a *BP* 1988 spectrometric method which measures only the total of parent compound and its sulfoxide. Thus, based on the needs of this study to quantitate both prochlorperazine salts and to separate them from their degradants, HPLC is considered to be the most effective technique.

4.2 HPLC method development

An important area in method development is the validation of the method for its effectiveness and suitability for the analysis to be undertaken. A number of tests are generally performed, including: linearity, range, specificity, accuracy and precision, detection limit and ruggedness. Method development is only complete when acceptable analytical performance has been demonstrated (Edwardson *et al.*, 1990).

A systematic approach, according to the ICH guidelines (1996) for the development of a method, includes: a) a literature review of the various methods used to analyse a particular drug product; b) the generation of background data (where initial experiments may be carried out to determine the amount of degradation taking place within a certain amount of time, e.g. a week); and c) the development and validation of the method. If analytical methods have been published, this information is usually used as a starting point, saving a great deal of time and effort. The following procedures are the assay methods recommended by the United States Pharmacopoeia (USP, 1995) for the analysis of prochlorperazine edisylate injectables and maleate tablets. The HPLC method which was eventually used for the analysis of the two prochlorperazine salts was based on a method used by Heyes and Robinson (1985), which was developed for the determination of fluphenazine metabolites in urine, because it is a simple method with no ion-pairing

reagents used. A method was developed by Drummond (1997) which involved the use of only orthophosphoric acid apart from the solvents. This method was not used because of the severe tailing encountered.

The assay (USP, 1995) for prochlorperazine edisylate is carried out with a mobile phase of acetonitrile : pH 2.0 sodium phosphate solution (65 : 35) and chlorpromazine HCl as an internal standard. A variable wavelength detector set at 254 nm, with a 0.25 x 25 cm CN column (3 to 10 μm) and a flow rate at 1.5 mL min^{-1} was used. An assay for prochlorperazine maleate tablets includes the following chromatographic parameters: a mobile phase consisting of acetonitrile : methanol : 5 x 10^{-3} M sodium 1-octane sulfonate (40 : 15 : 45) and a trifluoperazine HCl (140 μg mL^{-1}) internal standard. The detector was set at 254 nm, a 0.4 x 25 cm C_{18} column (3 to 10 μm) was used at a flow rate of 2 mL min^{-1}.

Most analytical chromatography is carried out on stationary phases which have been surface modified by the bonding or coating of a stable chemical phase. Of these the most common is the C_{18} bonded phase and although the most common and widely applicable, this may not always be the most suitable for a specific application. Reverse-phase column packing material such as C_{18} and C_8 columns deliver good performance in terms of separation, resolution, efficiency, column stability and repeatability (Kirkland, 1996). The majority of HPLC assay methods for the analysis of the piperazine subclass of phenothiazines have used reverse-phase C_{18} analytical columns (USP, 1995; Heyes and Robinson, 1985; Abdel-Moety *et al.*, 1996; Chagonda and Millership, 1989). Assay development was therefore initiated using a reverse-phase C_{18} column, however, this method was discarded due to severe tailing of the drug peak. A cyano hydroxy propyl (CN HP) column, for the analysis of prochlorperazine mesylate and edisylate, led to good peak symmetry, decreased retention times (2.5 and 5 minutes for sulfoxide and prochlorperazine, respectively) and limited tailing. CN HP columns are normally used for highly polar drugs because the more polar compounds will be retained on the column for longer. CN HP columns may be used in both normal- and reversed-phase HPLC. A CN column is generally applied where selectivity for double bonds is necessary.

4.2.1 Reference standards

Although the USP recommends that chlorpromazine be used as an internal standard, its stability, however, precluded its use. Because the sulfoxide is one of the main metabolites and is a major decomposition intermediate for all phenothiazines, due to their susceptibility to oxidation, it was decided that synthesised samples of the two sulfoxides would be used as standards in order to identify the sulfoxide presence during degradation studies.

The UV spectra of the two sulfoxides in water were recorded and have been shown in Chapter 2 (Figures 2.16 and 2.17) together with spectra of their parent compounds. The sulfoxides absorbed in the wavelength region below 375 nm with maxima occurring at 344, 301, 275, and 244 nm. Both the sulfoxides could thus be used in the proposed assay using a detection wavelength of 260 nm.

The retention features of each sample of prochlorperazine mesylate and edisylate and their respective sulfoxides were estimated on a Waters Novapak® CN HP column. Solutions of sulfoxide were spiked with edisylate or mesylate, injected and monitored at 260 nm. Because of the increased polarity of the sulfoxide compared to the parent and the resulting shorter retention time, the peaks corresponding to prochlorperazine and the sulfoxide are clearly resolved. The sulfoxide eluted from the column after approximately 2.5 minutes, whereas the less polar prochlorperazine salts eluted after 5.15 ± 0.05 minutes.

4.2.2 HPLC apparatus

The HPLC system consisted of a Spectraseries P100 isocratic solvent pump (Spectra Physics, U.S.A) , a M8125 20 µL fixed-loop Rheodyne injector (Rheodyne Inc., CA, U.S.A.), a 100 µL HPLC syringe (Hamilton Company, Reno, U.S.A), a Spectraseries UV100 variable wavelength UV detector (Spectra Physics, U.S.A.) and a Rikadenki flat-bed chart recorder (Kogyo Co. Ltd, Tokyo, Japan).

4.2.3 Reagents

Prochlorperazine edisylate and mesylate were kindly donated by Intramed (Port Elizabeth, South Africa) and Rhone-Poulenc Rorer (Pty.) Limited, respectively. All

chemicals were used as received and were of analytical grade. Potassium chloride and sodium hydroxide were obtained from UNILAB, SaarChem (Pty.) Limited. Orthophosphoric acid (Analar) was purchased from BDH Chemicals Limited, and methanol (HPLC grade) from Romil Ltd. (Cambridge, England). Water for chromatography was obtained using a Milli-Q Plus® water purification system (Millipore, Bedford, MA, U.S.A.), consisting of a super-C® cartridge, two Ion-X® exchange cartridges and an Organex-Q® cartridge.

4.2.4 Mobile phase

The decision to use acetonitrile : 1% aqueous KCl as a mobile phase was initially based on a HPLC method developed by Heyes and Robinson (1985) (mentioned earlier) for the separation of potential fluphenazine metabolites. A series of tests (which forms part of the method ruggedness) was performed to determine the effect of modifying the organic : aqueous ratio, KCl percentage, mobile phase pH, and flow rate.

4.2.5 Mobile phase preparation

1 L of a 1% aqueous solution of potassium chloride (KCl) was prepared. The required volumes of acetonitrile and buffer (85:15%) were mixed, the pH of the mobile phase adjusted to 2.5 with orthophosphoric acid and the solvent degassed using vacuum filtration through a 0.45 µm HVLP Millipore filter (Bedford, MA, U.S.A.). Further degassing was done using ultrasonication (Sonicor Instrument Corporation, Copiague, New Zealand) prior to use.

a) Effect of modifying the organic : aqueous ratio

To determine the effect of decreasing the polarity of the solvent system, the acetonitrile content was decreased from 15% to 5%. This increased the retention time of prochlorperazine from 5 minutes to approximately 11 minutes and that of the sulfoxide from 2.5 minutes to 5 minutes. This was attributed to the hydrophobic interactions between prochlorperazine and the sulfoxide and the CN HP stationary phase. An expected increase in tailing and a corresponding decrease in peak symmetry were also observed. Increasing the acetonitrile content from 15% to 25% resulted in a decrease in retention time from 5 minutes to 2.2 minutes for

prochlorperazine and from 2.5 minutes to 1.6 minutes for the sulfoxide. A change in peak shape and symmetry, with no tailing, was observed.

b) pH adjustment and the effect of KCl

When the CN HP packing material is used as a reverse-phase column, and KCl was omitted from the mobile phase, the retention times of prochlorperazine and sulfoxide increased to 26 and 11 minutes, respectively. Changes in pH were observed to affect the retention times and resolution of both prochlorperazine and the sulfoxide. The omission of pH adjustment from the procedure resulted in an increase in retention times of prochlorperazine and the sulfoxide to approximately 19.5 minutes and 8 minutes, respectively. This is to be expected because the acid protonates the nitrogen atoms resulting in a more polar molecule. The compound is therefore retained on the column for a shorter time resulting in improved peak resolution. Without the acid, the nitrogen atoms are not protonated, polarity is decreased and the compounds are retained for longer on the column with a loss of peak resolution. The adjustment of the pH from 2.5 to 4.08 resulted in an increase in the retention times of the sulfoxide and the prochlorperazine from 2.5 and 5.1 minutes to 4 and 8.9 minutes, respectively. Subsequent broadening of the peaks, decreased resolution and increased tailing were observed. Further adjustment in pH to 8.5 resulted in severe tailing and loss of resolution, while the retention times increased for the sulfoxide and prochlorperazine to 5 and 38 minutes, respectively.

c) Changing the flow rate

Changing the flow rate from 1 to 0.5 mL min^{-1} resulted in a decrease in resolution of the peaks and increased tailing. The results of changing the flow rate to 1.5 mL min^{-1} could not be obtained because the system was limited by pressure constraints.

d) Final chromatographic parameters

The mobile phase employed for the stability studies was thus acetonitrile : 1% aqueous KCl (15 : 85 % v/v) at a pH of 2.5 (orthophosphoric acid) and a flow rate of 1 mL min^{-1}. A typical chromatogram for prochlorperazine and its sulfoxide is shown in Figure 4.1.

Chromatographic conditions

Mobile phase:	Acetonitrile : 1% aqueous KCl pH 2.5 (15 : 85 % v/v)
Flow rate:	1 mL min^{-1}
Column:	Novapak® CN HP 4 μm 60Å steel cartridge column (3.9 x150 mm)
Column temperature:	ambient
Column back-pressure:	1780 p.s.i.
Detector wavelength:	260 nm
Injection volume:	20 μL
Chart speed:	30 mm min^{-1}
Retention times:	prochlorperazine, approximately 5.0 minutes the sulfoxide, approximately 2.5 minutes
Peak Responses:	Tailing Factor: < 1.5, Resolution > 3.292, RSD < 2.0%

Definitions of Tailing Factors, Resolution and Relative Standard Deviation (RSD) are given later in the chapter.

e) Variation of the detector wavelength on performance

The variable wavelength detector was set at 260 nm, instead of the usual 254 nm, in order to 'fit' the peak heights (where the drug is 100% intact) of the drug on the chart recorder paper without altering the sensitivity of the detector (so that both the solid state and solution studies could be carried out at the same sensitivity).

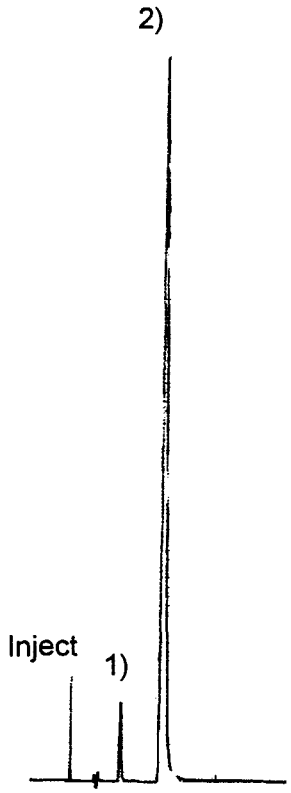


Fig. 4.1 Typical HPLC chromatogram of the reference standard, the sulfoxide at 2.5 minutes (1) and prochlorperazine at 5 minutes (2)

4.3 Method Validation

A method must be tested for its effectiveness and should be appropriate for that particular analysis. A number of general tests are usually performed: linearity, specificity, accuracy and precision, limit of detection and quantitation, and ruggedness / robustness. Thus, the method development is complete only when the method has been tested and shown to demonstrate acceptable analytical performance (Edwardson *et al.*, 1990). Analytical procedures refer to the way in which the analysis is performed where the steps necessary to perform each analytical test are often described in detail (Carstensen, 1995).

4.3.1 Preparation of calibration standards

The stock solution for calibration curves was prepared by accurately weighing out 0.625 g and 0.6249 g of prochlorperazine mesylate and edisylate, respectively, into a 50 mL volumetric flask, dissolving and making up to volume in water to achieve a concentration of 12.5 mg mL^{-1}. A 1 in 10 dilution in water yielded the working stock solution (1.25 mg mL^{-1}). Calibration standards containing 30, 50, 70, 90, 110 and 130 μg mL^{-1} were prepared by making the appropriate dilutions of the working stock solution.

The calibration standards were assayed in triplicate and calibration lines were constructed by linear regression of plots of peak height against concentration.

4.3.2 Linearity and Range

The linearity of an analytical procedure is its ability, within a given range, to obtain test results which are directly proportional to the concentration of analyte in the sample. Linearity is normally evaluated by visual inspection of a plot of signals as a function of analyte concentration or content (ICH Guidelines, 1996).

The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. The specified range is derived from linearity studies and depends on the application of the above procedure (ICH Guidelines, 1996).

Method:

Linearity was assessed over the same concentration range as the calibration curve i.e. 30 to 130 μg mL^{-1}.

Results:

Calibration curves, produced by plotting the prochlorperazine peak height against concentration (Tables 4.1 and 4.2), were found to be linear over the range studied (Figures 4.2 and 4.3). The linear regression equations are y = 169.21x + 4.93 (concentration range 30 - 130 μg mL^{-1}) for prochlorperazine mesylate with a correlation coefficient, r^2, of 0.9995, and y = 172.68x + 13.43 with a correlation coefficient, r^2, of 0.9992 for prochlorperazine edisylate. Regression analysis of the response against the prochlorperazine concentration demonstrated a proportional relationship in both cases, thus giving good confidence that the response and concentration are proportional and calculations may, therefore, be performed using a single reference standard rather than the equation of the calibration line.

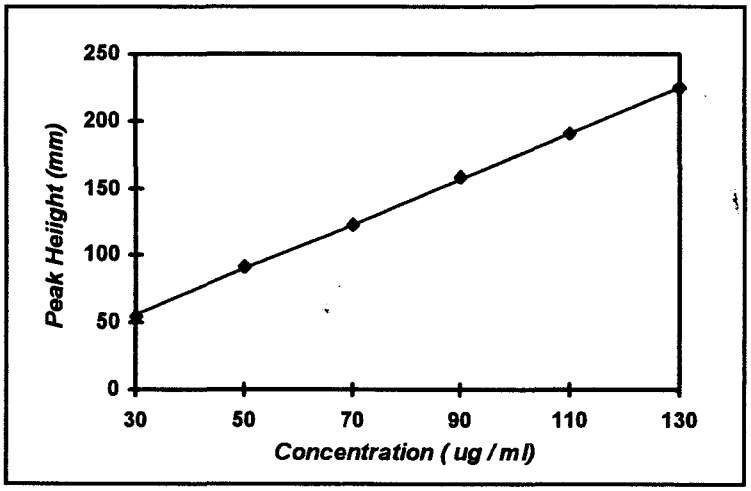


Fig. 4.2 Calibration curve constructed by plotting mean peak height against the concentration of replicate samples of aqueous prochlorperazine mesylate solutions

Table 4.1

Range of linearity of aqueous prochlorperazine mesylate solutions

Concentration (μg mL^{-1})	Mean Peak Height / mm	% RSD
30	54.5	1.8349
50	91.0	1.8388
70	122.5	1.4717
90	158.83	0.7922
110	190.67	0.8565
130	224.33	0.2574

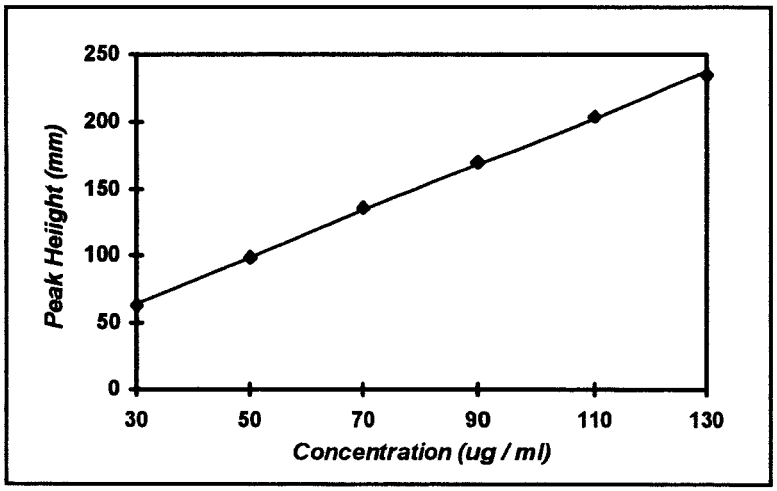


Fig. 4.3 Calibration curve constructed by plotting mean peak height against the concentration of replicate samples of the prochlorperazine edisylate solutions

Table 4.2

Range of linearity of aqueous prochlorperazine edisylate solutions

Concentration (μg mL^{-1})	Mean Peak Height / mm	%RSD
30	63.83	0.4522
50	99.42	1.0266
70	135.5	0.3690
90	170.7	0.3383
110	204.5	0.6740
130	235.5	0.2123

4.3.3 Specificity

This is the method's ability to assess, unequivocally, the analyte in the presence of components, including impurities, degradants, matrix, etc., which are expected to be present (ICH Guidelines, 1996).

A series of samples were subjected to the conditions listed below and injected onto the HPLC system, to determine whether the presence of other compounds or contaminants would interfere, or co-elute, with prochlorperazine or the sulfoxide which would then be determined from the subsequent chromatograms obtained.

Method:

1) mobile phase: A 60 µL (to overfill a 20 µL loop) injection of mobile phase.

2) solvent: The mobile phase used for sample preparation was injected.

3) acid / base hydrolysis: where a 12.5 mg mL^{-1} solution of prochlorperazine mesylate and edisylate was prepared in water (for comparative purposes), 0.1 M hydrochloric acid (HCl) and 0.1 M sodium hydroxide (NaOH) solutions and refluxed for one hour to determine whether any hydrolysis of the parent compound occurred under acidic or alkaline conditions (using HPLC). The NaOH was added to the prochlorperazine solution because alkaline conditions cause the drug to precipitate out of solution making it very difficult to manipulate.

4) Oxidation: solutions of prochlorperazine mesylate and edisylate at 12.5 mg mL^{-1} were prepared in 0.1 M hydrogen peroxide (H_2O_2) and left to stand overnight and the resulting solution was injected and chromatographed. Literature reports suggest that the accepted method for the assessment of the oxidative stability of compounds involves dissolving the respective derivatives in water with the addition of hydrogen peroxide (Owens *et al.*, 1989; Huang and Bhansali, 1968; Abdel-Moety, *et al.*, 1996). The solution is then either left to stand overnight with stirring, or heated for several hours. The oxidation test involved weighing out 125.8 mg and 125.4 mg of mesylate and edisylate, respectively, dissolving the sample in 100 mL each of the 0.1 M H_2O_2 solution prepared by diluting 3.4 mL of a 100% solution of H_2O_2 in 1 L.

5) UV irradiation: prochlorperazine mesylate and edisylate, both in the solution and solid states, were exposed to UV light at 254 nm and the resulting samples chromatographed on HPLC.

6) Light: prochlorperazine raw material and solutions were exposed to fluorescent light and subjected to chromatography.

7) Heat: both prochlorperazine salts, in solution and solid states, were heated at 60°C for 2 weeks and chromatograms for the products obtained .

The samples in solution were exposed in hermetically sealed ampoules, while the solid state samples were presented to the various conditions in thinly spread layers (no more than 3 mm thick) in petri-dishes and covered with a transparent lid.

Results:

In order to develop a suitable chromatographic system, a knowledge of the susceptibility of the drug to degradation and its degradation pathway is necessary. Any interference on the assay by possible degradants, or synthesis precursors, or by chemicals employed in sample preparation, and excipients present in the formulation needs to be investigated. This is a standard procedure in the development of a method (Edwardson *et al.*, 1990). Degradation products may be formed by a) acid / base hydrolysis; b) oxidation; c) UV irradiation (photolysis); d) light; e) heat, etc. (Edwardson *et al.*, 1990).

The chromatograms obtained for the series of samples injected are shown in Figures 4.4 - 4.11. Each chromatogram was examined for the presence of compounds which may interfere with or co-elute with prochlorperazine or its sulfoxide, the results of which, are summarised below.

1) Mobile Phase: The chromatogram of a 60 µL injection of mobile phase (on a 20 µL loop), used to overfill the loop, showed no observable peaks.

2) Solvent: The Milli-Q water used for sample preparation resulted in no discernible peaks and thus there is no interference with the sample peak.

3) Acid / base hydrolysis: The peak corresponding to prochlorperazine was the only observable peak after acid / base hydrolysis, so no interfering contaminants (Figure 4.4a, b and c) are produced. It is possible that prochlorperazine may not be susceptible to acid / base hydrolysis. After the period of refluxing, samples were cooled down and the pH was compared before and after refluxing. A general decrease of sample pH was observed after refluxing, which may be attributed to the liberation of HCl during hydrolysis and / or oxidation.

Table 4.3

Comparison of pH values before and after refluxing prochlorperazine mesylate and edisylate

Reflux Conditions	pH Before Reflux	pH After Reflux
Mesylate - water	3.57	3.09
Mesylate - 0.1 M HCl	1.56	1.18
Mesylate - 0.1 M NaOH	12.77	12.43
Mesylate - 0.1 M H_2O_2	3.11	2.93
Edisylate - water	2.93	3.32
Edisylate - 0.1 M HCl	1.76	1.14
Edisylate - 0.1 M NaOH	12.58	12.14
Edisylate - 0.1 M H_2O_2	3.06	2.94

4) Oxidation: Prochlorperazine, when dissolved in the hydrogen peroxide solution and left to stand overnight, formed the sulfoxide with approximately one-third of the drug remaining. Pawełczyk *et al.* (1975) reported that the thermal degradation of aqueous and buffered solutions of phenothiazine derivatives, in the presence of aerial oxygen, proceeded by oxidation of the ring sulphur atom in the phenothiazine nucleus (sulfoxidation). This occurred *via* an intermediate free-radical step for prochlorperazine and trifluoperazine. This reaction was dependent on the nature of the R_2 substituent, with the trifluoromethyl-derivative being more stable than prochlorperazine. The drug solutions in hydrogen peroxide were thus refluxed for comparative purposes. The major degradant formed during the oxidation reflux test was the 5-sulfoxide by comparison to the standards synthesised. The peak belonging to prochlorperazine decreased dramatically, while four other contaminants formed during reflux (Figures 4.5a and b). Prochlorperazine edisylate was found to degrade faster than the mesylate salt.

5) UV irradiation: Prochlorperazine in solution, exposed to UV light at 254 nm, showed the formation of four minor photoproducts, while for prochlorperazine in the solid state no degradants were detected (Figure 4.6a and b). The samples were placed in Pyrex glass containers at a distance of approximately 40 cm from the light source for 2 weeks.

6) Light: The prochlorperazine raw material and solutions were exposed to diffuse light for one week and seven detectable peaks were produced (Figure 4.7).

7) Heat: The chromatogram for a prochlorperazine solution which had been heated in an oven at 60°C for 4 weeks showed the start of the formation of one major degradant corresponding to the sulfoxide. These samples were spiked with a small amount of the synthesised sulfoxide. The growth of only one peak was evident (apart from the drug peak) and so it was assumed that this was the sulfoxide. This was later confirmed by the LC-MS studies in Chapter 6. The chromatograms of solid state samples of prochlorperazine (thinly spread (approximately 3 mm), in petri-dishes), were found to be free of detectable degradants (Figure 4.8) after heat treatment.

The above summary of the forced degradation studies indicates that the prochlorperazine peak in chromatograms was sufficiently resolved from all other degradants encountered. This method is thus specific for prochlorperazine mesylate and edisylate.

The need to ascertain the purity of the drug peaks in the exposed samples, in order to justify their use in the development of degradation profiles, suggests the use of photodiode array detection.

Two of the photodiode-array chromatograms, together with their corresponding superimposed UV spectra, showed a high degree of purity and superimposability of the corresponding UV spectra. Samples chosen for photodiode-array analysis included those exposed to direct sunlight and diffuse light, a fresh sample of prochlorperazine, the product resulting from forced oxidation, and a sample exposed to UV light in the solid state.

The chromatogram and the superimposed UV spectra of the fresh sample prepared demonstrates the purity of the prochlorperazine, indicating that there are no interfering co-eluting peaks. The superimposed UV spectra show the characteristic maxima at 307, 247 and 212 nm belonging to prochlorperazine. The sample product resulting from the forced oxidation produced a UV trace that was identical to that of the synthesised sulfoxides (shown in Figures 2.16 and 2.17). The characteristic peak maxima are observed at about 344, 301, 275 and 235 nm.

The solid state sample showed little degradation even after exposure for 5 months and photodiode-array analysis thus confirmed the integrity of the drug peak. The solution samples exposed to sunlight and diffuse light were evaluated. The purity of prochlorperazine peak was assessed in the presence of the degradants, all of which were adequately separated from the prochlorperazine peak. The good superimposability of the UV spectra displayed the integrity of the drug peak by the lack of co-eluting peaks, thus illustrating the specificity of the HPLC method. Photodiode array analysis is important for the conditions used in the kinetic studies in this investigation. Because the measurement of the drug peak height at various times is to be used in the kinetic interpretation of the data, it is important to be sure of the purity of the drug peak and that no degradant is formed at the same retention time under the drug peak.

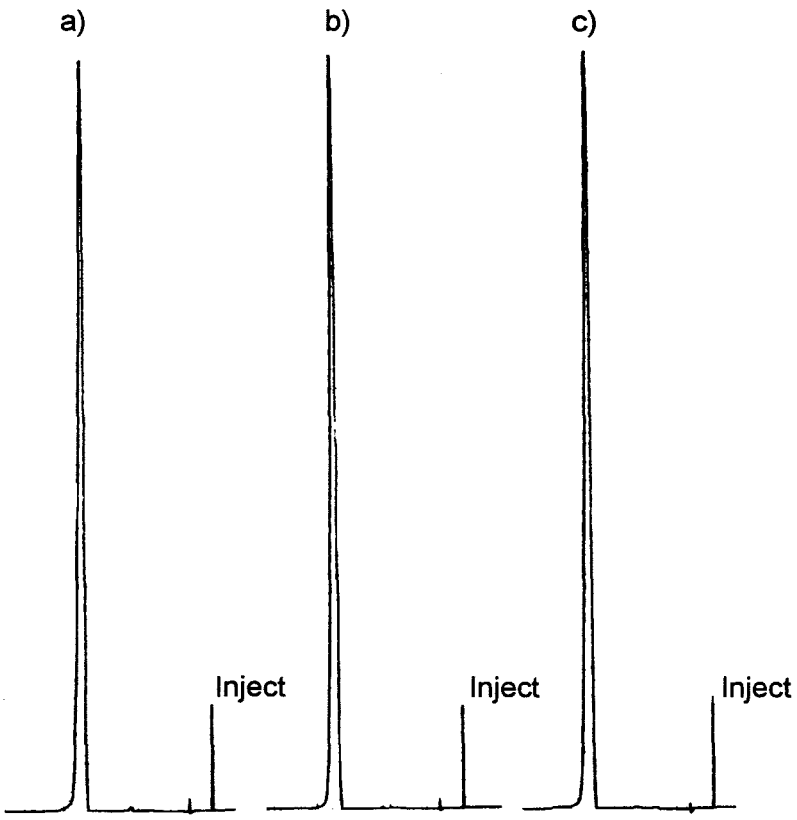


Fig. 4.4 Chromatograms obtained after refluxing prochlorperazine (retention time 5 minutes) in a) HCl, b) NaOH and c) water

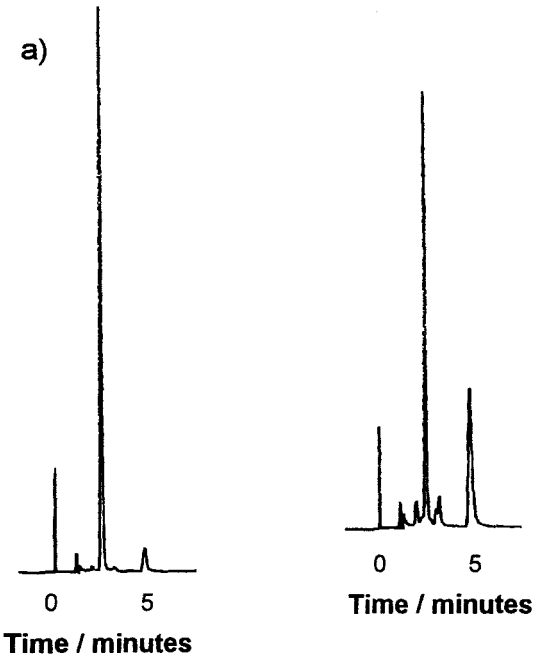


Fig. 4.5 Chromatogram showing the results of the oxidation test using a) room temperature conditions and b) reflux conditions

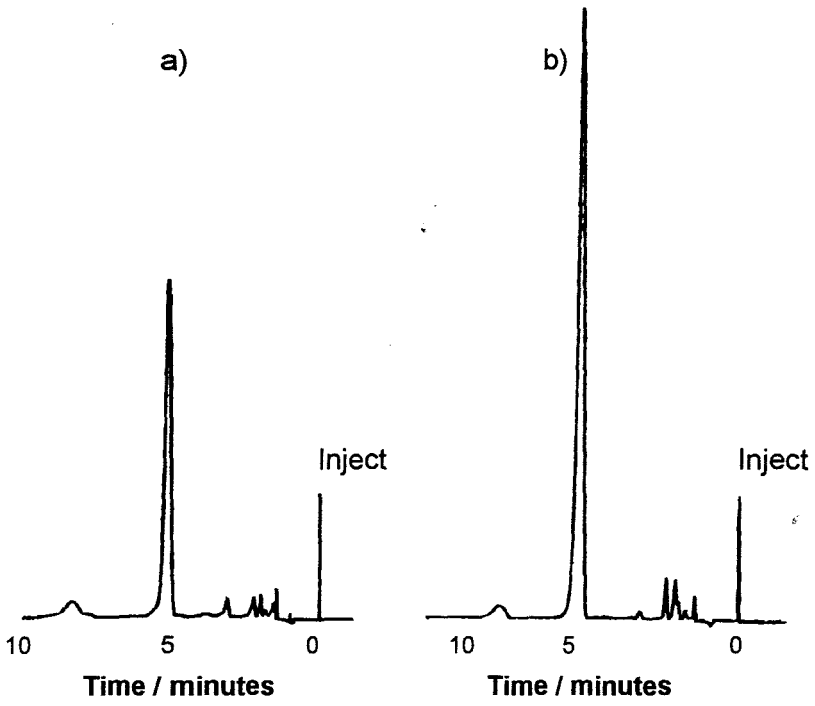


Fig. 4.6 The chromatograms obtained for prochlorperazine solutions exposed to UV light at 254 nm at a distance of a) 10 cm and b) 40 cm from the light source

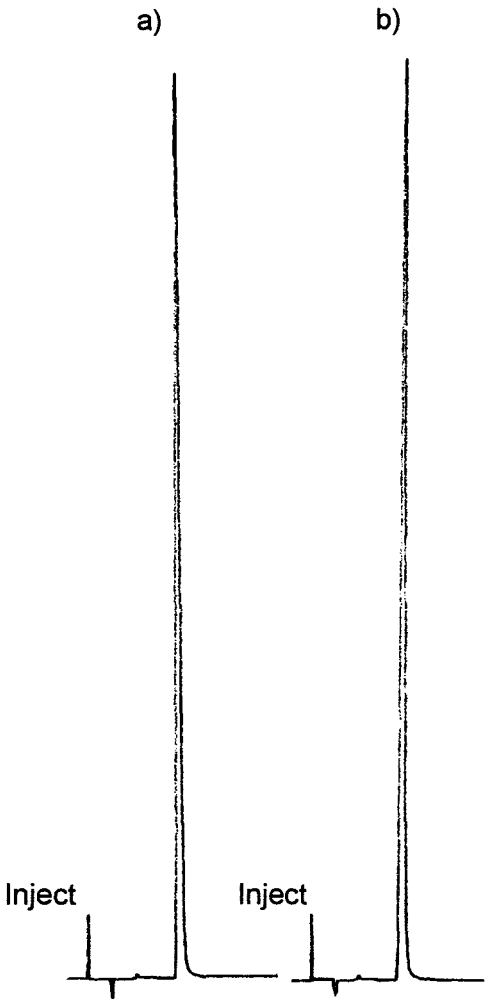


Fig. 4.7 Chromatograms of prochlorperazine (retention times at 5 minutes) exposed to UV light at a distance of a) 10 cm and b) 40 cm from the light source in the solid state

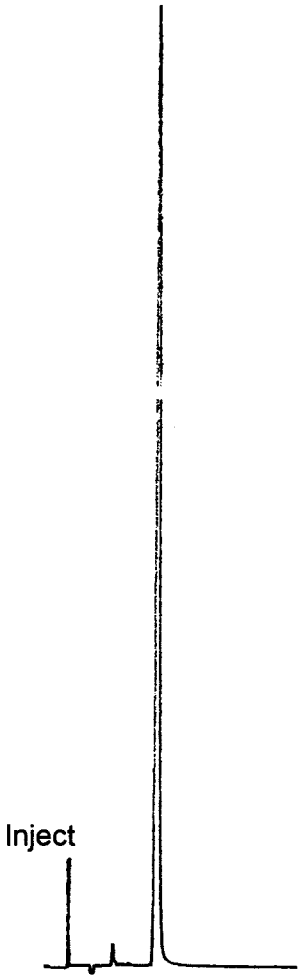


Fig. 4.8 Chromatogram of prochlorperazine solution (retention time at 5 minutes) heated at 60°C

4.3.4 Accuracy and precision

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy is usually established across the specified range of the analytical procedure (ICH guidelines).

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Repeatability expresses the precision under the same operating conditions over a short interval of time, intermediate precision expresses within-laboratories variations (for example different days, different analysts, different equipment, etc.) while reproducibility expresses the precision between laboratories (for example collaborative studies, usually applied to standardisation of methodology). Standard deviation, relative standard deviation (RSD) (coefficient of variation) and confidence interval have to be reported for each type of precision investigated (ICH Guidelines, 1996).

Accuracy is assessed using a minimum of 6 determinations over a minimum of 3 concentration levels covering the specified range, e.g. 3 concentrations and 3 replicates each of the total analytical procedure. Results are reported, together with their confidence levels, as percent recovery by the assay of a known added amount of analyte in the sample, or as the difference between the mean and the accepted true value (ICH Guidelines, 1996).

Method:

The precision of the analytical method was determined by assaying 6 spiked samples, each at the upper and lower limits of the concentration range studied (50 and 110 $\mu g\ mL^{-1}$) for each of the prochlorperazine salts. The accuracy of the method was determined by the mean concentrations obtained for the replicates and the percentage (%) difference. Samples were prepared by dilution of stock preparations with solvent for each phenothiazine derivative. The relative standard

deviations were then calculated for the prochlorperazine mesylate samples at the lower and upper limits of the 30 to 130 μg mL^{-1} concentration range.

The experimental procedure for the determination of precision is the same as that carried out for the assessment of accuracy. Precision was calculated as the RSD of the peak heights obtained divided by standard deviation of the sample. Precision experiments give a good indication of the method's performance and are normally repeated regularly. An RSD of 2% is considered acceptable and any increases above 2% should be investigated and the method is then completely revalidated (ICH Guidelines, 1996).

Results:

Results from the precision and accuracy studies, tabulated in Tables 4.4 and 4.5, show that acceptable precision and accuracy were obtained for the samples in the calibration range. The relative standard deviations calculated for the prochlorperazine mesylate samples at 50 and 110 μg mL^{-1}, were 1.56 % and 1.71 % respectively. The same procedure was done for edisylate giving a relative standard deviation (RSD) of 1.03 % and 0.67 % for the same upper and lower limits, respectively.

Table 4.4

Precision and accuracy data for quantification of prochlorperazine mesylate

Concentration (μg mL^{-1})	**Concentration found (μg mL^{-1}), Mean (n = 6)**	**% RSD**
50	51.60	1.56
110	110.16	1.71

Table 4.5

Precision and accuracy data for quantification of prochlorperazine edisylate

Concentration (μg mL^{-1})	**Concentration found (μg mL^{-1}), Mean (n = 6)**	**% RSD**
50	50.72	1.03
110	112.10	0.67

4.3.5 Detection and quantitation limits

The detection limit of an individual analytical procedure is the lowest amount of an analyte in a sample which can be detected, but not necessarily determined as an exact value. The quantitation limit is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in samples, and is used where the determination of impurities and / or degradation products is necessary. Detection limits are important in this study because the level of impurities (degradants or synthetic degradants) is required to be known. Similarly, quantitation limits are important in order to quantify the drug.

Several approaches for the determination of the detection and quantitation limits are available, depending on whether the analytical procedure is instrumental or non-instrumental.

Detection limits based on signal-to-noise ratios can only be applied to those procedures which exhibit baseline noise. Determination of the signal-to-noise ratio is performed by comparing measured signals from samples of known low analyte concentrations with those of blank samples, and by establishing the minimum concentration at which the analyte can be reliably detected or quantified. A signal-to-noise ratio between 3:1 and 2:1 is generally considered for the estimation of the detection limit while a typical signal-to-noise ratio for the quantitation limit is 10:1. This method was used in this study.

The standard deviation of the response and the value of the slope may be used to determine the detection limit. The detection limit (LOD) or quantitation limit (LOQ) may be expressed as:

$LOD = (3.3\ \sigma) / S$

or

$LOQ = (10\ \sigma) / S$

where σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S is estimated from the calibration curve of the analyte where the estimation may be based on the following ways: a) the standard deviation of the blank where the measurement of the magnitude of analytical background response is performed by analysing an appropriate number of blank samples and calculating the standard deviation of the responses; and b) the calibration curve where a specific calibration curve is studied using samples containing an analyte in the range of LOD or LOQ and the residual standard deviation of the regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

The detection or quantitation limits and the method used for determining these limits should be presented. If the LOD is determined from visual evaluation or from the signal-to-noise ratio, the relevant chromatograms need to be presented. If the limits are obtained by calculation or extrapolation, the values are then validated by the analysis of a suitable number of samples known to be near, or prepared, at the detection or quantitation limits (ICH Guidelines, 1996).

Method:

The limits of detection and quantitation were calculated for prochlorperazine mesylate in the calibration range 30 - 130 $\mu g\ mL^{-1}$. The method used was based on signal-to-noise ratio determinations.

Results:

The limits of detection (LOD) and quantitation (LOQ) for prochlorperazine mesylate in the calibration range 30 to 130 $\mu g\ mL^{-1}$, are shown in Table 4.6, to be 0.13 and 1.25 $\mu g\ mL^{-1}$, respectively.

Table 4.6

LOD and LOQ data for prochlorperazine mesylate over the 30 - 130 μg mL^{-1} range

Concentration Prepared (μg mL^{-1})	Mean Peak Height (mm)	Concentration found[©] (μg mL^{-1})	% RSD	% Bias[◇]	LOD[*]	LOQ
125.4	215.17	124.24	0	-0.925	accept	accept
12.54	25.0	11.86	1.25	-5.439	accept	accept
1.254	6.85	1.132	0	9.729	accept	accept
1.254×10^{-1}	2.0	-*	11.77	-*	accept	reject
1.272×10^{-2}	0.5	-*	22.22	-*	reject	reject
1.272×10^{-3}	no response	-*	-*	-*	reject	reject

*prochlorperazine concentration could not be determined since the peak height was less than the regression equation intercept.

© linear regression equation: $y = 169.214x + 4.9341$, $r^2 = 0.9995$

◇ the difference between the mean concentration measured and the prepared concentrations as a percentage of the prepared concentration.

* determined using an analyte response 3 times that of the noise (signal-to-noise ratio of 3 : 1) with the mean baseline noise = 0.5 mm ± 0.00 mm, n = 6.

4.3.6 Ruggedness / robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The evaluation of robustness has been considered during the development phase by varying the ratios of the organic phase to that of the aqueous phase in the mobile phase.

When measurements are susceptible to variations in analytical conditions, the analytical conditions must be controlled, or a precautionary statement may be included in the procedure. A consequence of the evaluation of robustness is that a series of system suitability parameters is established which ensures that the validity of the analytical procedure is maintained whenever used. Variations that may occur include the lack of stability of analytical solutions, differences in extraction times (tablets and their excipients) and differences in the analytical technique, e.g. variations of pH in a mobile phase; variations in mobile phase composition;

different columns (different lots and / or suppliers); temperature and flow rate, which were discussed earlier (refer to 4.2.5).

Method:

Variations of the parameters: pH in a mobile phase, mobile phase composition and flow rate were investigated during the evaluation of the specificity of the system (ICH Guidelines, 1996).

Results:

The stability of prochlorperazine in solution was evaluated after subsequent storage in a room without light at ambient temperature to determine the length of time a sample may be kept before degradation starts to take place. The raw data for the robustness test is presented in section 5.7.4 and shown in Figure 5.32 (sample solutions stored at 25 °C) since the data set is the same.

No relevant prochlorperazine instability was detected after 40 days of storage. Although notable increases in response were noticed after day 1 and day 6, the samples were still considered stable. Timm *et al.* (1985) proposed the following reasons: a) the compound may have produced a degradant having the same retention time as the parent compound, but a higher response to the detector system (which may, however, be excluded due to the specificity parameters investigated); and b) the systematic error being introduced during sample preparation, leading to increased responses, is the more probable explanation. This investigation showed that prochlorperazine stock solutions are stable and may be stored in the dark for at least 4 weeks at room temperature without degradation.

The results demonstrated that the method is reproducible because the percentage RSD is less than 2%.

4.3.7 System suitability testing

This testing is an integral part of analytical procedures and is based on the concept that the equipment, electronics, analytical operations and samples to be analysed constitute an integral system that can be evaluated (ICH Guidelines, 1996). This

includes the evaluation of the parameters by the methods discussed above i.e. 4.3.1.7)

Method:

System suitability parameters were calculated from the chromatograms obtained during oxidation and / or thermal stability studies.

Results:

System suitability parameters including tailing factors, capacity factors and resolution were calculated from the chromatogram shown in Figure 4.9 and are listed in Table 4.7. This is a typical chromatogram of prochlorperazine, obtained during oxidation and / or thermal studies, showing adequate separation of the sample from its main degradant. The UV spectrum obtained for each eluting compound was seen to correspond with the UV spectral characteristics of the synthesised sulfoxide and the pure prochlorperazine itself. The methods of calculation for these parameters are given in the notes below Table 4.7.

The conditions employed during the stability studies were thus confirmed to give good peak integrity, as shown by the superimposability of the UV spectra and the lack of other co-eluting peaks.

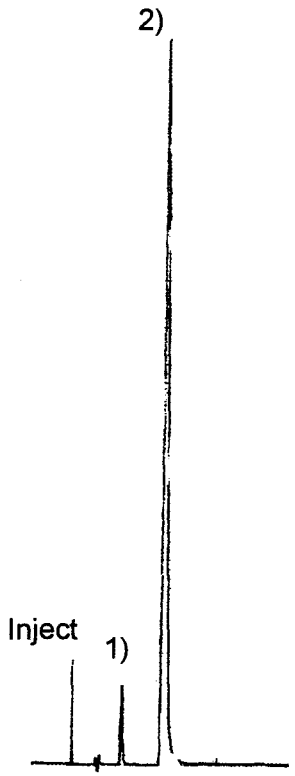


Fig. 4.9 Chromatograms obtained after injecting prochlorperazine (2) spiked with sulfoxide (1) to determine the system suitability parameters

Table 4.7

HPLC retention times and selected system suitability parameters for prochlorperazine and its sulfoxide

Sample	Retention time / minutes	k′ *	R_s ✧	T#
Mesylate	5.1	2.6428	3.250	1.50
Edisylate	5.2	2.7143	3.292	1.45
Sulfoxide (both salts)	2.5	0.9286	-	1.50

* Capacity factor: $k' = (t_a / t_\alpha) - 1$. Peaks should be well resolved from the void volume and k′ > 2 is acceptable

✧ Resolution: $R_s = 2 \times (t_2 + t_1) / (W_2 + W_1)$. $R_s > 2$ between the peak of interest and the closest potential interfering peak is desirable

Tailing factor: $T = W_{0.05} / 2f$. $T < 2$ is acceptable.

where:

t_α	=	elution time of the void volume or non-retained compounds
t_a	=	is the retention time from the time of injection to the time of the elution of the peak maximum
t_2 and t_1	=	are the retention times of the two components
W_1 and W_2	=	are the corresponding widths of the bases of the peaks, obtained by extrapolating the sides of the peaks to the baseline
$W_{0.05}$	=	peak width of the peak determined at 5% peak height (from the baseline)
f	=	distance from the front edge of the peak to the perpendicular at the peak maximum

The presence of the sulfoxide degradant was not quantitatively measured.

4.4 Conclusions

The analysis of drug substances and of finished products within the pharmaceutical industry is performed to satisfy both the manufacturer and the regulatory authority about the quality, integrity and stability of the medicine to be administered to the patient. A survey conducted by the Pharmaceutical Analytical Sciences Group (*PASG*) in 1992 determined the current practice employed in the validation of analytical methods for drug substances and drug products in pharmaceutical laboratories in the United Kingdom (UK). The survey was divided into method

validation parameters employed for drug substances and those for drug products. The investigation indicates that the highest degree of consistency is seen in the application of validation parameters to chromatographic techniques, specifically in the case of HPLC, where there was good agreement over which parameters should be applied to method validation. This reflects the widespread application and dependence on this technique within the pharmaceutical industry (Clarke, 1994).

The survey included the following validation parameters: accuracy, precision, linearity, limit of detection / quantitation, ruggedness, selectivity, and system suitability which were the most frequently applied to the validation of bulk active and finished products by all the pharmaceutical laboratories. Ruggedness, and sensitivity determinations were only applied in a few laboratories. The method for the validation of prochlorperazine considers the validation parameters reported as used by pharmaceutical industries in the UK, which are acceptable to regulatory authorities (Clarke, 1994).

In order to facilitate the evaluation of the validation parameters, acceptance criteria should be universally applicable, mathematically explicit, complete and achievable. The acceptable limits for a number of validation parameters such as accuracy, precision, specificity, linearity and sensitivity (LOD and LOQ) were reported by Jenke (1996) (shown in Table 4.8).

Table 4.8

Summary of acceptable limits of validation parameters (Jenke, 1996)

Validation parameters	**Acceptable limits**
Linearity	Correlation coefficient should be between 0.98 - 1.00
Specificity	Should have baseline separation between peak of interest and all other analytical responses
Accuracy	98 to 102% of the theoretical value
Precision	2.0% RSD
LOD and LOQ	No specific criteria exist in the literature

The results from the validation study reported here demonstrate excellent precision and accuracy for prochlorperazine, with no RSD values above 2% for precision study. The range over which to perform linearity experiments is subject to some discussion. Pharmaceutical industries in the UK report that linearity is determined

over a range of 50 - 150% for bulk drug and bulk drug impurity assays, while for finished product assays, the range is 75 - 125%, with a wider range for finished product degradant assays (Clarke, 1994). However, Edwardson *et al.* (1990) report that it is usual practice to perform linearity experiments over a range of 25 - 200% of the nominal concentration of analyte.

The range chosen for this investigation was 24 to 104% of the nominal analyte concentration to allow for valid quantitation of the active drug during the degradation studies. Regression analysis of mean peak height *versus* concentration for prochlorperazine demonstrated proportional relationships, with correlation coefficients for the regression lines of above 0.999 for both prochlorperazine salts. These values are within the acceptable limits for linearity.

The lowest amounts of analyte which can be reproducibly quantitated above baseline noise (for which duplicate injections which resulted in a RSD 2%) is 1.25 $\mu g\ mL^{-1}$ for prochlorperazine mesylate. The lowest amount of analyte which can be detected above baseline noise is 0.13 $\mu g\ mL^{-1}$ for prochlorperazine mesylate.

The chromatograms of representative exposed samples containing degradation products for prochlorperazine, Figures 4.5, 4.6, 4.8 and 4.9, indicate the separation of the parent compound from degradants is adequate. No peaks were observed to interfere with the parent compound in the chromatograms. The method is therefore specific for prochlorperazine.

System suitability procedures, such as tests for precision, selectivity, and chromatographic performance (tailing factor and column efficiency) are extensively used in the UK pharmaceutical industry. The results obtained for the system suitability tests conducted with prochlorperazine demonstrate the suitability of the method with different systems for the compounds under investigation. In addition the experimental results were within the limits set for the variables. Capacity factors (k') calculated showed that the drug peaks (both mesylate and edisylate) were well resolved from the void volume. Capacity factor values were found to be above 2.6, where k' values above 2 are deemed to be acceptable. The k' value obtained for the sulfoxide was found to be below 1, indicating that this peak is not well resolved from the void volume. Thus the sulfoxide should not be quantitatively analysed, but the method may be used for qualitative studies. In all cases the tailing factor (T)

was found to be less than 2, which is deemed to be acceptable. The resolution (R_s) of the drug peak from the closest interfering peak (sulfoxide) was found to be acceptable for both the mesylate and the edisylate.

It can therefore be concluded that this initial validation of the analytical method for prochlorperazine provides preliminary assurance of the reliability of the analytical procedures. This information provides the foundation from which a new validation protocol can be prepared for subsequent investigations. Development and validation of the HPLC method for the assay of prochlorperazine has provided some information on the susceptibility of the drugs to degradation. Thus the foundation for the determination of the minor components / degradants has been provided.

CHAPTER 5 . STABILITY STUDIES IN SOLUTION

In this chapter the kinetics of the photolytic and thermal degradation of aqueous solutions of prochlorperazine mesylate and edisylate in ampoules, sealed under aerobic and anaerobic conditions, are presented. The effects of various light conditions on the degradation of the above two salts were examined.

5.1 Introduction

The effect of light is an important factor in drug instability and various official compendia and licensing agencies have taken this into account by recommending appropriate storage conditions, such as light-resistant containers.

The absorption of electromagnetic radiation, resulting in the excitation of an electron from a lower to a higher molecular quantum state, is the first step in a sequence of events leading to the final photochemical product. The electronically excited molecule is unstable and may undergo further reactions, or lose its excitation energy and return to its ground state. There are a number of different possible physical de-excitation pathways which are dependent on the type of molecule and the nature of the electronic states involved. These de-excitation pathways are very rapid and may be classified as 1) *radiative processes,* where electromagnetic radiation is emitted by the excited molecule as it returns to the lower ground state; 2) *non-radiative processes,* where the populations of the initially excited quantum states are transferred into others without accompanying emission; and 3) *quenching processes,* which are bi- or trimolecular de-excitation processes that involve the transfer of energy from the initially excited molecule to other molecules that are in collision with it (Gilbert and Baggott, 1995).

Sunlight is the most readily available source of light, but is unsuitable for quantitative photochemical studies. It is more quantitative to use a source of light where the wavelength and intensity are readily controlled. The rate of a photochemical reaction is dependent on the wavelength and the intensity of the irradiating source, as well as the shape and position of the reaction vessel in relation to the light source. Thus, the number of quanta of the relevant wavelength region being absorbed per unit time is only one of the factors which determines the

rate at which a photochemical reaction occurs. Another important factor is the photochemical efficiency, or quantum yield, of the reaction. Moore *et al.* (1990) pointed out that caution should be used in the interpretation of rate constants derived from the rate equations for photochemical reactions.

The experimental determination of the quantum yield constitutes one of the main aspects of the study of photochemical changes. In order to establish the quantum efficiency for photodegradation, the wavelength employed should be restricted by using filters or monochromators (Shahjahan and Enever, 1996 a). The number of moles of a light-absorbing substance that reacts within a given time may be determined by a suitable analytical procedure. This process is called actinometry. The measurement of quanta or the energy absorbed is necessary since this energy is dependent on the wavelength (Shahjahan and Enever, 1996 b).

Photochemical reactions can be induced and promoted by different organic compounds (sensitisers) which can act in the following ways:

1) *Photoinitiators* - are compounds which are excited by absorbed light and subsequently decompose into free radicals which may initiate secondary reactions.
2) *Photosensitisers* - are compounds which are excited by light and transfer their kinetic energy to other molecules or to oxygen molecules forming singlet oxygen, e.g. prochlorperazine, benzophenone.
3) *Photo-optical sensitisers* - are compounds which extend the optical sensitivity of other compounds to radiation of longer wavelength.

Depending on the conditions of the experiment, an appropriate chemical compound may behave either as a photoinitiator or as a photosensitiser (Rabek, 1982).

5.2 Light sources

Although there are purposes for which the continuum from incandescent lamps is useful, much higher intensities of near-monochromatic radiation are obtained by filtering radiation from lamps that emit most of their energy in a small number of bands or lines. Several types of gas-discharge lamp may be employed for this purpose. They may contain inert gases or vapours of volatile (often metallic) elements that produce the appropriate atomic emission lines. At low pressures,

most of the emitted energy is concentrated in the resonance lines (those due to transitions from the first excited state to the ground state), and essentially monochromatic light can be obtained without the use of filters. Typical of such lamps are those containing low pressure Xe (λ=147.0 nm) or Hg (λ= 184.9 nm, 253.7 nm). A little inert gas is usually present in the Hg lamps, but contributes little to the emitted radiation. At higher pressures, which can be created in discharges through metal vapours by operating the lamp at high temperature, more emission lines appear, and they show pressure broadening. The resonance line itself is often reversed as a result of absorption by cooler metal vapour near the walls of the lamp. Mercury discharge lamps are very frequently used in photochemical experiments, Table 5.1 shows the intensities of the main lines from typical low pressure (intensities relative to λ = 253.7 nm) and medium pressure (intensities relative to λ = 365.0 nm) mercury discharge lamps.

Table 5.1

Relative intensities of some lines from mercury discharge lamps (Calvert and Pitts; 1966)

λ (nm) (UV)	**Relative Intensity Low Pressure**	**Relative Intensity Medium Pressure**	**Energy carried by 1 mol of photons (einstein) at a particular λ (Gilbert and Baggott, 1995)**
365.0	0.54	100.0	328 kJ mol^{-1}
253.7	100.0	reversed	471 kJ mol^{-1}

The intensity of a low-pressure discharge lamp is low but the efficiency is high. The filling gas for a low-pressure lamp for efficient UV production must have the following properties:

1) A fairly low ionisation energy, so that the avalanche effect will take place readily. This implies a metallic element.
2) Sufficient vapour pressure at a reasonable temperature (i.e. at ambient or slightly above ambient) to provide the optimum pressure for production of the resonance radiation.
3) Although the ionisation energy must be reasonably low, the lowest excited state which is not metastable must be at a level above the ground state such that the resonance radiation appears at a useful wavelength.

4) The element must be chemically inert towards the envelope and the electrode materials.

Mercury fulfils all these requirements excellently. The low-pressure mercury lamp forms the basis of the common fluorescent lighting tube. The envelope is of glass which is coated on the inside with a fluorescent powder which converts the resonance mercury radiation at 253.7 nm into white visible light. Low-pressure mercury lamps are also used as sources for germicidal radiation (Phillips, 1983).

5.3 Photodecomposition of Cl-substituted phenothiazines

Certain substances increase the sensitivity of the skin to solar radiation. While it is possible to avoid such side effects (by staying away from light), a photochemical basis is needed for classifying a drug as a potential photosensitiser (Moore, 1977).

Chlorine-containing drugs, such as chlorpromazine and prochlorperazine, are among a number of chemicals capable of photosensitising oxidative reactions *in vitro*. The adverse reaction is believed to be initiated by the photosensitised oxidation of skin components, where the drug functions as the photosensitising agent (Moore,1980). Drugs with a significant light absorption in the 280-800 nm region are involved in this process. The photosensitising effects of several drugs are significantly decreased by window glass, which is opaque to radiation below 310 nm (Moore,1977). Mechanisms by which photooxidation may occur are classified as follows: *Type I* is a free-radical chain process generally termed autoxidation, while *Type II* involves singlet molecular oxygen and is termed oxygenation. Those drugs which photosensitise oxidation by a Type I (free-radical) mechanism have been shown by Moore *et al.* (1977) to yield a free chloride ion.

A number of cases of photosensitivity or photoallergy, such as abnormal skin reactions and ocular changes, have been associated with chlorpromazine use. Skin discolouration occurs on the exposed portions of the bodies of patients receiving long-term large doses of phenothiazines, especially chlorpromazine and prochlorperazine. *In vivo* photosensitivity studies of phenothiazines have indicated that both photoallergy and phototoxicity reactions are mainly due to the decay products formed and not to the parent compound (Huang and Sands,1967).

The decomposition of chlorpromazine catalysed by UV light gives rise to different products under aerobic and anaerobic conditions (Huang and Sands, 1967; Pawełczyk and Marciniec, 1977a). Under aerobic conditions, oxidation products, such as sulfoxides and *N*-oxides, are formed. Under anaerobic conditions, a polymerisation process predominates. The biological activity of the polymer was tested on human volunteers where a bluish purple colour, resembling that of the skin discolouration observed in patients receiving chlorpromazine, was detected. This discolouration faded within three days, indicating that the polymer is transported from the site of administration, or is metabolised.

Light-induced decomposition of chlorpromazine in aqueous solution is also accompanied by a shift in pH from 4.5 to 1.9. Potentiometric studies showed that one mole of chloride ion is produced per mole of drug irradiated in deoxygenated solution (anaerobic), with subsequent equimolar formation of hydrogen ion (Moore and Tamat 1980). Both the liberation of HCl and the subsequent formation of a polymer of chlorpromazine are thus implicated as possible causes of the photosensitivity and skin discolouration of patients (Nejmeh and Pilpel, 1978). The HCl could be responsible for the skin rash observed in patients exposed to sunlight. Similar results have been reported for thioproperazine where cleavage of the $SO_2N(CH_3)_2$ functionality forms sulfuric acid (Huang and Sands, 1967).

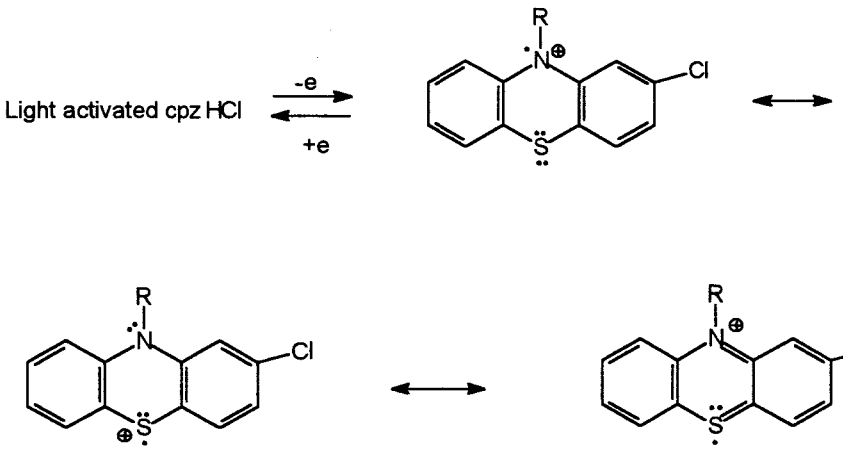
Formation of the polymer is initiated when the chloride is cleaved to give rise to a radical (or carbonium ion) at position 2 (Figure 1.2). This is followed by free-radical attack (or electrophilic attack) on another chlorpromazine molecule at the carbon in position 7 (Figure 1.2) giving rise to a 2-7 linkage. The formation of a dimer, with a 2-2 linkage, from chlorpromazine also occurs. These free-radicals are only present in solvent media and not in the solid state (Huang and Sands, 1967). Forrest *et al.* detected free-radicals in solution using electron spin resonance (ESR).

The formation of HCl in methanol solutions of the drug was strongly inhibited upon saturation of the solutions with oxygen (aerobic conditions), but only partial inhibition occurred in aqueous solution. A photodissociative process occurs predominantly in methanol, while photoionisation occurs in aqueous media. In "non-phenothiazines", such as chlordiazepoxide and hexachlorophane, no chloride ion was detected after extended periods of irradiation. The photolability of the chlorine in phenothiazines thus correlates with their ability to photosensitise oxidation by the Type I (free-

dimethylfuran, respectively. Phenothiazine derivatives were found to be capable of photosensitisation by both mechanisms, with promethazine and trifluoperazine showing high activity.

Roseboom and Perrin (1977) studied the rate of phenothiazine degradation in an acidic oxygen-saturated medium where 3*H*-phenothiazine-3-one and phenothiazine-5-oxide were produced by parallel reactions. The overall degradation rate of phenothiazine was found to be independent of pH. After isolation and identification of their degradation products, 10-methylphenothiazine-5-oxide and 3*H*-phentohiazine-3-one, the degradation kinetics were studied. The main degradation product was found to be the 10-methylphenothiazine-5-oxide, but at low pH values and high temperatures the formation of 3*H*-phentohiazine-3-one was increased.

Felmeister and Discher studied the photodecomposition of chlorpromazine HCl at 253.5 nm to determine the quantum efficiency under normal (aerobic) and oxygen-free (anaerobic) atmospheres. The quantum efficiency was found to be constant, but increased with pH above 4.5, and the photodegradation was found to follow zero-order kinetics. Infrared studies showed that ring hydroxylation also occurs upon irradiation. Spectral evidence showed that the semiquinone free-radical was formed as an intermediate on irradiation of the chlorpromazine HCl aqueous solutions at 253.5 nm. Chlorpromazine-5-oxide was found to form by disproportionation of the free-radical in aqueous media in the presence or absence of dissolved oxygen (Figure 5.2). The phenolic compounds formed on irradiation are thought to result from the degradation of the photolabile chlorpromazine-5-oxide. Photodegradation of chlorpromazine HCl was found to be more dependent on the presence of water than dissolved oxygen and the elimination of oxygen was not expected to have a marked effect on the degradation of solutions of chlorpromazine HCl irradiated at 253.5 nm (Felmeister and Discher, 1964).



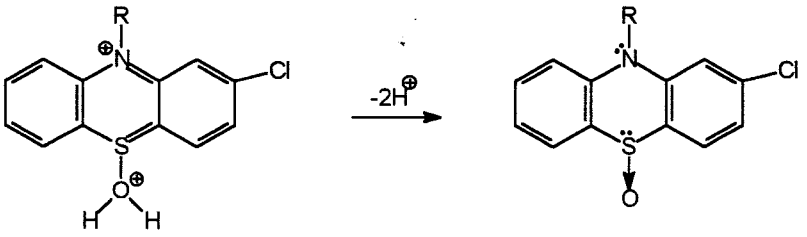
Resonance forms of the semiquinone free-radical of chlorpromazine (cpz) HCl

Two molecules of the semiquinone free-radical of cpz HCl ⟶ disproportionation

cpz HCl

cpz HCl phenazathionium ion

cpz phenazathionium ion $\xrightarrow{H_2O}$



postulated intermediate

cpz sulfoxide HCl

R = $CH_2CH_2CH_2N(CH_3)_2.HCl$

Fig. 5.2 The light-activated disproportionation of chlorpromazine HCl (Felmeister and Discher, 1964)

Felmeister and Schaubman (1969 b) established that UV irradiation modified the interaction of some phenothiazine drugs with a lecithin monolayer film. The chlorine-substituted derivatives (chlorpromazine and prochlorperazine) showed a marked increase in reactivity towards the film as a result of irradiation. Trifluoperazine, in contrast, exhibited a decrease, while triflupromazine and promazine showed no change in reactivity following irradiation. The ability of UV-irradiated phenothiazine drugs to interact with a lecithin monolayer may be a measure of their *in vivo* membrane-penetrating and phototoxic properties. In the absence of chlorpromazine, the lecithin films were unaffected by UV light. Thus the expansion observed was due primarily to the second reaction product, 2-hydroxypromazine. The 2-hydroxypromazine could be formed (Figure 5.3) as follows (where the liberated HCl would again contribute to the observed decreases in pH):

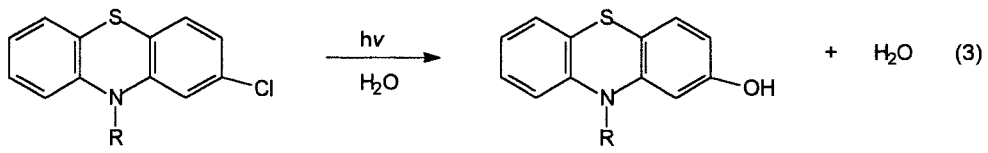


Fig. 5.3 The formation of 2-hydroxypromazine (Nejmeh and Pilpel,1978)

Schaubman and Felmeister (1969 a), using monolayers of lecithin film, studied the effects of UV irradiation and pH on aqueous solutions of chlorpromazine HCl. The degree of penetration of the chlorpromazine into the lecithin film was found to be dependent on the surface pressure, the pH and the UV irradiation.

Other degradants are formed by the removal of the terminal N-alkyl group. Metabolites resulting from the partial degradation of the piperazine ring to form ethylenediamine structures have been found in tissues by Underberg *et al.* (Breyer *et al.*; 1974)

Pawełczyk *et al.* (1975) have shown, using Hammet-type plots, that a relationship exists between the basicity of the compound and the rate of degradation. The basic properties of the compound increase with increasing volume of the substituent at the C_2 position, with the same applying to the pKa_1 values. For example, butaperazine and thioproperazine, which have high basicities (with correspondingly

high substituent volumes), showed the lowest degradation under the influence of light. Also, the rate of photochemical degradation of perazine derivatives, where the first step of photodegradation involves the formation of free-radicals in the presence of oxygen, increases with increasing acidity of the medium. Derivatives with Cl, CF_3 and $SO_2N(CH_3)_2$ substituents degrade differently (photooxidation) from derivatives with H and SC_2H_5 substituents (photooxidation and photolysis) and those with $COCH_2CH_2CH_3$ (photolysis only), thus providing further evidence of the relationship that exists between the basicity of the compound and the rate of degradation. Photooxidation of the phenothiazine ring was shown to be inhibited by the presence of the $COCH_3$ substituent. Pawełczyk *et al.* (1975) concluded, from the actinometric values obtained, that only a small amount of the radiation energy at λ=254 nm takes part in the photooxidation process. This also proves that the rest of the energy is consumed in other photophysical processes and that light plays the role of a catalyst in the reaction (Pawełczyk and Marciniec, 1977).

There is little information available about the thermal decomposition of phenothiazine derivatives. The number, direction and kind of reactions proceeding under the influence of temperature are dependent on the structure of the particular derivatives being investigated. Since phenothiazines vary mainly at the C_2 and N_{10} positions, the direction of thermal degradation is dependent on the substituents present at these positions. Thermal degradation of the majority of these compounds has been found to proceed, in the presence of air (oxygen), by oxidation of the sulfur atom in the ring *via* an intermediate step involving free-radicals (Pawełczyk *et al.*, 1975). The main reaction may, in some cases, be the hydrolytic degradation or oxidation of substituents at both the C_2 and N_{10} positions (Pawełczyk *et al.*, 1975).

The lack of publications on the subject of the kinetics of thermal degradation of phenothiazine derivatives may be due to a greater interest in the far more sensitive and faster photooxidation of phenothiazines. Pawełczyk *et al.* (1975) discussed the dependence of the stability on the kind of substituent present in the C_2 position in perazine derivatives of phenothiazines in terms of the Hammet equation. The rate of thermal degradation was found to be first-order and was then verified using the Ostwald-Zawidzki expression (Pawełczyk *et al.* 1975). All the perazine derivatives degraded due to oxidation of the sulfur atom on the ring. The substituents and other parts of the molecule were not involved in the thermal oxidation. From

Hammet plots and calculated thermodynamic parameters, it was found that substituents such as Cl, CF_3 and $SO_2N(CH_3)_2$ decrease the reactivity of the molecule in relation to the unsubstituted compound, while SC_2H_5 enhanced the reactivity minimally, and COC_3H_3-n enhanced the reactivity markedly. Thus it was concluded that the halogen substituents and $SO_2N(CH_3)_2$ in the C_2 position influence the sulfur atom in the ring by induction and resonance, which results in an increase in electron density on the atom with a corresponding decrease in its acidity. This results in the inhibition of oxidation and a corresponding decrease in the reactivity of the molecule. SC_2H_5, causes a small decrease in electron density on the sulfur atom, resulting in an increase in acidity and an accelerated reaction occurs. A decrease in reactivity thus results in an increase in stability. Thus the substituent at C_2 determines the stability of perazine derivatives (Pawełczyk et al., 1975).

The presence of oxygen usually has a detrimental effect on photochemical reactions, because of its ability to quench both singlet and triplet excited states and to react readily with radical intermediates. Photooxidation is a principal cause of degradation in many compounds, resulting in their discolouration and loss of properties. Oxidation may be inhibited by the presence of UV screening compounds or radical scavengers (e.g. ascorbic acid).

There are two general methods by which oxygen can be removed from a liquid sample: 1) freeze-pump-thawing, which involves the use of a vacuum system to pump all gases from above the liquid sample which has been frozen to solid (thawing of the frozen sample releases dissolved gases into the vacuum above it); and 2) the use of an inert gas, e.g. nitrogen, which is bubbled through the liquid until all the oxygen has been replaced (Gilbert and Baggott, 1995).

Moore and Tamat (1980) stated that, in the presence of oxygen, the chlorpromazine triplet-state energy is efficiently transferred with the formation of singlet state molecular oxygen:

$$^3cpz + {}^3O_2 \rightarrow cpz + {}^1O_2$$

where cpz is chlorpromazine. This was verified by the subsequent oxidation of 2,5-dimethylfuran, both in propanol (Davies *et al.*, 1976) and methanol (Moore, 1977), and the strong inhibition of the Cl^- production.

5.4 Actinometry

Photochemical processes may lead to chemical change. The nature of the products, and the rates of their formation, may be determined by standard analytical techniques. The irradiation of a sample with monochromatic light of constant intensity is generally known as steady-state or continuous photolysis. The reaction quantum yield (Φ) is defined as (Favaro, 1998):

$$\Phi = \frac{\text{no. of molecules undergoing the process of interest per unit time}}{\text{no. of photons absorbed by the photoreactive substance per unit time}}$$

where the number of molecules undergoing the process is measured by an analytical technique.

Determination of the quantum yield for a photochemical reaction depends on a) analytical determination of the amount of reactant consumed, or of product formed, during a given period of irradiation, and b) accurate measurement of the total light absorbed by the system at the wavelength used during this period. The energy of monochromatic radiation is determined most accurately by means of a thermopile, but for most purposes the accuracy of the thermopile is not required and so the energy of the absorbed light may be determined by means of a chemical actinometer. The principle of an actinometer is to substitute for the system under investigation a suitable, clearly defined photochemical reaction and to measure its response in a comparable time under identical experimental conditions. Several such systems (gaseous or liquid), responsive to different wavelength regions, have been characterised.

A potassium ferrioxalate actinometer has been widely used and was chosen for this study because of its suitable wavelength range (Shahjahan and Enever, 1996).

Advantages of an actinometer include reproducibility, insensitivity to intensity fluctuations during exposure and applicability to a wide range of irradiation geometries (Gilbert and Baggott, 1995).

The quantum yields of a number of photo-processes have been accurately measured and are sufficiently independent of the experimental conditions that they

can be used as standards in the determination of light intensities. The intensity of the absorbed light, I_a, per unit time, is determined from the rate of product formation (d[P]/dt) in the actinometer divided by the known quantum yield, ϕ_a, of the actinometer:

$$1/\phi_a \cdot (d[P]/dt)_{actinometer} = I_a$$

If the concentration of product depends linearly on irradiation, as in most actinometers, this equation may be simplified to:

$$[P] / \phi_a t = I_a$$

For I_a to be an accurate measurement of the absorbed light intensity that must be used in determining the quantum yield, the conditions for irradiation of the actinometer must be as close as possible to those used in the irradiation of the sample being studied. The reaction cells must, therefore, have the same volume and geometry, and the same solvent and the same optical density must be used (Ferraudi, 1988).

Assuming a reaction of the type:

$$A \xrightarrow{h\nu} B$$

in the actinometer of volume, V, the intensity of light absorbed can be determined (Rabek, 1982): either by measuring the concentration of unreacted actinometer species [A], i.e.

$$d[A] / dt = -I_a \bullet \Phi_A / V$$

where Φ_A = quantum yield for the photolysis reaction of species A, or by measuring the concentration of products [B] formed in the actinometer:

$$d[B] / dt = I_a \bullet \Phi_B / V$$

Φ_B = quantum yield of formation of stable product B.

There are some important requirements for any type of chemical actinometer:

1) The product of time and intensity should remain constant.
2) Quantum yields (Φ_A and Φ_B) should be independent of light intensity, concentration of the actinometer and of the temperature.
3) For measurement of polychromatic radiation, the actinometer species must possess a broad absorption spectrum and quantum yields (Φ_A and Φ_B) must be constant over a wide range of wavelengths. A system which has a narrow absorption range can only be used for specific wavelengths. If the quantum yields (Φ_A and Φ_B) are not constant over the range of wavelengths considered, such an actinometer can be used only for measuring intensities of radiation with wavelengths for which values of Φ_A or Φ_B are known (Rabek, 1982).

The sensitivity of an actinometer depends both on its quantum yields (Φ_A and Φ_B) and the method of analysis. Improved sensitivity can be achieved by proper selection of the analysis procedure (Rabek, 1982).

Uranyl oxalate has often been used for solution work in the conventional wavelength range of 250-435 nm, although recently potassium ferrioxalate actinometers have gradually been replacing them. Potassium ferrioxalate offers greater sensitivity, and an extended range into the visible region up to 578 nm. Both systems, however, have the limitation of being usable only in the ultraviolet and near-ultraviolet region. For actinometry, solutions in dilute mineral acid are used and, under these conditions, the main photoactive species present are the mono- and di-oxalato compounds. Taking the mono-oxalato ions as an example, the mechanism below involves excitation and dissociation of the excited ion to give the oxalate radical and reactions of this radical with more ferrioxalate ion:

$$Fe^{3+}(C_2O_4)^{2-} \xrightarrow[\text{primary reaction}]{h\upsilon} Fe^{2+}(C_2O_4)^{-} \qquad \text{excitation} \qquad [1]$$

$$Fe^{2+}(C_2O_4)^{-} \xrightarrow[\text{excited complex}]{\text{Dissociation of}} Fe^{2+} + (C_2O_4)^{-} \qquad [2]$$

$$(C_2O_4)^{-} + Fe^{3+}(C_2O_4)^{2-} \rightarrow 2CO_2 + Fe^{2+} + (C_2O_4)^{2-} \qquad [3]$$

$$2(C_2O_4)^{-} \rightarrow 2CO_2 + (C_2O_4)^{2-} \quad [4]$$

Under normal conditions the radical is apparently consumed almost exclusively by reaction [1]. The radical-radical reaction [4] occurs only at very high intensities and low ferrioxalate concentrations (Parker and Hatcher, 1959).

Reaction [3] is monitored by measuring Fe^{2+} absorptiometrically as its red 1,10-phenanthroline complex. The complexing agent (in this instance phenanthroline) is added following exposure. $\varphi(Fe^{3+})$ is 1.25 for 253.7 nm light, but decreases gradually to about unity for 436 nm light and 0.86 for 509 nm light. $\varphi(Fe^{3+})$ is also slightly sensitive to ferrioxalate concentration. The very small dependence of the quantum yields on reactant and product concentrations, intensity of the incident light, and temperature over a considerable range adds to the utility and popularity of this actinometer which is considered to be the best solution-phase chemical actinometer (Calvert and Pitts, 1966; Rabek, 1982).

5.5 Photochemical kinetics

The rate of a photochemical reaction is in general dependent upon the concentration of the initial component and the magnitude of the total radiant flux which is absorbed by the reacting system. The following assumptions are made in formulating the rate equations:

1) The reaction system is closed, i.e. there is no exchange of mass with the environment.
2) Radiation energy is transferred in a certain direction in space by a beam of parallel rays.
3) The agitation of the reaction mixture is sufficiently intense to prevent a concentration gradient along the path of radiation.
4) The volume of the irradiated system does not change during the reaction.
5) The length of the radiation path in the reacting system is constant (Rabek, 1982).

The rate of a single-step (thermal) chemical reaction, e.g. $X + Y \rightarrow P$, is given by:

$$\text{Rate of product formation} = d[P]/dt = k[X][Y] = A\, e^{-(Ea/RT)}[X][Y]$$

where k is the rate constant, A the Arrhenius pre-exponential factor and E_a the activation energy.

The proper definition of the rate of a photochemical reaction requires caution. The rate constant which defines the chemical transformation of the excited state is usually the only significant one

$$M^* + N \xrightarrow{k} P$$

and the rate of product formation, d[P]/dt, is a function of the quantum yield Φ of the reaction and the intensity of the light absorbed (Suppan, 1982):

$$d[P] / dt = \Phi I_{abs}$$

5.6 Experimental

Preliminary actinometric studies were carried out to examine the factors influencing the photolability of the drug, such as wavelength and intensity of light, and aerobic and anaerobic conditions. The photodecomposition of prochlorperazine at 254 nm (nearest to the drug absorbance peak) was evaluated to determine the quantum efficiency and compared with the photodecomposition of prochlorperazine in sunlight and diffuse light.

5.6.1 Materials

Prochlorperazine mesylate and edisylate salts were obtained from Rhone-Poulenc (Port Elizabeth, South Africa) and Intramed (Port Elizabeth, South Africa), respectively. Sodium acetate and ferric chloride were obtained from Saarchem (Krugersdorp, South Africa). Potassium oxalate and 1,10-phenanthroline were obtained from PAL Chemicals and Aldrich (South Africa), respectively. All chemicals were of analytical grade.

5.6.2 Light sources

Three different light conditions were used.

1) A 254 nm Philips 30W medium pressure Hg lamp in a light cabinet kept at 25 ± 2°C. Samples were placed at distances of either 40 cm or 10 cm from the lamp.
2) Sunlight with samples placed behind glass (wavelength cutoff below 310 nm) at 35 ± 2°C.
3) Diffuse fluorescent laboratory light at 20 ± 2°C.

5.6.3 Sample preparation, placement and analysis

Samples were prepared by dissolving the appropriate quantity of the edisylate and mesylate salts in water in volumetric flasks to give a final concentration of 12.5 mg mL^{-1} and stored for one day in the dark. 1.0 mL of this stock solution was then pipetted into clear or amber glass ampoules which were then sealed. These samples were then subjected to the various light and heat conditions for given lengths of time. Ampoules for use as controls were prepared, stored in dark cupboards and removed when required. Samples to be irradiated were consistently placed relative to the light sources. Samples without packaging were placed in open petri-dishes and irradiated. To allow for evaporation, the sample solutions were reconstituted to their original volumes.

In preparation for the HPLC analyses, 0.1 ml of the irradiated and control solutions were pipetted into 10 mL volumetric flasks. Analyses were then performed using a UV detector at 260 nm (as described in Chapter 3). Decomposition of the drug was then measured as a percentage of the drug peak height divided by the control peak height.

5.6.4 The actinometer and its use

Potassium ferrioxalate was chosen as the chemical actinometer because it is sensitive to wavelengths from 253.4 to 577 nm and is easy to use. It was prepared using the method described by Calvert and Pitts (1966). The $K_3Fe(C_2O_4)_3 \cdot 3H_2O$ salt was produced by mixing 3 volumes of 1.5 *M* $K_2C_2O_4$ (potassium oxalate) solution and one volume of 1.5 *M* $FeCl_3$ solution by agitation. 2.43 g of $FeCl_3$ (iron (III) chloride) was weighed out into a 10 mL volumetric flask and made up to volume using water. 30 mL of water was used to dissolve 8.29 g of $K_2C_2O_4 \cdot H_2O$ to make up the 3 volumes described previously. The preparation was carried out in a darkroom using a red photographic safelight. The reaction was left to stir for two hours and

the precipitated $K_3Fe(C_2O_4)_3 \cdot 3H_2O$ was then recrystallised twice from warm water and dried. This reagent was stored in a foil-covered vial to protect it from light.

For wavelengths up to 430 nm, a 0.006 *M* solution of $K_3Fe(C_2O_4)_3 \cdot 3H_2O$ (0.74 g of the solid was dissolved in 200 mL of water, 25 mL of a 0.5 *M* sulfuric acid was added and the solution diluted to 250 mL) is prepared. In a 1 cm path it absorbs 99% or more of the light up to 390 nm and 50% at 430 nm. Equal irradiation of sample and actinometer solutions can be obtained when they are placed and rotated in a 'merry-go-round' device (Rabek, 1982).

To determine the intensity of a source, 11 mL (V_1) of the ferrioxalate solution was pipetted into clear glass or amber ampoules and carefully positioned relative to the light source. The samples were irradiated for a time (t) and analysed. After irradiation, a 10 mL (V_2) aliquot was pipetted into a 25 mL (V_3) volumetric flask. 2 mL of the 1,10-phenanthroline solution (prepared by dissolving 0.1 g in 100 mL of water) and 5 mL of the buffer solution (6.80 g of sodium acetate in 100 mL of water) are added and mixed well. These solutions were added to 60 mL of a 0.5 *M* H_2SO_4 solution and a further 7 mL of water was added. The transmission of the solution in a 1 cm cell was then measured at 510 nm in a spectrophotometer using a blank solution as a reference.

5.6.5 Calculations

The number of Fe^{2+} ions formed (n_{Fe2+}) during photolysis can be calculated from:

$$n_{Fe2+} = \frac{1000 N_A V_1 V_3 \log 10(I_0/I)}{V_2 L \epsilon}$$

where:

N_A = Avogadro's constant = 6.023×10^{23} mol^{-1}

V_1 = volume of the actinometer solution irradiated (mL)

V_2 = volume of aliquot taken for analysis (mL)

V_3 = final volume to which the aliquot V_2 is diluted (mL)

$\log_{10}(I_0 / I)$ = measured optical density of the solution

L = path length of the spectrophotometer cell used (cm)

ϵ = experimental value of the molar extinction coefficient of the Fe^{2+} complex as determined from the slope of the calibration curve (equal to 1.11×10^4 L mol^{-1} cm^{-1}). The number of quanta absorbed by the actinometer (n_a) is obtained from:

$$n_a = n_{Fe2+} / \Phi_\lambda t$$

where:

t = the irradiation time in seconds (Rabek, 1982; Calvert and Pitts, 1966)

and

Φ = 1.21 (for potassium ferrioxalate).

Both the actinometer and the sample were assumed to absorb the same number of quanta.

The irradiated drug solutions were analysed using the HPLC method described in detail in Chapter 3. The average of three readings was taken and the concentration of the drug was determined by reference to the control samples. From the difference between the control and irradiated samples, the amount of drug decomposed in the sample volume could be determined. The number of molecules of drug decomposed in the sample volume could be determined by multiplying the amount of drug decomposed by factors of 1.0823×10^{18} and 1.0671×10^{18} for mesylate and edisylate, respectively. These values were obtained from:

$$\frac{(12.5 - y \text{ mg mL}^{-1})}{\text{molar mass (g mol}^{-1})} \times \frac{6.0 \times 10^{23} \text{ mol}^{-1}}{1000 \text{ mg m}^{-1}} \rightarrow \text{factor}$$

where:

y = value obtained from HPLC analyses (peak height and calibration curves)

12.5 = the undegraded drug concentration in mg ml^{-1}

6.0×10^{23} = Avogadro's constant (mol^{-1})

The quantum yield for the disappearance of prochlorperazine at the particular wavelength used was obtained by dividing the number of molecules of drug decomposed in a given time by the number of quanta absorbed in that time.

5.7 Results and Discussion

5.7.1 Photodegradation of aqueous solutions of the prochlorperazine salts

From the studies carried out under the various light and atmospheric conditions, the initially colourless solutions, developed a brownish colour after exposure to irradiation when sealed under normal atmospheric conditions (as well as those unpackaged). For those samples sealed under nitrogen conditions, a bright yellow colour was observed. For the kinetic studies, more samples were taken initially, because, with the development of colour, the degradation rate slows down considerably and appears to achieve an equilibrium.

The chromatographic patterns of the products of the photo-induced reaction of prochlorperazine in a aqueous solutions under anaerobic conditions were quite different to those of the products under aerobic conditions. Sulfoxide formation was observed for solutions sealed under air but not for samples sealed under nitrogen. A lack of sulfoxide formation under anaerobic conditions has previously been noted by Huang and Sands (1967), as well as Pawełczyk and Marciniec (1977 a). Sulfoxide formation under aerobic conditions was, however, not the major degradation process. An unidentified product was also found to form in greater quantities under anaerobic conditions than in aerobic conditions.

Photodegradation of pharmaceuticals often follows zero-order kinetics. This can be expected when the rate is limited by factors such as absorption of light (Shahjahan and Enever, 1996). Zero-order behaviour is immediately apparent from plots of % drug remaining (100 D/D_o) against time, under constant intensity irradiation which should be linear (D represents the analytical (HPLC) peak height of the drug as a percentage of the control at a given time (t) while D_o is the initial analytical peak height). In this study, plots of 100 D/D_o against t were usually curved and so they were examined for higher-order (1 or 2) behaviour. Linear plots of ln (D/D_o) against time indicate first-order behaviour, while plots of (D_o/D) or $(1/D)$ against time are linear for second-order reactions. Apparent first-order or second-order rate constants were obtained from the values of the slopes of the appropriate plots. Best straight lines were determined using linear regression. The results obtained are shown in Tables 5.2 - 5.8 and in Figures 5.4 - 5.15.

a) Sunlight

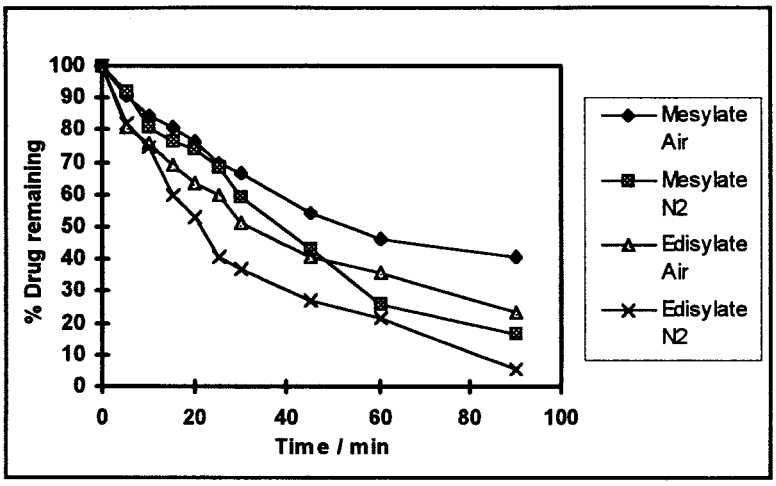


Fig. 5.4 Photodegradation of prochlorperazine salts under sunlight in clear ampoules (in the atmospheres specified)

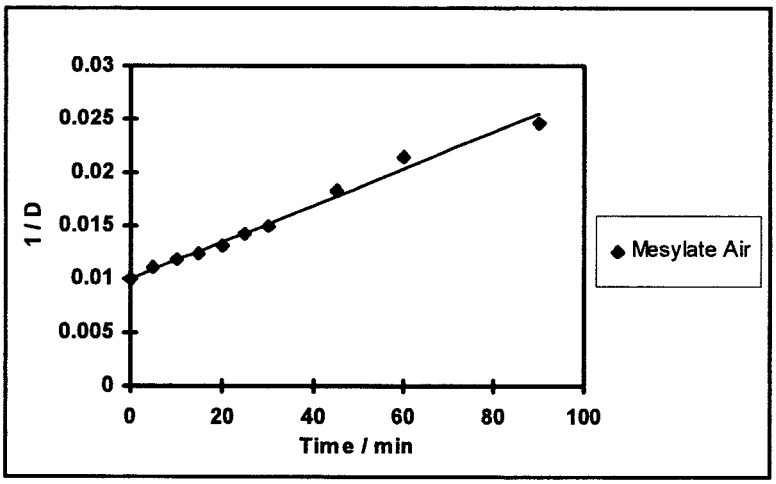


Fig. 5.5 Second-order plot of the photodegradation of prochlorperazine mesylate under aerobic conditions and sunlight

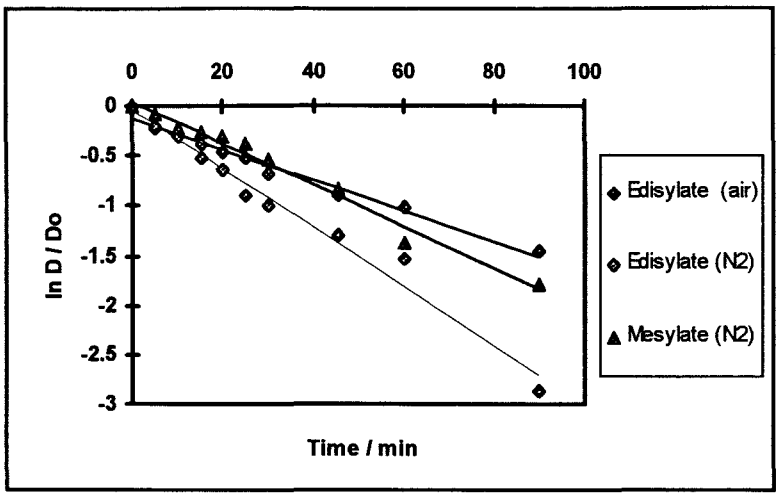


Fig. 5.6 First-order plots of prochlorperazine salts subjected to sunlight in clear glass ampoules (in the atmospheres specified)

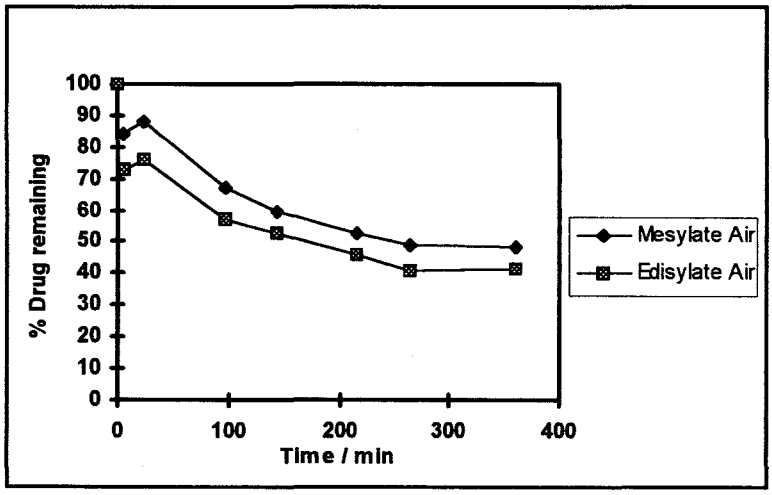


Fig. 5.7 Photodegradation of prochlorperazine salts under sunlight and aerobic conditions in amber ampoules. A small increase in the percentage of drug is observed in both mesylate and edisylate in the early stages of photodegradation

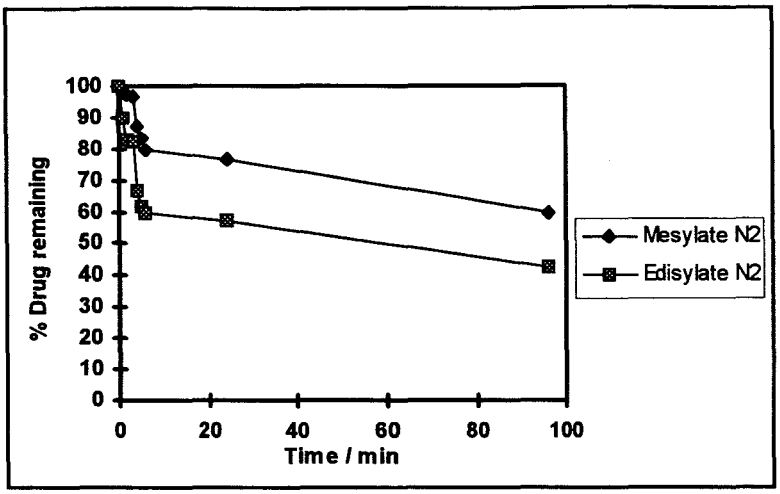


Fig. 5.8 Photodegradation of prochlorperazine salts under sunlight and anaerobic conditions in amber ampoules

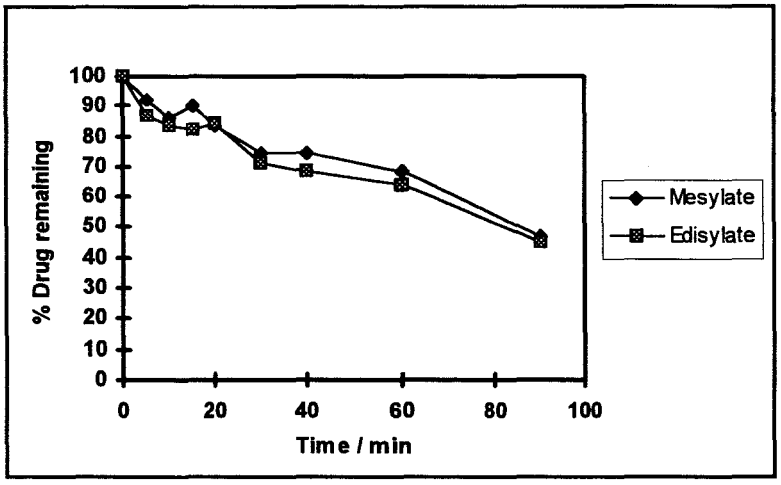


Fig. 5.9 Photodegradation of unpackaged prochlorperazine salts, under sunlight

From the sunlight studies (Figures 5.4 to 5.9), it can be seen that the fastest degradation occurred with those samples sealed under nitrogen, while the mesylate salt sealed under air seemed to be the most stable of all four samples. After 90 minutes in clear ampoules, 45% of the mesylate sealed under air remained, while only 5% of the edisylate sealed in nitrogen remained.

In amber ampoules, after 360 hours (15 days) of exposure to sunlight, more than 40% of the drug remained intact under aerobic conditions, while after only 96 hours under anaerobic conditions, approximately 60% and 43% of mesylate and edisylate

remained intact. Thus the screening effect of the coloured glass is superimposed on the effect of the atmosphere.

Table 5.2

First-order kinetic data obtained for samples tested under sunlight

Sample	Rate constant / min^{-1}	R^2
Me N_2 Cl	(2.09 ±0.11) x 10^{-2}	0.983
Ed Air Cl	(1.54±0.06) x 10^{-2}	0.988
Ed N_2 Cl	(2.95±0.17) x 10^{-2}	0.976

Table 5.3

Second-order kinetic data obtained for samples tested under sunlight

Sample	Rate constant / min^{-1}	R^2
Me Air Cl	(5.07±0.24) x 10^{-10}	0.985
Me Air Am	(3.08±0.34) x 10^{-5}	0.932
Me N_2 Am	(4.81±0.90) x 10^{-3}	0.805
Ed Air Am	(3.82±0.52) x 10^{-5}	0.899
Ed N_2 Am	(6.13±0.26) x 10^{-4}	0.722
Me UP	(1.10±0.12) x 10^{-4}	0.920
Ed UP	(1.19±0.12) x 10^{-4}	0.956

- *apparent reaction orders where:
- Cl = clear ampoules
- Am = amber ampoules
- UP = unpackaged samples
- Me = mesylate
- Air = air in the ampoules
- N_2 = nitrogen in the ampoules
- Ed = edisylate

From the kinetic results described above (Tables 5.2 and 5.3, and Figures 5.7 and 5.8) it can be seen that the degradation of prochlorperazine solutions seems to approximate second-order kinetics for both edisylate and mesylate for amber ampoules. For those solutions irradiated in clear glass ampoules and unpackaged conditions, the degradation had apparent first-order kinetics for all samples, except for the mesylate sample irradiated under air which approximated second-order kinetics.

b) UV light at 254 nm

The effects of 254 nm radiation on prochlorperazine solutions are shown in Figures 5.10, 5.11 and 5.12.

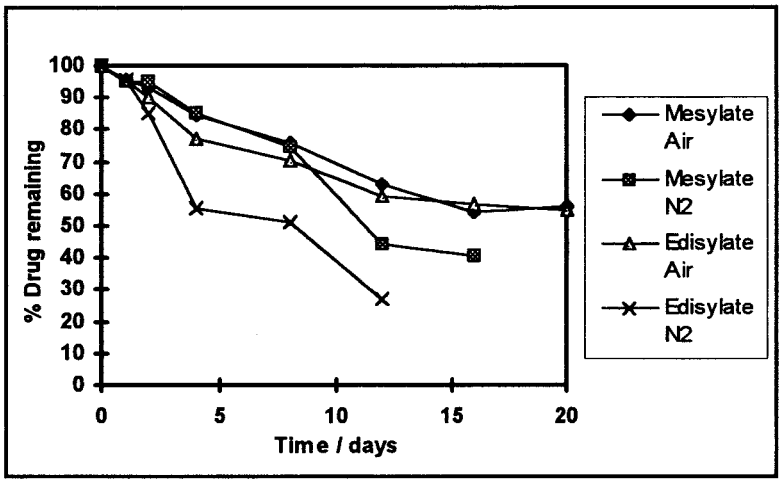


Fig. 5.10 Photodegradation of prochlorperazine salts under 254 nm UV light in clear ampoules (in the atmospheres specified)

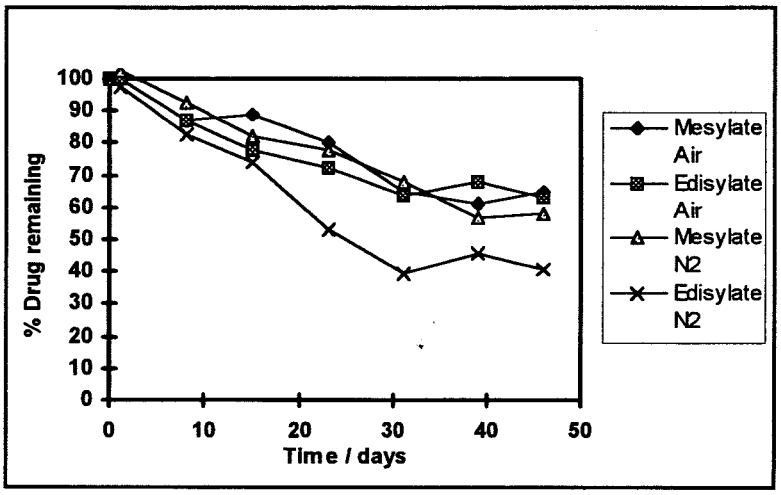


Fig. 5.11 Photodegradation of prochlorperazine salts under 254 nm UV light in amber ampoules (under the specified atmospheres)

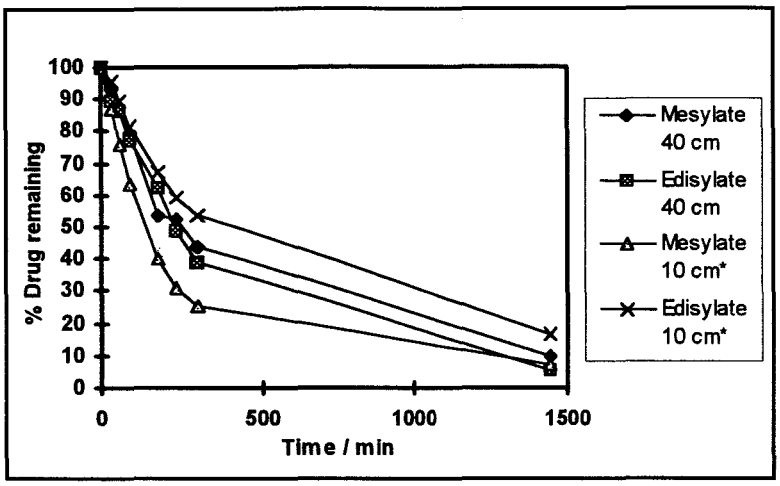


Fig. 5.12 Photodegradation of unpackaged prochlorperazine salts under 254 nm UV light (samples placed 10 cm* and 40 cm from the irradiation source)

In clear ampoules, after 12 days, less than 50% of prochlorperazine remained intact under anaerobic conditions, while samples sealed under aerobic conditions had approximately 60% of the drug intact. In amber ampoules, more than 60% of the drug (except for edisylate under anaerobic conditions) remained intact after 46 days, compared to the 12 days obtained with clear ampoules. The data obtained from the unpackaged sample studies showed that edisylate had the fastest degradation rate under 254 nm irradiation. Almost complete degradation occurred within 24 hours, while mesylate had only 10% of its initial amount intact.

Table 5.4

First-order kinetic data obtained for samples tested under UV light at 254 nm

Sample	Rate constant / days^{-1}	R^2
Me Air Cl	(3.15±0.31) x 10^{-2}	0.953
Me N_2 Cl	(5.52±1.21) x 10^{-4}	0.839
Me Air Am	(1.10±0.13) x 10^{-2}	0.919
Me N_2 Am	(1.32±0.08) x 10^{-2}	0.976
Ed Air Cl	(2.98±0.38) x 10^{-2}	0.924
Ed N_2 Cl	(1.06±0.16) x 10^{-1}	0.935
Ed N_2 Am	(2.14±0.27) x 10^{-2}	0.915

Table 5.5

Second-order kinetic data obtained for samples tested under UV light at 254 nm

Sample	Rate constant / $days^{-1}$	R^2
Me UP	$(6.67\pm0.22) \times 10^{-5}$	0.993
Ed Air Am	$(1.31\pm0.17) \times 10^{-4}$	0.909
Ed UP	$(1.17\pm0.06) \times 10^{-4}$	0.983

- *apparent reaction orders where:

- Cl = clear ampoules
- Am = amber ampoules
- UP = unpackaged samples
- Me = mesylate
- Air = air in the ampoules
- N_2 = nitrogen in the ampoules
- Ed = edisylate

The kinetic data obtained for the prochlorperazine solutions irradiated with 254 nm (samples placed 40 cm from the light source) light are consistent with the results obtained using samples placed 10 cm from the irradiation source. Apparent first-order kinetics were observed, except for the mesylate solution exposed unpackaged conditions and the edisylate sample under aerobic conditions.

Table 5.6

First-order kinetic data obtained for samples tested under UV light at 254 nm (samples placed 10 cm from the light source)

Sample	Rate constant / $days^{-1}$	R^2
Me Air Am	$(7.49\pm0.08) \times 10^{-2}$	0.941
Me N_2 Am	$(6.87\pm0.89) \times 10^{-3}$	0.909
Ed Air Cl	$(1.75\pm0.19) \times 10^{-1}$	0.944
Ed UP	$(1.21\pm0.09) \times 10^{-3}$	0.983

Table 5.7

Second-order kinetic data obtained for samples tested under UV light at 254 nm (samples placed 10 cm from the light source)

Sample	Rate constant / $days^{-1}$	R^2
Me Air Cl	$(2.34\pm0.20) \times 10^{-3}$	0.960
Me N_2 Cl	$(1.37\pm0.13) \times 10^{-2}$	0.966
Me UP	$(9.05\pm0.15) \times 10^{-5}$	0.997
Ed N_2 Cl	$(3.00\pm0.38) \times 10^{-2}$	0.925
Ed Air Am	$(1.33\pm0.11) \times 10^{-4}$	0.957
Ed N_2 Am	$(2.24\pm0.19) \times 10^{-4}$	0.958

- *apparent reaction orders where:
- Cl = clear ampoules
- Air = air in the ampoules
- Am = amber ampoules
- N_2 = nitrogen in the ampoules
- UP = unpackaged samples
- Ed = edisylate
- Me = mesylate

Prochlorperazine exposed in clear ampoules to 254 nm UV light (10 cm away from the light source instead of 40 cm) (Table 5.7) was found to be almost completely degraded after 20 days while after 46 days in amber ampoules approximately 50 to 70% of the drug was found to be intact. With the unpackaged sample studies carried out (Figure 5.12), after 24 hours less than 10% of the drug remained when samples were placed 10 cm from the light source. Thus, the protection offered by both clear glass and the amber ampoules is evident.

Those samples (Tables 5.6 and 5.7) sealed under anaerobic conditions follow mostly second-order kinetics in both amber and clear glass ampoules, except for the mesylate solution irradiated in an amber ampoule.

c) Diffuse light

The effect of diffuse light on prochlorperazine solutions at approximately 20 ± 2°C is presented in Figures 5.13, 5.14 and 5.15.

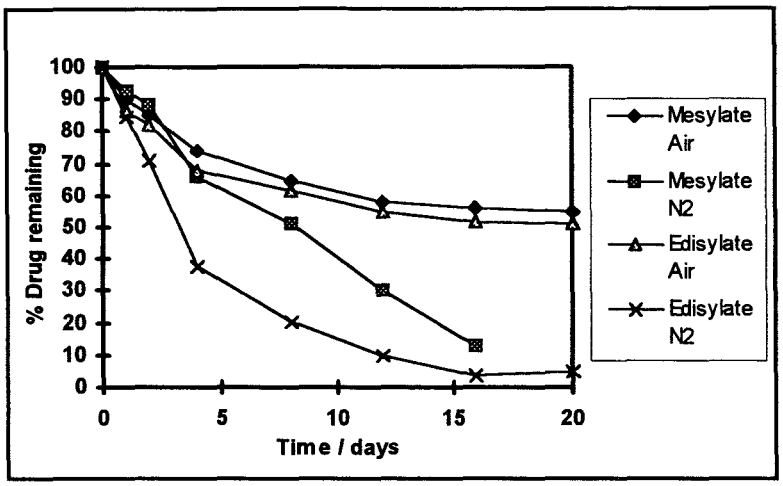


Fig. 5.13 Photodegradation of prochlorperazine salts under diffuse light in clear ampoules (in the atmospheres specified)

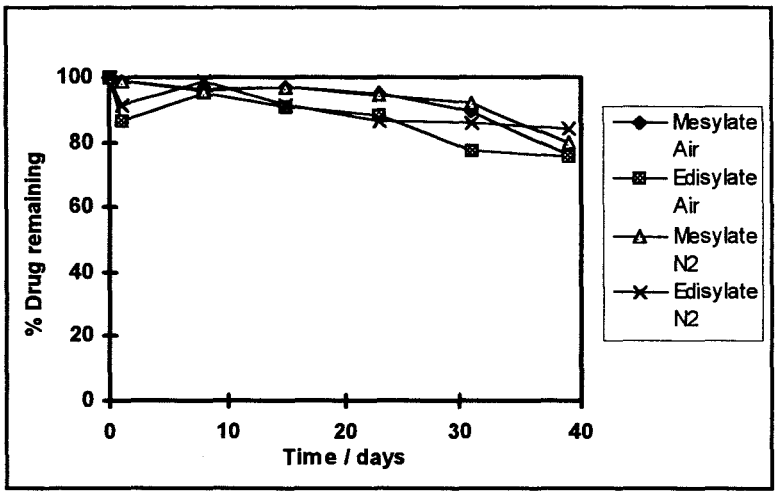


Fig. 5.14 Photodegradation of prochlorperazine salts under diffuse light in amber ampoules

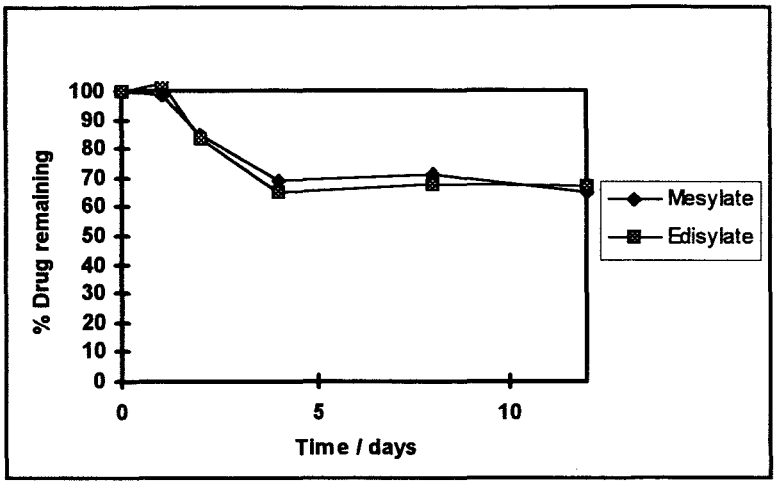


Fig. 5.15 Photodegradation of prochlorperazine salts in unpackaged conditions under diffuse light

In clear glass ampoules the drug was found to degrade to 50% under aerobic conditions after a period of 20 days, while for the same time under anaerobic conditions the drug remaining was decreased to less than 10%. Mesylate was found to be more stable than edisylate under diffuse light, as was found with all other light conditions. In amber ampoules more than 80% of the drug remained intact after 39 days indicating that amber ampoules offer significant protection for longer wavelengths. The amounts of photodegradation under diffuse light conditions was too small to allow for more than approximate kinetic data to be estimated.

Table 5.8

Approximate kinetic data obtained for samples tested under diffuse light

Sample	Reaction order	Rate constant / days^{-1}	R^2
Me Air Cl	second	$2.01\pm0.27 \times 10^{-4}$	0.918
Me N_2 Cl	second	$1.80\pm0.41 \times 10^{-3}$	0.831
Me Air Am	zero	$4.83\pm1.13 \times 10^{-1}$	0.786
Me N_2 Am	zero	$3.97\pm0.94 \times 10^{-1}$	0.780
Me UP	second	$4.34\pm1.18 \times 10^{-4}$	0.772
Ed Air Cl	first	$2.76\pm0.45 \times 10^{-2}$	0.884
Ed N_2 Cl	second	$6.11\pm1.13 \times 10^{-3}$	0.853
Ed Air Am	second	$6.83\pm1.87 \times 10^{-5}$	0.728
Ed N_2 Am	second	$4.31\pm1.08 \times 10^{-5}$	0.760
Ed UP	second	$4.36\pm1.71 \times 10^{-4}$	0.620

- *apparent reaction orders where:
  - Cl = clear ampoules
  - Am = amber ampoules
  - UP = unpackaged samples
  - Me = mesylate
  - Air = normal atmosphere in the ampoules
  - N_2 = nitrogen atmosphere in the ampoules
  - Ed = edisylate

The reproducibility of an experiment refers to how closely the results may be duplicated on repetition under identical conditions. Such information is important in assessing whether observed differences are due to a lack of reproducibility, or to real causes, e.g. air and nitrogen, etc.

The photodegradation experiments were repeated at least twice and the following results were obtained:

1) The edisylate salt was found to degrade faster than the mesylate under all conditions except for irradiation under a 254 nm light.
2) Samples sealed (both in amber and clear glass ampoules) under nitrogen were found to degrade faster than those sealed under air.
3) Samples were found to be more stable in amber ampoules than in clear glass ampoules under all the conditions studied.
4) For both amber and clear glass ampoules, the drug was found to decompose (under air or nitrogen) in the following order from fastest to slowest:

Sunlight >> UV light at 254 nm > diffuse light.

5) For drug samples in clear glass ampoules (sealed in air or nitrogen), samples degraded 5 - 10% after 1 day under UV light irradiation at 254 nm (samples placed 40 cm from the light source), 10% after 5 minutes under sunlight, more than 20% under UV irradiation with samples 10 cm from the light source under 254 nm light and approximately 10% under diffuse light.
6) For amber ampoules: Under all conditions except for diffuse light, edisylate was found to degrade faster than mesylate, with those samples sealed under nitrogen degrading faster than those sealed under air. 10-15% of the sample was found to have degraded after 1 week for those irradiated with the 254 nm UV light (where samples were placed 10 and 40 cm from the light source). 20-30% degradation of the sample occurred upon exposure to sunlight in amber ampoules for both air and nitrogen atmospheric conditions. Less than 5% of the drug degraded when irradiated under diffuse light
7) For unpackaged samples exposed, mesylate was found to be slightly more stable than edisylate except during exposure to UV light at 254 nm.

5.7.2 UV spectroscopy

UV spectra (Figures 5.16, 5.17 and 5.18) of all the degraded samples were obtained. The spectra show that during degradation, the lambda max bands increase and become less resolved. The development of a shoulder at approximately 345 nm in the absorption spectra of aqueous solutions of degraded phenothiazines is indicative of the corresponding phenothiazine sulfoxides (Davidson, 1978). This shoulder is not very clearly seen in the spectra below, but, in Figure 5.18, a small shoulder is beginning to develop after 24 hours in the unpackaged samples.

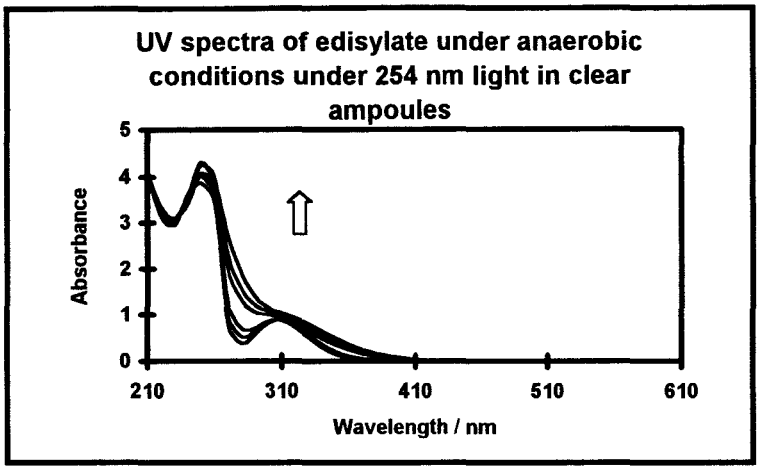


Fig. 5.16 UV spectra of prochlorperazine mesylate samples irradiated in 254 nm light under air in clear ampoules

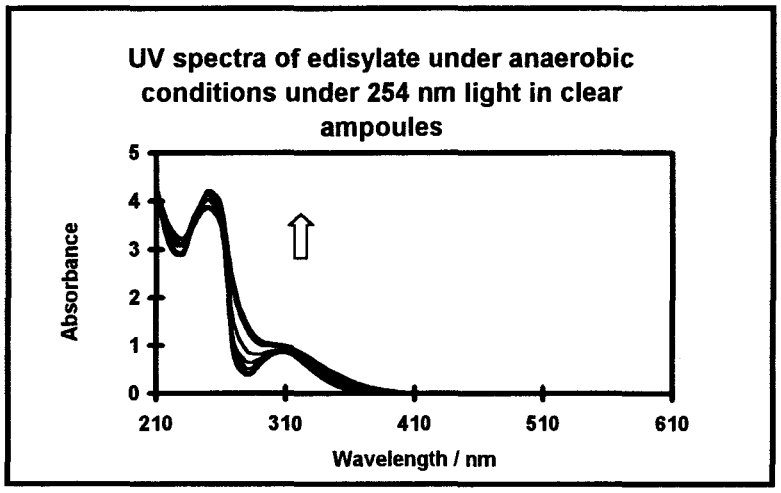


Fig. 5.17 UV spectra of prochlorperazine mesylate samples in 254 nm light under nitrogen in clear ampoules

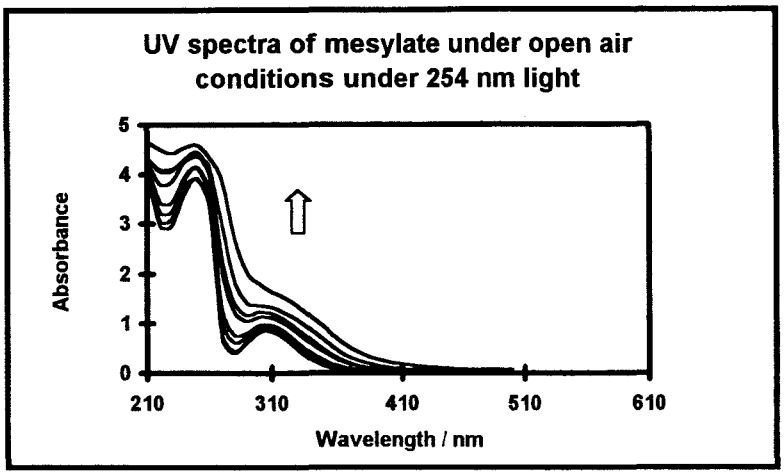


Fig. 5.18 UV spectra of prochlorperazine samples irradiated in 254 nm light in the unpackaged samples

Ordinary window glass transmits only about 50% of the incident radiation at wavelengths about 320 nm (Wayne, 1988) and cuts out all radiation below 310 nm (Moore, 1977). Prochlorperazine, having a lambda max at 250 nm, is expected to have a faster degradation rate under 254 nm UV radiation than under sunlight. The degradation rate was, in fact, found to be faster under sunlight, taking only 90 minutes to degrade to 45% under normal atmospheric conditions and 5% in anaerobic systems. This may be due to a combination of light and heat with temperatures reaching almost 40°C in winter on a window sill. It will be shown later, however, that prochlorperazine solutions are quite stable when exposed to heat only. The slower degradation of prochlorperazine under the UV light may be explained by the fact that there is some protection offered by glass. In the degradation patterns obtained by those samples irradiated unpackaged, the protection offered by the glass is absent. Consequently the degradation of the prochlorperazine salts is faster when samples are irradiated unpackaged than for the solutions sealed in clear glass or amber ampoules.

5.7.3 Effect of wavelength on quantum yield of prochlorperazine mesylate and edisylate

Preliminary actinometric studies were carried out to examine the factors influencing the photolability of the drug (such as wavelength and intensity of the light, and aerobic and anaerobic conditions) on the quantum efficiency of prochlorperazine under the various light conditions.

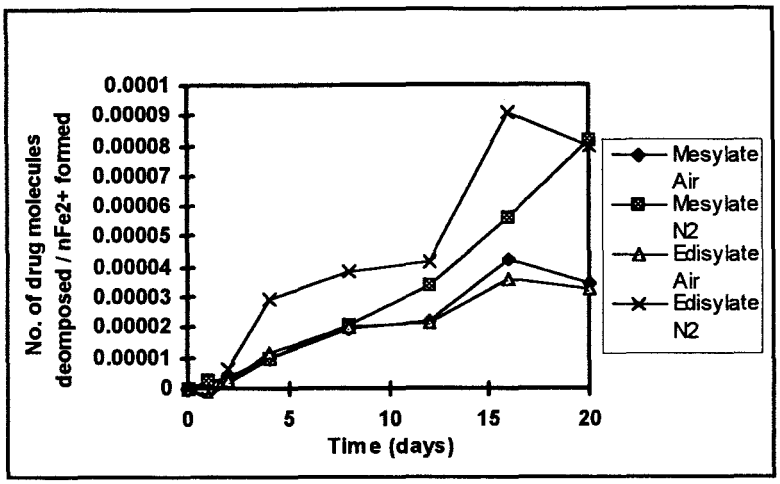


Fig. 5.19 The effect of 254 nm UV light on the quantum yield of prochlorperazine solutions in clear glass ampoules

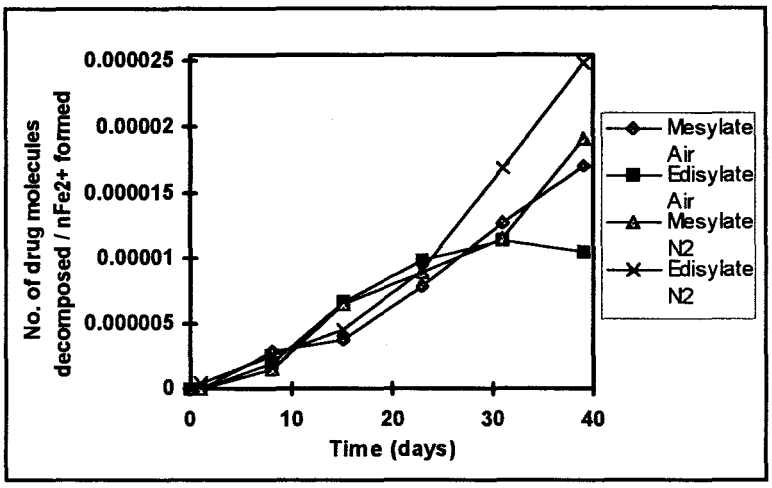


Fig. 5.20 The effect of 254 nm UV light on the quantum yield of prochlorperazine solutions in amber ampoules

Table 5.9

Quantum yield data obtained for both prochlorperazine salts irradiated under 254 nm UV light

Sample	Clear glass ampoules	R^2	Amber ampoules	R^2
Mesylate Air	$(2.44\pm0.24) \times 10^{-6}$	0.955	$(3.89\pm0.34) \times 10^{-7}$	0.957
Mesylate N_2	$(3.39\pm0.24) \times 10^{-6}$	0.976	$(4.43\pm0.32) \times 10^{-7}$	0.895
Edisylate Air	$(2.24\pm0.25) \times 10^{-6}$	0.942	$(2.82\pm0.39) \times 10^{-7}$	0.969
Edisylate N_2	$(5.16\pm0.73) \times 10^{-6}$	0.909	$(6.19\pm0.82) \times 10^{-7}$	0.905

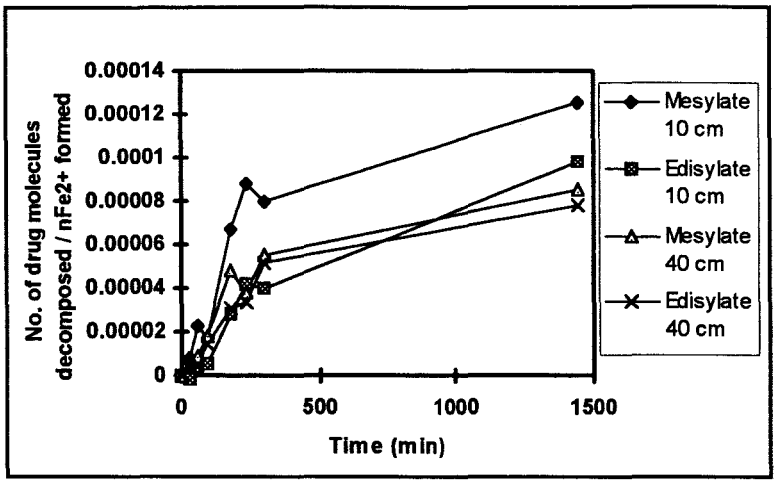


Fig. 5.21 Effect of UV light (samples placed 10 and 40 cm from the light source) on the quantum yield of unpackaged prochlorperazine solutions

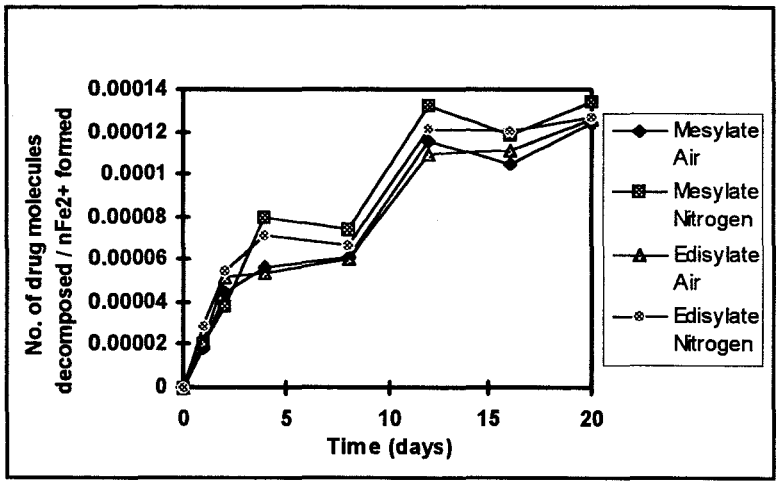


Fig 5.22 Effect of 254 nm UV light (samples placed 10 cm from the irradiation source) on the quantum yield of prochlorperazine in clear glass ampoules

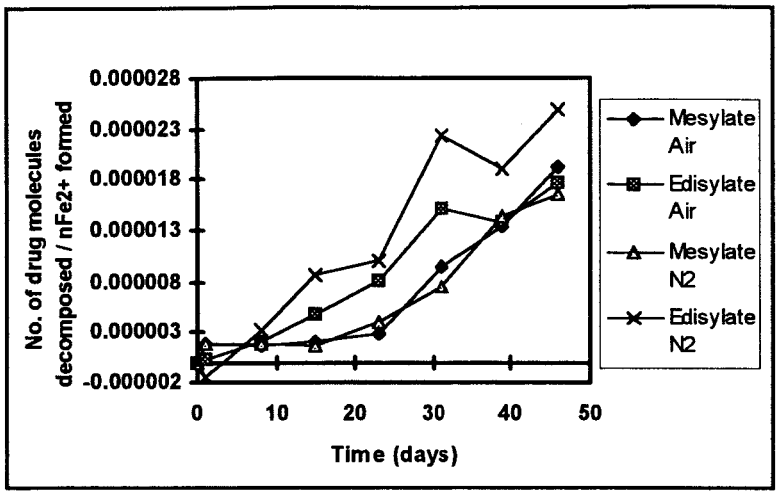


Fig. 5.23 Effect of 254 nm UV light (samples placed 10 cm from the irradiation source) on the quantum yield of prochlorperazine solutions in amber ampoules

Table 5.10

Quantum yield data obtained for both prochlorperazine salts irradiated under 254 nm UV light (samples placed at 10 cm from the light source)

Sample	Clear glass ampoules	R^2	Amber ampoules	R^2
Mesylate Air	$(5.74\pm0.86) \times 10^{-6}$	0.883	$(3.74\pm0.60) \times 10^{-7}$	0.865
Mesylate N_2	$(6.36\pm1.12) \times 10^{-6}$	0.842	$(3.41\pm0.50) \times 10^{-7}$	0.885
Edisylate Air	$(5.73\pm0.79) \times 10^{-6}$	0.897	$(3.98\pm0.33) \times 10^{-7}$	0.961
Edisylate N_2	$(5.28\pm1.30) \times 10^{-6}$	0.732	$(5.73\pm0.59) \times 10^{-7}$	0.941

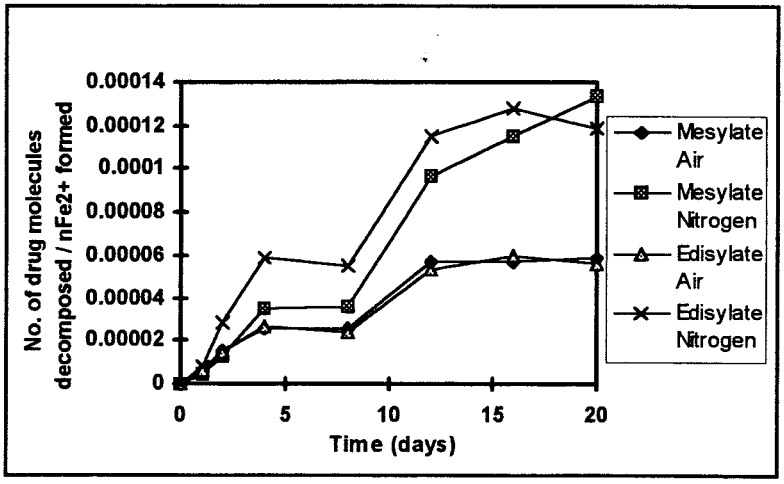


Fig. 5.24 Effect of diffuse light on the quantum yield of prochlorperazine solutions in clear glass ampoules

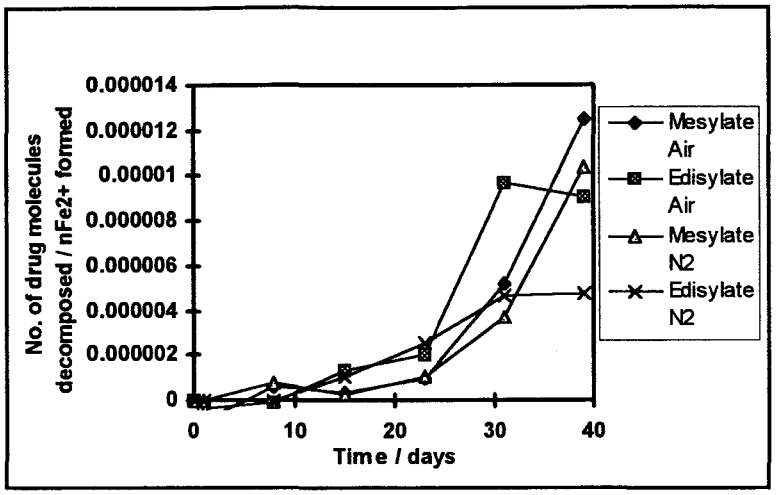


Fig. 5.25 Effect of diffuse light on the quantum yield of prochlorperazine solutions in amber ampoules

Table 5.11

Quantum yield data obtained for both prochlorperazine salts irradiated under diffuse light in clear glass and in amber ampoules

Sample	Clear glass ampoules	R^2	Amber ampoules	R^2
Mesylate Air	$(3.01\pm0.42) \times 10^{-6}$	0.897	$(2.72\pm0.72) \times 10^{-7}$	0.740
Mesylate N_2	$(6.98\pm0.52) \times 10^{-6}$	0.967	$(2.11\pm0.62) \times 10^{-7}$	0.700
Edisylate Air	$(2.98\pm0.42) \times 10^{-6}$	0.895	$(2.66\pm0.55) \times 10^{-7}$	0.824
Edisylate N_2	$(6.45\pm0.95) \times 10^{-6}$	0.885	$(1.39\pm0.16) \times 10^{-7}$	0.935

The results obtained from the actinometric studies conducted in amber ampoules were somewhat erratic as evidenced by the R^2 values obtained (Table 5.11). This experiment would have to be repeated in order to obtain reproducible results.

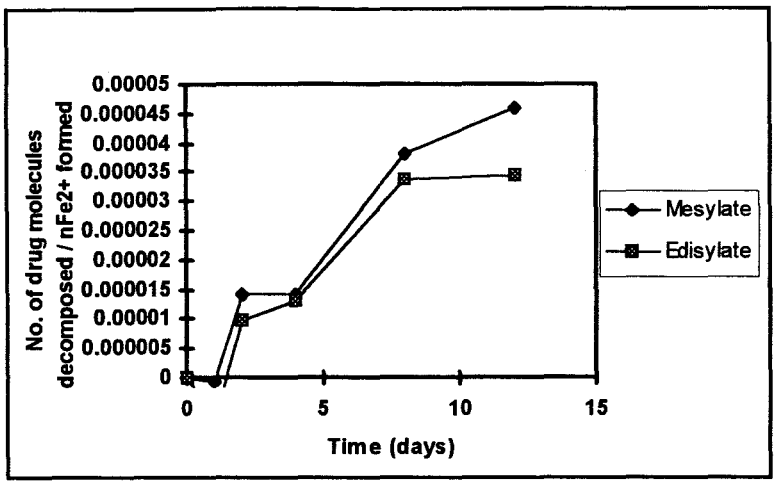


Fig. 5.26 Effect of diffuse light on the quantum yields of unpackaged prochlorperazine solutions

The effect of wavelength of irradiation on the quantum yield for photodegradation of prochlorperazine shown in Figures 5.19 to 5.26. Quantum yields obtained varied from (2.24±0.25) x 10^{-6} to (6.98±0.52) x 10^{-6} for those samples sealed in clear glass ampoules. The quantum yield was found to decrease at longer wavelengths. Prochlorperazine salts in aqueous solution exhibit wavelength maxima at approximately 307 and 250 nm and is thus more likely to be susceptible to decomposition in these wavelength regions. This may be due, in part, to glass ampoules used in the actinometric and photodegradation studies. Yoshioka *et al.* (1994) showed that only about 10% of the 254 nm radiation is transmitted to the sample, compared to the 90% transmission occurring at 365 nm radiation. Shahjahan and Enever (1996) showed, with the use of 1 cm silica cells, that the quantum yield decreased with increasing wavelength.

From the results obtained for the quantum yield studies done on the two types of ampoules, it can be seen that those samples sealed under nitrogen have the greatest quantum yields. For the unpackaged samples and those samples sealed under normal atmospheric conditions, mesylate seems to degrade only slightly faster than the edisylate samples, with the degradation being more pronounced in the unpackaged samples.

The effect of the type of ampoules on the quanta absorbed by the actinometer is shown in Figure 5.27, the number of ions formed in the actinometer in clear glass ampoules and in amber ampoules, are similar within experimental error. This

suggests that amber ampoules do not offer significant protection to the high energy 254 nm wavelength radiation.

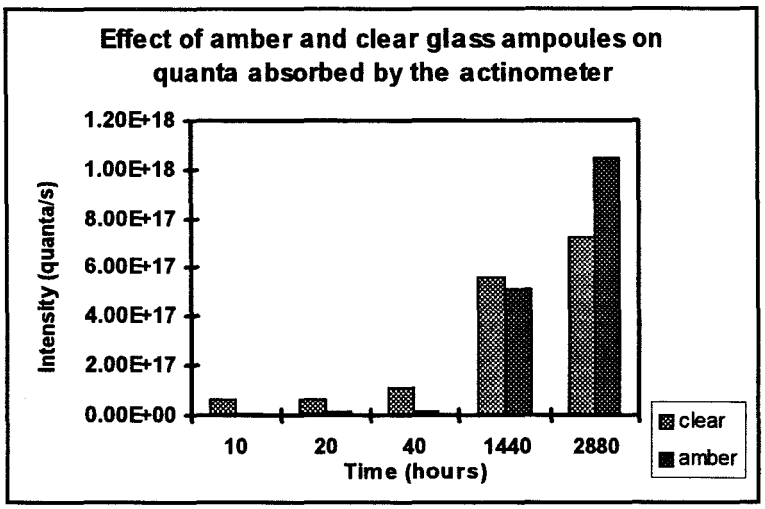


Fig. 5.27 Effect of amber and clear glass ampoules on the quanta absorbed by the ferrioxalate actinometer

5.7.4 Thermal degradation of prochlorperazine solutions under aerobic and anaerobic conditions

The thermal degradation of prochlorperazine salts at a series of different isothermal temperatures was studied. The usually colourless solutions developed a pink colour under aerobic conditions and stayed either colourless, or developed a pale beige colour, under anaerobic conditions. The pink colour has been attributed to the formation of the semiquinone free-radical.

The thermal degradation of aqueous prochlorperazine salt solutions at 100°C is shown in Figure 5.28.

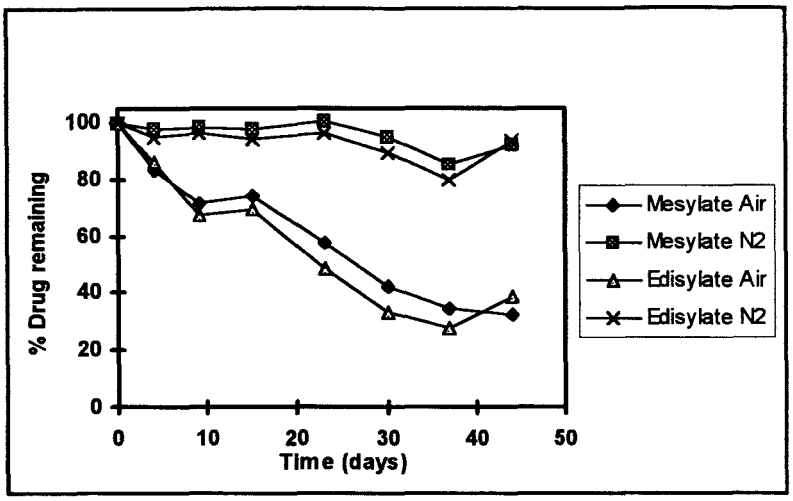


Fig. 5.28 Thermal degradation of prochlorperazine solutions at 100°C

The samples sealed under aerobic conditions are seen to degrade faster than those samples sealed under anaerobic conditions. After 44 days, more than 90% of the drug remained intact under anaerobic conditions while under aerobic conditions less than 40% remained. The chromatographic patterns of the products obtained showed the sulfoxide to be the major product of thermal degradation. Little or no sulfoxide formation was observed in the samples heated under anaerobic conditions. This is to be expected because sulfoxides are formed by aerial oxidation of the parent phenothiazine. Small amounts of sulfoxide formation under anaerobic conditions were attributed to incomplete removal of either the atmospheric oxygen or the residual oxygen present in the solution itself. Pawełczyk and Marciniec (1975) found that 10-substituted phenothiazines degrade, primarily, due to oxidation of the ring S-atom and that the substituents or any other part of the molecule are not involved in the thermal oxidation. Once again, the mesylate was found to be more stable than the edisylate under both anaerobic and aerobic conditions.

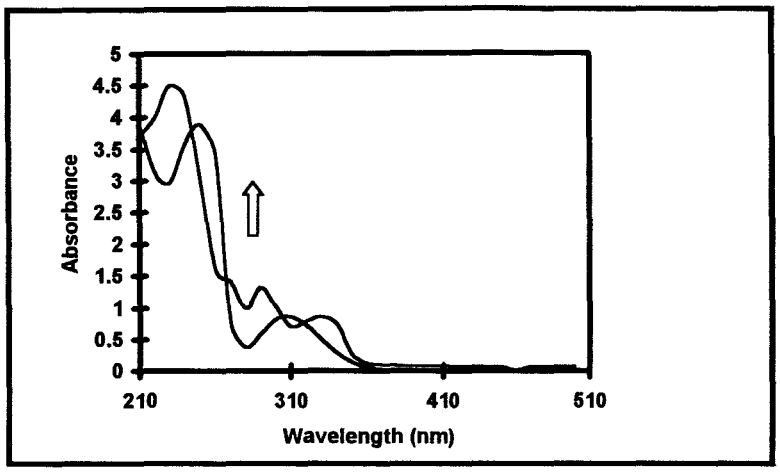


Fig. 5.29 UV spectra of prochlorperazine mesylate and the product mixture after heating under aerobic conditions at 100°C

The UV spectra in Figure 5.29 shows the shift of the UV absorption bands upon degradation of the prochlorperazine mesylate solutions under aerobic conditions at 100°C. The development of a broad shoulder at 330 nm and the narrowing of the original peak at 307 nm is clearly indicated. The bands also shift to shorter wavelengths and three clear peaks were observed instead of the usual two. Sulfoxide derivatives have a high molar absorptivity for UV radiation but at wavelength maxima different from those of unchanged phenothiazine thus giving very distinct UV absorption spectra.

The UV spectra obtained during the attempted thermal degradation of prochlorperazine for 32 days under anaerobic conditions at 100°C, confirmed the stability of the drug at this relatively high temperature. The removal of oxygen prevents the formation of sulfoxide and the spectra remained unchanged.

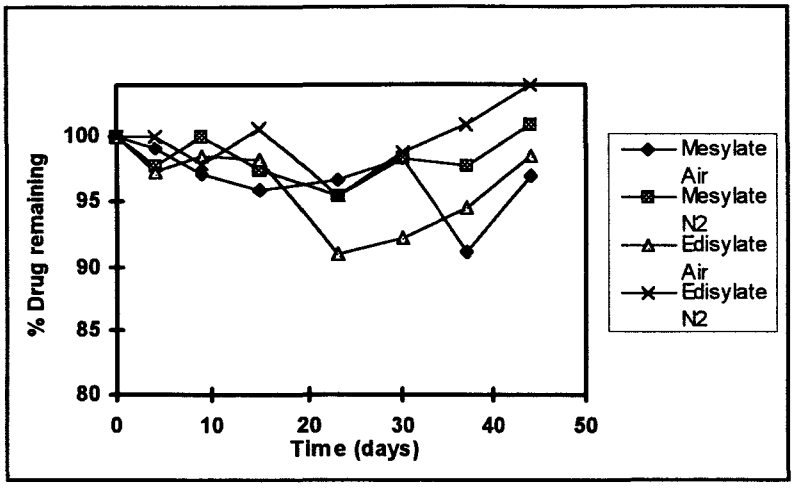


Fig. 5.30 Thermal degradation of prochlorperazine salt solutions at 60°C.

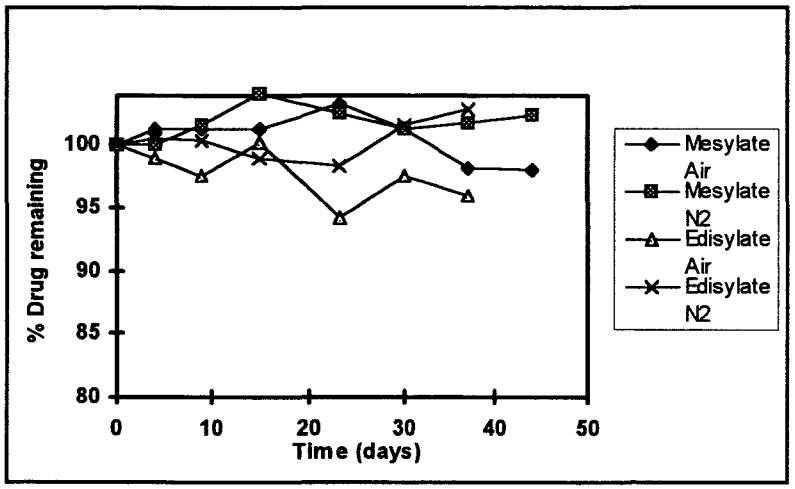


Fig. 5.31 Thermal degradation of prochlorperazine salt solutions at 40°C

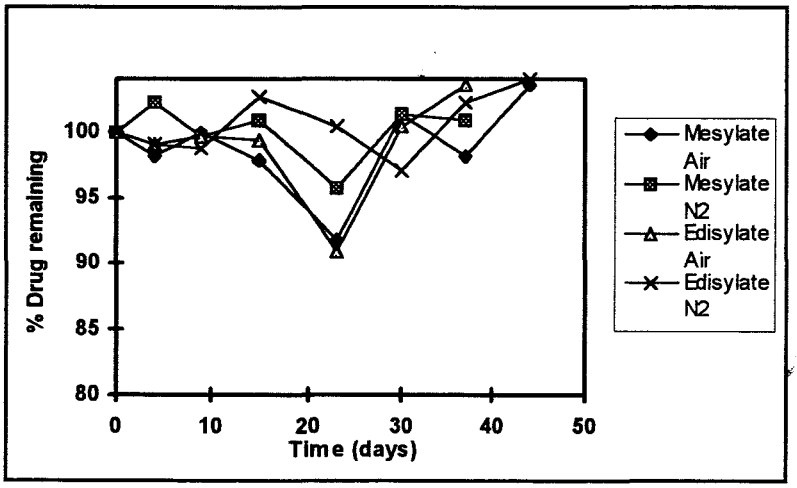


Fig. 5.32 Thermal degradation of prochlorperazine salt solutions at 25°C

The thermal degradations of aqueous prochlorperazine salt solutions at 60°, 40° and 25°C are shown in Figures 5.30, 5.31 and 5.32. The data indicate that the aqueous solutions of prochlorperazine are reasonably stable with more than 90% of the drug remaining intact after 44 days at 60°C under both aerobic and anaerobic conditions. An approximate kinetic analysis suggested that the thermal degradations of the mesylate and edisylate were second-order processes with under aerobic conditions at 100°C. The apparent rate constants and Arrhenius parameters obtained are listed in Table 5.12.

Table 5.12

Kinetic data for the thermal degradation of aqueous solutions of prochlorperazine salts

Sample	Apparent reaction order*	Rate constant / days^{-1}	R^2
100°C Me Air	second	(4.86±0.45) x 10^{-4}	0.969
100°C Me N_2	second	(2.62±0.11) x 10^{-5}	0.518
100°C Ed Air	second	(5.25±1.13) x 10^{-4}	0.781
100°C Ed N_2	second	(2.98±1.55) x 10^{-5}	0.381
60°C Me Air	second	(1.07±0.63) x 10^{-5}	0.329
60°C Me N_2	second	(3.80±4.77) x 10^{-7}	0.001
60°C Ed Air	second	(9.52±8.40) x 10^{-6}	0.176
60°C Ed N_2	second	(5.80±5.95) x 10^{-6}	0.136
40°C Me Air	second	(5.63±3.91) x 10^{-6}	0.257
40°C Me N_2	second	(3.70±3.06) x 10^{-6}	0.198
40°C Ed Air	second	(1.07±5.67) x 10^{-5}	0.415
40°C Ed N_2	second	(5.40±4.33) x 10^{-6}	0.236
25°C Me Air	zero	(4.68±8.52) x 10^{-2}	0.048
25°C Me N_2	second	(1.75 ±6.91) x 10^{-6}	0.013
25°C Ed Air	zero	(3.41±1.21) x 10^{-2}	0.015
25°C Ed N_2	zero	(6.84±0.05) x 10^{-2}	0.226

Using the data in Table 5.12 it is possible to estimate apparent Arrhenius parameters (pre-exponential factors, A, and activation energies, E_a) for the thermal

degradations of the two salts in air and in nitrogen, by plotting ln k versus 1/T (where T is in Kelvin).

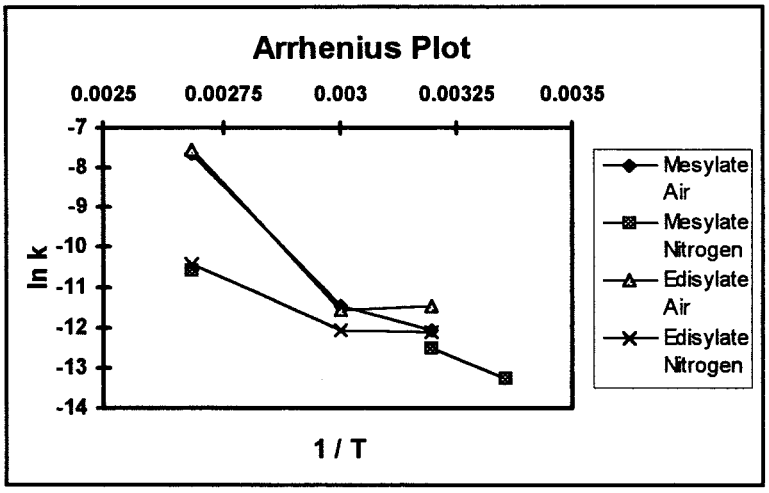


Fig. 5.33 Arrhenius plot for the thermal degradations of prochlorperazine salt solutions

where:

intercept = ln (A / time units^{-1}) slope = $-E_a / R$

Although there are few data points the Arrhenius parameters, even with their large uncertainties, appear to depend upon the atmosphere (Figure 5.33). Both E_a and ln A values are smaller in nitrogen than in air. This supports the occurrence of different reactions.

5.7.5 pH changes taking place during thermal and photolytic studies

The pH's of the mesylate and edisylate solutions were measured before and after exposure to various heat and light conditions. The results obtained are shown in Table 5.13.

Table 5.13

Effect of photolytic and thermal degradation on pH

Sample	pH
Me (undegraded)	3.57
Ed (undegraded)	2.93
Sun Me Air Cl	2.51
Sun Me N_2 Cl	1.51
Sun Me Air Am	1.62
Sun Me N_2 Am	1.45
$UV_{254\ nm}$ Me Air Cl	2.15
$UV_{254\ nm}$ Me N_2 Cl	2.21
$UV_{254\ nm}$ Me Air Am	2.35
$UV_{254\ nm}$ Me N_2 Am	2.72
$UV_{254\ nm}$ Ed Air Cl	2.32
$UV_{254\ nm}$ Ed N_2 Cl	2.22
100°C Me Air	2.86
100°C Me N_2	2.78

A general decrease in pH is observed with all the degraded samples. This decrease in pH has been observed by previous investigators (Felmeister and Discher, 1964; Huang and Sands, 1964, 1967). The greatest decrease in pH is observed with the samples exposed to sunlight.

A less discernible drop is observed for the two samples thermally treated at 100°C. This was expected because, in the absence of light, free-radical formation does not occur and a chloride ion does not form. Thus the formation of HCl is probably inhibited, with no liberation of H^+ and Cl^- to cause the decrease in pH. Pawełczyk *et al.* (1975) concluded that halogen substituents of a type X or CX_3 in position 2 of the phenothiazine molecule influence the sulfur atom in the ring by induction and resonance. This results in an increase of the electron density on that atom, corresponding with a decrease in its acidity and a general decrease in reactivity of the molecule.

5.4 Conclusions

The photolytic degradation studies showed that, for both salts, samples sealed under nitrogen degraded faster than those sealed under air. Molecular oxygen (in air) has a triplet ground state, $^3O_2$, and acts as a quencher. It has the ability to quench both singlet and triplet excited states and to react readily with radical intermediates. Drugs, on irradiation, yield free-radicals that are scavenged, with a 1:1 stoichiometry, by oxygen molecules. The degradation of prochlorperazine follows a free-radical pathway (Type I) and reaction of this triplet excited drug molecule with a molecule which possesses a triplet ground state (O_2) leads to quenching. Quenching thus removes the drug's molecular excitation via a physical process which leaves it unchanged with respect to its nuclear configuration. The presence of oxygen can thus inhibit and / or significantly modify photoprocesses. The absence of oxygen was thus found to enhance the rate of degradation. Huang and Sands (1967) found that polymerisation processes predominate under anaerobic conditions. Upon irradiation chlorine free-radicals are formed and, as a result, a carbonium ion is created in position 2. A carbon in position 7, however, having a high electron density, is susceptible to free-radical attack which is triggered by the carbonium ion in position 2. A polymer with a 2-7 linkage results.

The photolytic studies have shown that the use of amber ampoules does not significantly retard photolytic degradation. This would be advantageous to the industry because the solutions could therefore be packaged in cheaper clear glass ampoules. The prochlorperazine solutions irradiated in both the amber and clear glass ampoules showed the formation of the same degradants. The degradation rates, under the various light conditions observed, were in the following order from fastest to slowest: Sunlight > 254 nm UV light > diffuse light for both amber and clear glass ampoules. The studies carried out on the unpackaged samples showed the same order.

As found for the photo-degradation, thermal studies carried out under aerobic and anaerobic conditions showed that oxygen plays an important roles. The thermal stability studies indicated that prochlorperazine is quite stable in aqueous solutions up to 60°C.

The energy of 1 mol of photons of wavelength is given by:

$$E = N_A \frac{hc}{\lambda}$$

Thus, the 254 nm radiation used to excite the $\pi \rightarrow \pi^*$ transition in prochlorperazine has an energy of 471 kJ mol^{-1}. This indicates the amount of energy involved in a typical photochemical conversion. UV radiation has energies in excess of many bond energies (for example, the bond energy of the Cl-Cl bond in molecular chlorine is 240 kJ mol^{-1} and a typical C-C σ-bond energy is 350 kJ mol^{-1}). Low energy (long wavelength) radiation is sufficient to excite molecular rotation and vibration which involve displacements of nuclei. With higher energy (short wavelength) radiation the electrons in bound molecular energy states are excited causing chemical change (Gilbert and Baggott, 1995).

The quantum yields for the degradation of prochlorperazine solutions under various conditions were of the order of 4.95×10^{-7} to 1.27×10^{-4}. These low quantum yields may be due to: 1) loss of absorbed energy through collisions of drug molecules with solvent molecules; 2) the gain of an electron by the photoproduced radical, resulting in the reformation of prochlorperazine; and / or 3) fluorescence, the emission by the excited molecule at a higher wavelength of part of the absorbed energy. Felmeister and Discher (1964) proposed that a disproportionation reaction (Figure 5.3) would also favour a low quantum efficiency because the starting material is continually being regenerated.

The increase in quantum yields for those samples sealed under nitrogen suggests that nitrogen did not exert any quenching effect on the photochemical reaction but may even have a sensitising effect. Samples were initially sealed under nitrogen in order to prevent degradation of prochlorperazine under light because sulfoxide formation has been found to be the major pathway of degradation. De-aeration of the system was thus shown to be insufficient to prevent the photodecomposition of the prochlorperazine aqueous solutions. This may be due to water taking part in the reaction.

The decrease in pH observed for the degraded solutions of prochlorperazine, which implies the liberation of HCl, has been suggested as the possible cause of the photosensitivity and skin discolouration of patients receiving chlorpromazine

medication for a prolonged period. The chloride function at C_2 of the molecule is thought to be cleaved easily under these conditions (since chlorine is photolabile) and this is supported by the decrease of the pH.

The deep brownish colour which develops in the irradiated samples under aerobic conditions and the UV spectra obtained for these samples (Figure 5.16) suggest the absence of semiquinone free-radicals, because solutions containing the semiquinone free-radical are pinkish and the UV spectra would have the three characteristic absorption peaks present (with the middle peak attributed to the radical). This absence may be due to the rapid disproportionation of the semiquinone free-radical at low hydrogen ion concentrations (Felmeister and Discher, 1964). Higher pH values (Table 5.13) were obtained for the samples undergoing heat treatment only and these solutions showed the pink colouration and the distinctive UV spectra (Figure 5.29) associated with the semiquinone free-radical. The semiquinone free-radical would then disproportionate and form the phenazathionium ion and eventually the sulfoxide (Figure 5.3). This may explain why sulfoxide was not found to be the major degradant in the chromatographic patterns obtained from the studies carried out under aerobic conditions.

CHAPTER 6. SOLID STATE STABILITY STUDIES

This chapter is concerned with solid state stability studies of prochlorperazine mesylate and edisylate. In these studies, the stabilities of both salts were evaluated using HPLC.

6.1 Introduction

Degradation of solids may have a thermal as well as a photolytic origin. At sufficiently high temperatures intramolecular bonds may be disrupted resulting in the formation and subsequent growth of nuclei of degradation product(s). It is possible that partial and / or localised melting may accompany degradation. The products and reactant may sometimes form a eutectic phase. Mechanisms of thermal and photodegradation may be similar or vastly different.

In addition, there is always the possibility of reaction of the initially solid reactant with residual moisture causing hydrolysis on a more localised scale than observed in solution. Reaction may also occur with the surrounding gaseous atmosphere.

6.2 Solid state stability

The mechanisms involved in the solid state decompositions of drugs are complex and the kinetic factors affecting the decompositions are of both theoretical and practical importance (Yoshioka and Uchiyama, 1986). Photochemical mechanisms of solid state reactions have been reviewed (Byrn, 1976). Because of the complexity of photochemical reactions, very little on the photostability of solid dosage forms has been reported (Matsuda and Masahara, 1983).

Since aqueous solutions of prochlorperazine are not stable under various light conditions, the objective of this investigation was to obtain information on the stability of the solid prochlorperazine salts (prochlorperazine mesylate and edisylate) in the presence of light and heat, under ordinary and accelerated storage conditions. The lack of reported information may be due to the slow rates of decomposition in the solid state relative to solutions.

6.3 Experimental

6.3.1 Materials

Both prochlorperazine mesylate and edisylate were used for the solid state studies.

6.3.2 Kinetic procedures for the solid state studies

Individual powder samples of about 80 mg each were stored in plastic petri-dishes and were exposed to the various photolytic and thermal (in constant temperature ovens) conditions in a thin layer. Samples were removed periodically for assay. The accurately weighed samples were dissolved in water in a 10 mL volumetric flask and 20 μL of the aqueous solution was injected onto the HPLC column.

6.3.3 Analytical procedures for stability studies

HPLC

The HPLC method validated in Chapter 3 was used to follow the degradation of prochlorperazine in the solid state. IR and UV spectroscopy, as well as the use of photodiode array detection, were used to determine whether any degradation had occurred. The IR data were obtained using a silver chloride disc with the samples dissolved in HPLC grade methanol.

LC-MS

LC-MS was used to aid the detection of degradants not picked up by UV detection at a sensitivity of 0.5 AUFS. The method used is described in detail in Chapter 8. A C_{18} μ-Bondapak steel cartridge column with a mobile phase consisting of 80 : 20 methanol : 20 mM ammonium acetate at a flow rate of 1.0 mL min^{-1} was used. The parameters used on the mass spectrometer are identical to those used for structural elucidation, also described in Chapter 8. Samples used included solid state samples exposed to the various light conditions for 6 months and samples stressed at 100° and 60°C.

6.4 Results and discussion

6.4.1 HPLC

The results obtained from the photolytic and thermal studies are shown in Figures 6.1 and 6.2 and are discussed below.

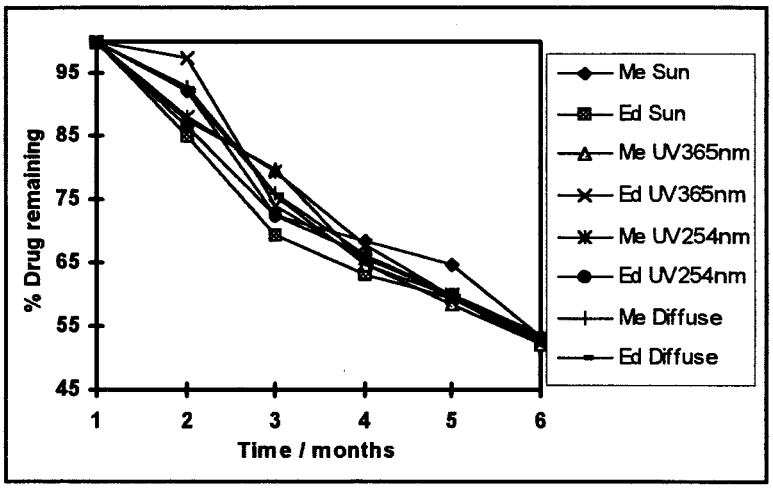


Fig. 6.1 The effect of various light conditions on the degradation of prochlorperazine mesylate and edisylate

The photodegradation of prochlorperazine mesylate and edisylate in the solid state appeared to follow mostly first-order kinetics under all light conditions. First-order rate constants were used as an empirical method of comparison.

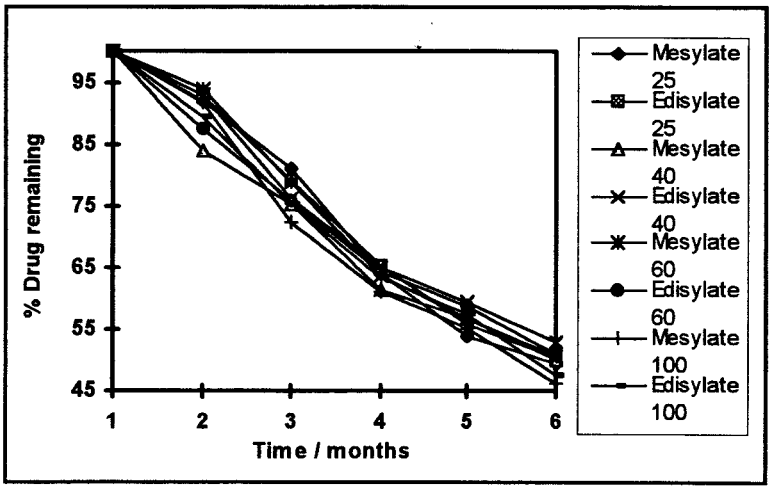


Fig. 6.2 The effect of various heat conditions on the degradation of prochlorperazine mesylate and edisylate

Accelerated stability testing of the amorphous (mesylate) and crystalline (edisylate) prochlorperazine solids was carried out by exposing samples to various photolytic and elevated temperature conditions. The amount of drug remaining after treatment was determined using HPLC. HPLC chromatograms did not reveal the nature of the degradants. An approximate kinetic analysis was carried out on plots of percentage drug remaining against time (Figures 6.1 and 6.2). Both the photo- and thermal degradations were found to be approximately first order with respect to the drug. The reaction mechanism is undoubtedly more complex than an equivalent homogenous first-order reaction.

The degradation of prochlorperazine in the solid state at 25°C in the absence of additional light, shows loss of about 45% of the drug almost linearly over 6 months. This rate of degradation is not increased by increasing the temperature to as high as 100°C, or by exposing the sample to various types of illumination. The lack of sensitivity to temperature indicates a low value of the apparent activation energy for thermal degradation, but it is not possible to establish the nature of the rate-controlling process.

In general, the thermal degradation of the mesylate was slightly faster, than that of the edisylate at the same temperature. This could be explained by the crystalline state of the edisylate salt as opposed to the amorphous state of mesylate or by slight differences in the influences of the mesylate and edisylate constituents. Comparisons of the thermal decomposition data for crystalline and amorphous solids have demonstrated that crystalline material is usually more stable, e.g. crystalline cefoxitin sodium and amorphous cefoxitin sodium, and the amorphous forms of penicillin potassium, epicillin and cephalosporins are less chemically stable than their crystalline counterparts (Oberholtzer and Brenner, 1979).

Solid state samples are generally more stable than aqueous solutions of the same compound. Prochlorperazine is thought to degrade via a free radical pathway, due to Cl^-, but because this ion is present only in trace amounts in the solid state, the powders are expected to be more stable than the solutions of the drug. Byrn (1967) suggested that a reaction occurring in an inert solvent at a reasonable rate at 60 to 100°C below the melting point of the substances present, will probably occur in the solid, although this rule-of-thumb is not generally recognised. In solid state chemistry, the Tamman temperature is defined as 0.5 T_m, where T_m is the melting

point in Kelvin. Solids are more reactive above their Tamman temperatures. Since the prochlorperazine salts have such a high melting points they could be expected to be thermally stable at room temperature.

Upon ageing, amorphous prochlorperazine (mesylate) developed a more intense colour than edisylate upon irradiation. This is another indication that the crystalline edisylate was slightly more stable than the mesylate in the solid state.

The colours that developed due to exposure to radiation were found to develop only in the thin layers on top of the exposed powders. The colour changes observed in both edisylate and mesylate were greatest for those samples exposed to sunlight while the least colour change was observed with diffuse light. The UV spectrum of the original sample before irradiation gave an absorption maximum and minimum at 247 and 275 nm respectively. Grottus-Draper's law states that only light absorbed by a substrate can cause chemical change, absorption of light does not necessarily produce degradation (Matsuda and Matahara, 1983). After a molecule has absorbed a quantum of energy, collision with other molecules may result in transfer of kinetic energy, seen as an elevation of the system temperature.

The IR data obtained for the solid state studies of both mesylate and edisylate were compared at the end of the six month period and found to be identical. The IR data were confirmed by the UV spectroscopy and photodiode array analyses. These techniques did not show any features which could be associated with the formation of degradation products. Although HPLC showed a reduction in peak height, no peaks of degradation products were present. Since a UV detector was used for the HPLC analyses, this may indicate that the degradation products are not UV absorbing, rendering both photodiode array (which also uses UV detection) and UV spectroscopy unusable. Other techniques which could be used include the use of a refractive index (RI) detector (which has the disadvantage of being less sensitive than UV), or electrochemical techniques. The use of internal standards in solid state stability studies would also be useful in order to ascertain whether the drug is degrading or whether it is the HPLC method that is at fault. Mass spectrometry coupled with an HPLC system is also a very good alternative because it is reliant on the detection of the molecular mass of a molecule. The development of the colour (observed during the exposure to irradiation) may be due to photochroism. Photochroism is usually a reversible occurrence and the drug does not degrade.

For example, some sydnones are photochromic and at least one derivative of morphine has thermochromic properties. *N*-(3-pyridyl)sydnone gives colourless crystals which turn blue on exposure to sunlight. The IR spectra of the blue and colourless crystals were identical. Similar results were obtained with salicylideneanilines where the changes in the crystals were due to small concentrations of highly coloured species (Byrn, 1976).

In the solid state, contributions from photolysis are generally restricted to the limited amount of sample in the surface layers. Such contributions to the overall degradation rate are probably too small to be significant in this study, in spite of the observed colour changes on photolysis.

6.4.2 LC-MS studies

Three to seven degradants (listed in Table 6.1) were found in small quantities in the samples analysed using LC-MS. Mesylate, in the solid state, was found to have a greater number of degradants compared to edisylate, especially in samples exposed to sunlight. Some of the degradants found were the same in all samples, i.e. the samples had the same molecular ions. Common ions found include *m/z* 358.7, 298.8, 429.0 and 355.4 (Table 6.1). The degradants found in the mesylate sample exposed to sunlight also had the following molecular masses: *m/z* 307.3, 333.2 and 285.0. The elucidation of the structures of these degradants will be attempted in Chapter 8. From the above, the degradation of the drug peak observed in the HPLC analyses of the solid state samples may be explained.

Table 6.1

Results obtained using LC-MS

Sample	Molecular Ions (*m / z*)
Me Sun	429.0, 358.7, 355.1, 333.2, 307.3, 298.8, 285.0
Ed Sun	429.2, 359.1, 355.5, 299.3
Me UV 254* nm	447.1, 429.0, 359.1, 355.0, 299.2
Ed UV 254* nm	429.0, 359.0, 355.4, 299.1
Me UV 254 nm	371.4, 359.0, 355.4, 299.3,
Ed UV 254 nm	447.1, 359.1, 355.4, 299.3,
Me 100°C	371.4, 359.0, 299.2
Ed 100°C	371.5, 359.1, 299.3,
Me 60°C	447.7, 371.6, 359.1, 355.4, 299.3,
Ed 60°C	371.5, 359.1, 299.2

notes:

Me	= mesylate	60°C	= samples stressed at 60°C
Ed	= edisylate	100°C	= samples stressed at 100°C
UV 254 nm	= UV light at 254 nm samples placed 40 cm from the light source		
UV 254* nm	= UV light at 254 nm samples placed 10 cm from the light source		

6.5 Conclusion

The thermal and photostability studies conducted on samples of prochlorperazine edisylate and mesylate in the solid state showed that the drugs were significantly more stable to both heat and light than in aqueous solution. The samples were found to degrade mostly by first-order kinetics while the degradation rates were approximately equal in all light and heat conditions.

Very small quantities of degradants were found in samples exposed for six months using LC-MS, indicating that prochlorperazine is relatively stable in the solid state. This would explain the decrease in peak height of the drug peak in the HPLC analyses. The results of the experimental photolytic and thermal studies, with prochlorperazine in the solid state, indicates its greater stability in solid dosage forms.

CHAPTER 7. COMPATIBILITY TESTING

This chapter is concerned with the evaluation of the prochlorperazine mesylate in combination with standard tabletting excipients. Only the mesylate salt was used due to its greater availability. The potential incompatibilities of several common tabletting excipients with prochlorperazine mesylate were examined using DSC as a screening technique.

7.1 Introduction

For drug substances susceptible to various environmental factors, preformulation testing is of fundamental importance in the development of dosage forms. In designing a solid dosage form, it is necessary to know the inherent stability of the drug substance (Matsuda and Masahara, 1983).

The interaction of water with powdered materials is a major factor in formulation, processing and product performance of solid pharmaceutical dosage forms. The effects of environmental conditions on the solid state chemical stability are therefore important considerations in the design of pharmaceutical preparations. In attempts to improve drug stability, the decomposition kinetics of drugs in the solid state e.g. aspirin, nitrazepam, have been investigated by many researchers.

7.2 The use of thermal analysis in the detection of excipient-drug interactions in solid dosage forms

The formulation of a stable, effective dosage form requires careful selection of excipients for compatibility. The administration, consistent release and bioavailability of the drug and the protection of the active moiety from the environment must be ensured. Excipients are often assumed to be inert, but chemical and physical interactions between drugs and excipients are common (Fassihi and Persicaner, 1987; Monkhouse, 1984). Excipients may decrease the stability of a drug by chemical reactions, or by sorption of moisture, or by catalysis (Botha and Lötter, 1989). Unless incompatibility is glaringly evident, such as the formation of eutectic melting below room temperature, it is necessary to carry out a stability study which may take weeks or months (Jacobsen and Reier, 1989). A compatibility study should be based on good experimental design and minimum

experimental effort. The most commonly used methods in determining drug-excipient interactions are differential scanning calorimetry (DSC) and quantitative isothermal stress tests.

DSC is also often used for the characterisation of active ingredients and excipients. Properties such as melting points, purity, specific heat capacities, heats of fusion, glass transitions, vapour pressures and solubilities can be examined (Pyramides *et al.*, 1995). This information may indicate potential physical and chemical incompatibilities which can be studied further using DSC on drug-excipient mixtures (Durig and Fassihi, 1993).

Physical interaction between a drug substance and excipients through eutectic formation does not necessarily mean incompatibility, but may explain difficulties experienced with a given composition during processing. DSC is regarded as a first choice method, but the evaluation of results is often difficult and firm conclusions are rarely obtained. Further investigation is usually required because of the elevated temperatures and the high heating rates used. Moisture stress testing is also important. Thus DSC should be used in combination with other complementary techniques such as HPLC, diffuse reflectance spectroscopy (DRS), and IR which may provide information on chemical interactions by the appearance or disappearance of peaks (Malan *et al.*, 1997; Hartauer and Guillory, 1991; van Dooren, 1983; Durig and Fassihi, 1993). Use of Plackett-Burman designs (Plackett and Burman, 1946) have also been recommended by Durig and Fassihi (1993) in the preformulation stages of tablet design.

Examples of the use of DSC in predicting drug-excipient compatibility include studies of ketoprofen, aminophylline and lactose, chlorhexidine diacetate, naproxen and pyridoxal hydrochloride with various excipients. DSC correctly predicted those excipients likely to be incompatible with these drugs (Botha and Lötter, 1989; Hartauer and Guillory, 1991; Pesonen *et al.*, 1995; Botha and Lötter, 1990; Durig and Fassihi, 1993). Interactions discovered may not be detrimental, but DSC can indicate excipients with interaction potential. DSC is thus suitable for rapid preliminary screening (Jacobsen and Reier, 1969; Botha and Lötter, 1989). Isothermal accelerated stress testing of drug-excipient mixtures involves the

exposure of the mixture to elevated temperatures and to moisture. Interactions are then assessed visually or by chromatographic analysis (Durig and Fassihi, 1993).

Advantages of thermal analysis over stress testing include: 1) long duration experiments and subsequent chromatographic analysis are not required, 2) only small amounts of drug are needed for individual experiments and 3) there is no need for the development and validation of a chromatographic method 4) costs, other than equipment capital, are lower (Durig and Fassihi, 1993; Ager *et al.*, 1983). DSC cannot replace classical stability programs involving long term observation, but it may provide an alert to incompatibility and indicate the most favourable directions to pursue for a successful formulation.

The present study was undertaken to establish the compatibility of prochlorperazine mesylate with a number of commonly used tablet excipients. Tablets typically contain diluents, binders, disintegrants and lubricants. Excipients from each group were selected and screened for incompatibilities.

7.3 Design and formulation of compressed tablets

7.3.1 Tabletting

The most common solid dosage forms are tablets. Most formulations are composed of one or more medicaments plus excipients of various types.

Tablets are prepared by placing the appropriate powder in a metal die on a tablet press and the powder is then compressed into a tablet. Making tablets of uniform mass is a significant factor in the tabletting process. The tablet also needs to have sufficient mechanical strength to withstand packaging and processing, yet be capable of rapid breakdown on administration to release the drug.

The first step in tabletting is to decide whether a powdered mixture is to be compressed directly or whether an intervening moist granulation step is to be introduced. Factors which have to be considered are: 1) the stability of the medicament to heat and moisture, 2) the flow properties of the mixed ingredients, and 3) the tendency of the mixture to segregate. Direct compression, where the ingredients are dry mixed and then compressed, has an economic advantage over

the traditionally used method of wet granulation, where the ingredients are mixed with a granulating liquid such as water or ethanol to give readily compressible granules.

Important criteria in the formulation of a tablet include: 1) each component must be uniformly dispersed within the mixture, 2) the mixture must have certain minimum flow characteristics and must be cohesive when compressed, and 3) the most suitable chemical form of the active ingredient must be selected (Banker and Rhodes, 1979).

There are two main types of formulation used for direct compression: 1) where the active ingredient is the major component and 2) where the active ingredient is the minor component (10 to 20% of the compression mass).

'Compressional aids' are sometimes added to improve the mechanical strength and release characteristics of the drug, for example, microcrystalline cellulose. These aids should not adversely affect the drug.

7.3.2 Diluents

If the drug is present in small amounts, a suitable diluent is needed to produce tablets of a reasonable size. Diluents can stabilise formulations and decrease ageing effects. Direct compression diluents include lactose, microcrystalline cellulose, Encompress® and Starch 1500®. These diluents are inexpensive and unreactive.

In addition to enhancing the tablet's mechanical strength, diluents must be non-toxic and have an acceptable taste. They should also have reasonable solubility profiles. Carbohydrates, including lactose, starch and Encompress®, are examples of suitable diluents (Banker and Rhodes, 1979).

7.3.3 Binders

When the powder formulation lacks sufficient cohesion to form a suitably hard tablet, binders or granulating fluids are added. Excipients may be added as dry powders or may need to be mobilised by water during the moist granulation process

before they can exert a binding action. Some diluents exert a binding effect in a mixture. Microcrystalline cellulose and the methylcelluloses are effective in this role (Banker and Rhodes, 1979).

7.3.4 Disintegrants

Disintegrants are added to tablets to overcome the cohesive strength that is introduced by compression and by any binders present. These excipients are used for those tablets that are swallowed whole and are required to disintegrate on entering the stomach, or for those that are dispersed in water before administration. There are several types of disintegrants: 1) those which enhance the capillary forces in producing a rapid uptake of aqueous liquids, 2) those which swell in contact with water, 3) those which release gases to disrupt the tablet structure, 4) those which melt at body temperature and 5) those which destroy the binder by enzymatic action. Examples include the Avicels®, starch (such as Explotab®), Primogel® etc. (Banker and Rhodes, 1979).

7.3.5 Antifrictional agents (lubricants and glidants)

Antifrictional agents such as lubricants and glidants are added to a formulation when the components adhere to the punches and dies of the press and generate frictional forces between the die and the tablet. Lubricants are added to reduce this die-wall friction. Where frictional interaction between individual particles is large enough to hinder the flow and packing of the material in the die, a glidant is added.

Lubricants, for example Lubritab®, are added at the end of a precompression stage. Lubricants are hydrophobic and may also affect the release of a medicament. Their proportions should thus be kept to a minimum (approximately 1 - 4%). Lubricants may also reduce the mechanical strength of the tablet. Soluble lubricants, which are more hydrophilic, include sodium lauryl sulfate which is also a good flow inducer. Stearates as lubricants usually lead to the best compromise in terms of lubricity, tablet strength and disintegration. Suitable glidants include starch and talc (Banker and Rhodes, 1979)

7.4 Experimental

7.4.1 Materials

Prochlorperazine mesylate was used as the active ingredient. The excipients used are grouped below as a) diluents, b) binders, c) lubricants and d) disintegrants.
a) *Diluents:* lactose, modified lactose (Ludipress®), crosscarmellose sodium (Ac-Di-Sol®), dextrose-combination-maltose (Emdex®), dibasic calcium phosphate (Encompress®) and pregelatinised starch (Starch 1500®).

b) *Binders:* microcrystalline cellulose (Avicel PH 101®, Avicel PH 102®, Encocel®), different grades of methylcellulose (Methocel®: A4M, A15LV, E4M, E5, E5M, E10M CR, E15LV, E50LV, K4M, K4M CR, K15M CR, K100LV CR, K100M CR)

c) *Lubricants:* magnesium stearate, sodium lauryl sulfate, stearic acid hydrogenated vegetable oil (Lubritab®).

d) *Disintegrants:* sodium starch glycolate (Explotab® and Primogel®)

7.4.2 Methods

Sample preparation:

Preliminary screening was done using mixtures of prochlorperazine mesylate with the excipients in 1:1 mass ratios. Those excipients that showed the least interactions were then selected and combined in a 3:3:4 (drug : lubricant : diluent) mass ratio for further screening. Mixtures were prepared by blending approximately 100 mg of the powders in a glass vial for 20 minutes using a Gallenkamp shaker.

The final formulations for testing were prepared as tablets containing 25:4:71 mass ratios of drug : lubricant : diluent, using direct compression, as opposed to wet granulation. The lubricant was only added before compression.

Compression of tablets: A 100 mg sample of each mixture was placed in a 13.1 mm diameter die of the hydraulic press. The batch of tablets was prepared using a compaction load of 2 tons.

Equipment:

A Perkin-Elmer DSC 7 differential scanning calorimeter was used. Samples (2 to 8 mg) were weighed into standard aluminium pans, covered with lids, and heated in flowing nitrogen at 10°C min^{-1} over the range 50 to 300°C.
The following samples were examined using DSC:
1) The drug and the excipients individually.
2) Mixtures of drug and excipients immediately after compression.
3) Mixtures of the drug and excipients after 3 months storage under diffuse light conditions at room temperature.

TLC analysis:

Samples dissolved in a mobile phase of 9 : 1 acetonitrile : ammonia were run on silica gel $60F_{254}$ TLC plates to determine whether any degradation had taken place.

7.5 Results and Discussion

The DSC curve of prochlorperazine is shown in Figure 7.1, curve a). A sharp melting endotherm is observed with an extrapolated onset temperature of 240°C as well as a broad endotherm, characteristic of adsorbed moisture, below 100°C. Above about 290°C an exotherm is observed, probably due to decomposition of the molten drug. Figure 7.1 also shows the DSC trace of an 'aged' mesylate tablet that was exposed to diffuse light for 3 months at 25°C (Figure 7.1, curve b). The peak is observed to shift only slightly downwards with the endotherm remaining quite sharp. The trace also shows the development of additional endotherms that may be due to decomposition.

Several types of behaviour were observed when prochlorperazine mesylate was heated in separate binary mixtures with the excipients. Some drug-excipient mixtures showed no solid state interaction, while others showed major changes in the DSC curves signifying possible interactions in the solid state. If there are no interactions between the drug and excipient, the thermal properties of the physical mixture are expected to be approximately the sum of the properties of individual components. Interactions are indicated by: large shifts in melting point, changes in

peak shape and area (corresponding to changes in enthalpy), disappearance of peaks or appearance of peaks not present in the separate components.

The 1:1 drug-excipient mixtures are a much higher ratio than would ever be used in practice. This 'worst case scenario' was used as a screening method for ensuring that potential interactions would be observed.

No significant thermal events in the range 50 to 300°C were shown by crosscarmellose sodium (Ac-Di-Sol), microcrystalline cellulose (Avicel PH 101 and Avicel PH 102, Encocel), the methylcelluloses (Methocel: A4M, A15LV, E4M, E5, E5M, E10M CR, E15LV, E50LV, K4M, K4M CR, K15M CR, K100LV CR, K100M CR) and pregelatinised starch (Starch 1500) apart, perhaps from the broad endotherms resulting from adsorbed moisture. Tabletting of prochlorperazine mesylate resulted in a broadening and shifting of the melting endotherm to slightly lower temperatures. Interactions in the mixtures or tablets of the drug and the excipients were expected to result in lowering of the melting endotherm of prochlorperazine mesylate due to formation of solid solutions.

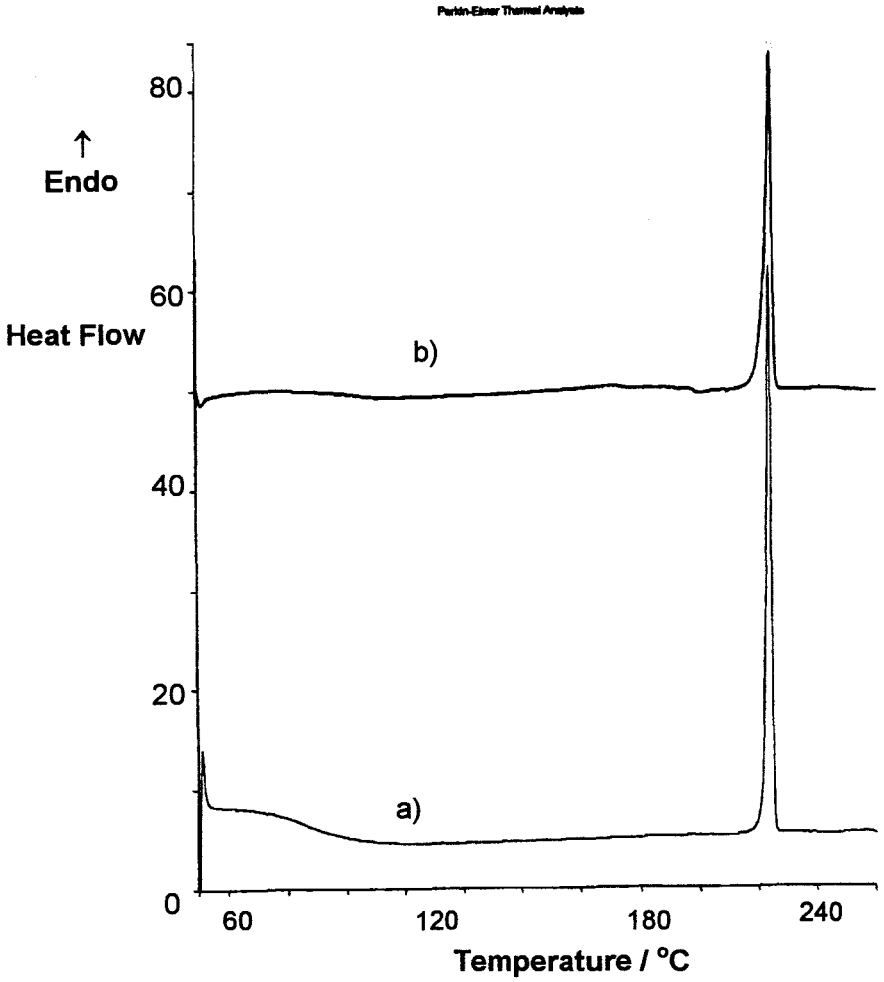


Fig. 7.1 DSC trace of a) prochlorperazine mesylate and b) an aged (3 months) tablet of prochlorperazine mesylate. Samples were heated at 10°C min^{-1} under a nitrogen atmosphere.

Significant thermal events were observed in the individual DSC traces of magnesium stearate, sodium lauryl sulfate, Emdex, Explotab, Primogel, Encompress and lactose.

The DSC curve of lactose (Figure 7.2, curve b) showed two main endotherms, at about 140°C and 205°C. The DSC trace of a lactose-prochlorperazine mixture, c), showed a significant shift and decrease in the melting endotherm of prochlorperazine (more than 40°C) which indicates interactions between the drug and lactose. Lactose - prochlorperazine preparations were greyish brown. Maillard type condensation reactions are known to occur between lactose and compounds with primary amino groups to form brown coloured products. Prochlorperazine has tertiary amino groups. This 'browning' reaction is known to be base catalysed and is accelerated by the presence of alkaline lubricants. Ludipress (a modified lactose with a DSC trace similar to normal lactose) (Figure 7.2, curve e) had a similar effect to lactose on the drug's melting endotherm.

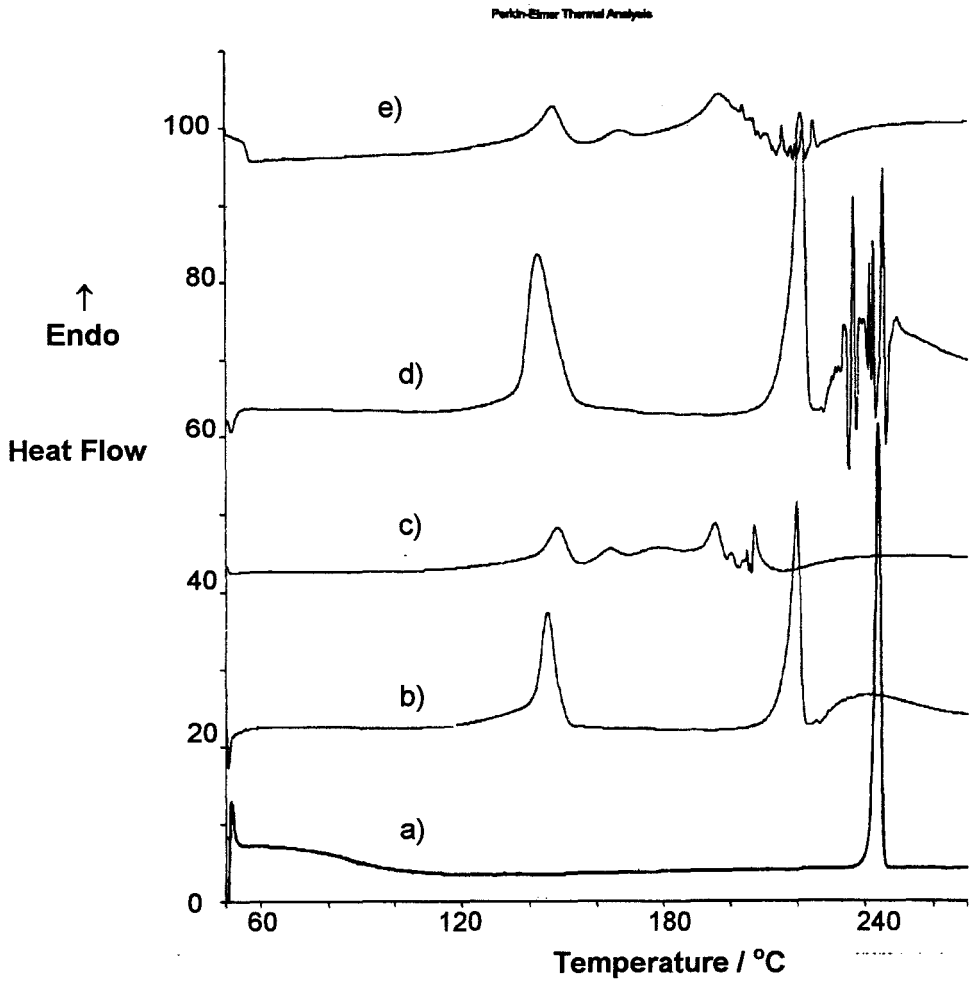


Fig. 7.2 DSC traces of a) prochlorperazine mesylate, b) lactose, c) mesylate and lactose tablet, d) Ludipress, e) mesylate and Ludipress tablet. The samples were heated at a rate of 10°C min^{-1} under a nitrogen atmosphere

The DSC curve of sodium lauryl sulfate (Figure 7.3, curve b) shows a broad, shallow endotherm at about 80°C corresponding to loss of adsorbed water. There are small well-defined endotherms at about 140 and 270°C, with other more complex peaks inbetween. Upon mixing of the drug with sodium lauryl sulfate, c), a total suppression of the drug's endotherm was observed.

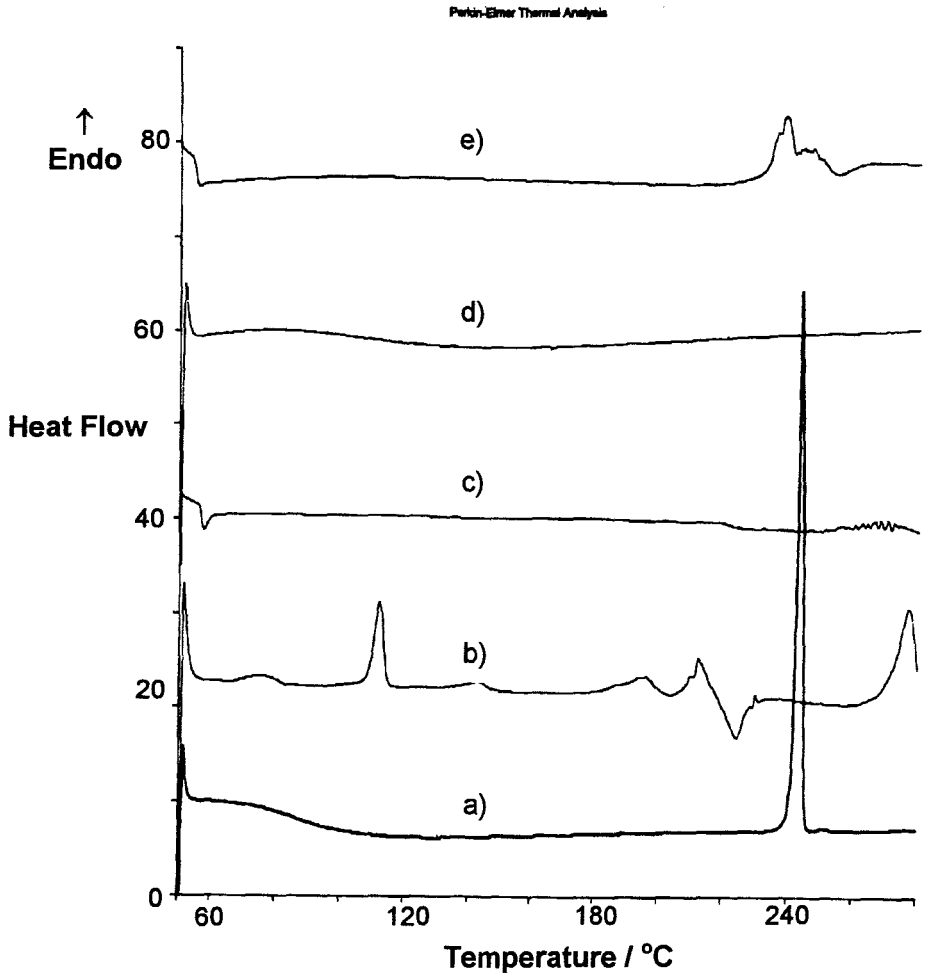


Fig. 7.3 The DSC curve of a) prochlorperazine mesylate, b) sodium lauryl sulfate, c) mesylate and sodium lauryl sulfate, d) Avicel PH 101, e) mesylate and Avicel PH 101 tablet. The samples were heated at a rate of 10°C min^{-1} under a nitrogen atmosphere

Magnesium stearate (Figure 7.4, curve b), a commonly used lubricant, shows two broad endotherms in the 80 to 120°C range, possibly corresponding to different hydrated states or pseudo-polymorphs (Durig and Fassihi, 1993). The DSC trace for the tablet showed the disappearance of all endotherms (Figure 7.4, curve c). Some small endotherms may indicate loss of adsorbed moisture and small amounts of hydrolysis. Incompatibilities involving magnesium stearate are frequently observed, even though antifrictional agents only make up 1 to 4% of the tablet. Magnesium stearate is stated in the Handbook of Pharmaceutical Excipients (1986) to be incompatible with acidic or alkaline substances and more than a 0.5% content is warned to lead to softening of the tablet.

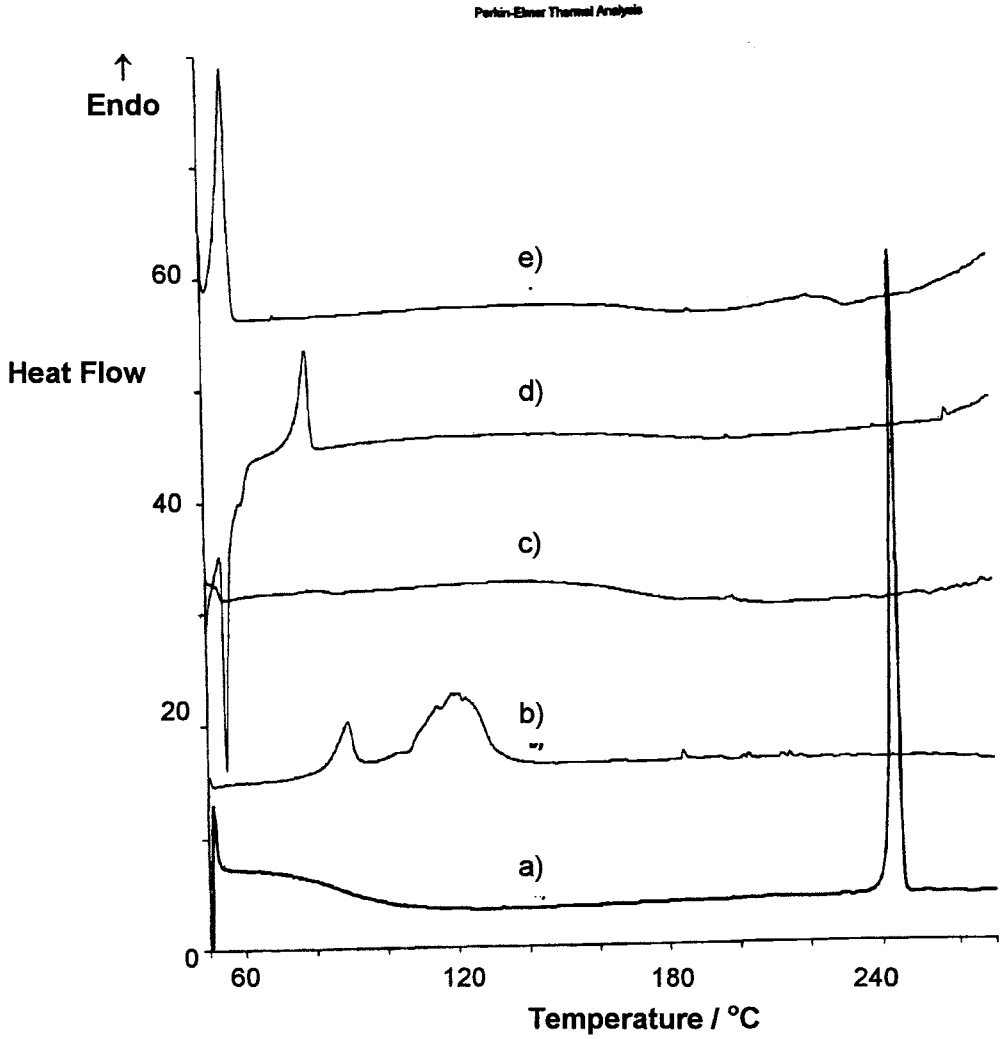


Fig. 7.4 DSC curves of a) prochlorperazine mesylate, b) magnesium stearate, c) mesylate and magnesium stearate, d) stearic acid and e) mesylate and stearic acid. The samples were heated at a rate of 10°C min^{-1} under a nitrogen atmosphere

Stearic acid (Figure 7.4, curve d) and Lubritab (Figure 7.5, curve d), both used as lubricants, have low melting endotherms at approximately 64°C and 75°C, respectively. The drug peak is absent in the DSC curve of the mixture with stearic acid due to the molten acid dissolving the drug (Figure 7.4, curve e). The alternative lubricant, Lubritab, was found not to interact with the drug, however. The melting endotherm of the drug in the DSC trace for the mixture with Lubritab had an onset at approximately 238°C and was only slightly broadened. Lubritab is a hydrogenated vegetable oil and thus mesylate, with its polar groups, probably did not dissolve in the Lubritab melt.

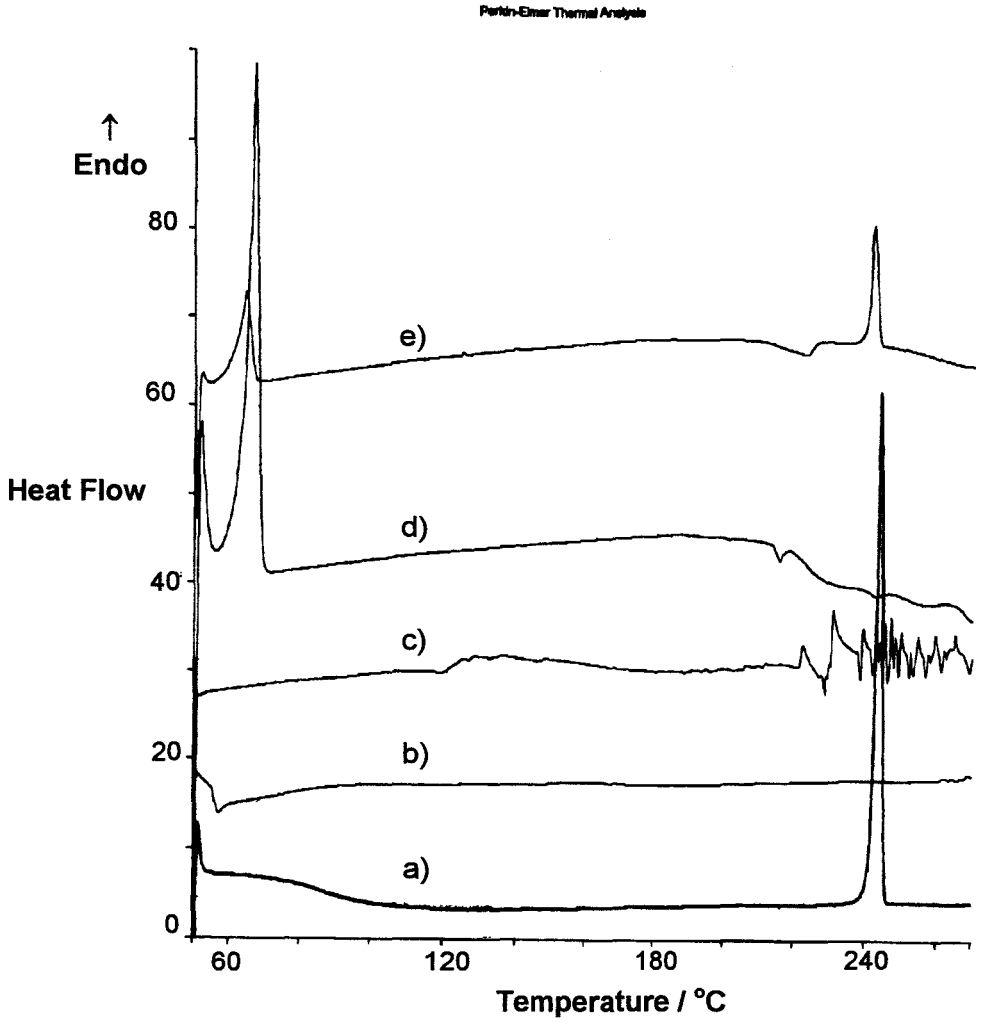


Fig. 7.5 DSC curve of a) prochlorperazine mesylate, b) Explotab, c) mesylate and Explotab, d) Lubritab, e) mesylate and Lubritab. The samples were heated at a rate of 10°C min^{-1} under a nitrogen atmosphere

Explotab (Figure 7.5, curve b) showed virtually no thermal activity below 300°C. The melting endotherm of the drug was markedly changed in mixtures with Explotab (Figure 7.5 curve c). Primogel (Figure 7.6, curve b and c) contributed to a slight shift in the melting endotherm of the drug in the mixtures, by the presence of two broad endotherms in the 60 to 200°C range.

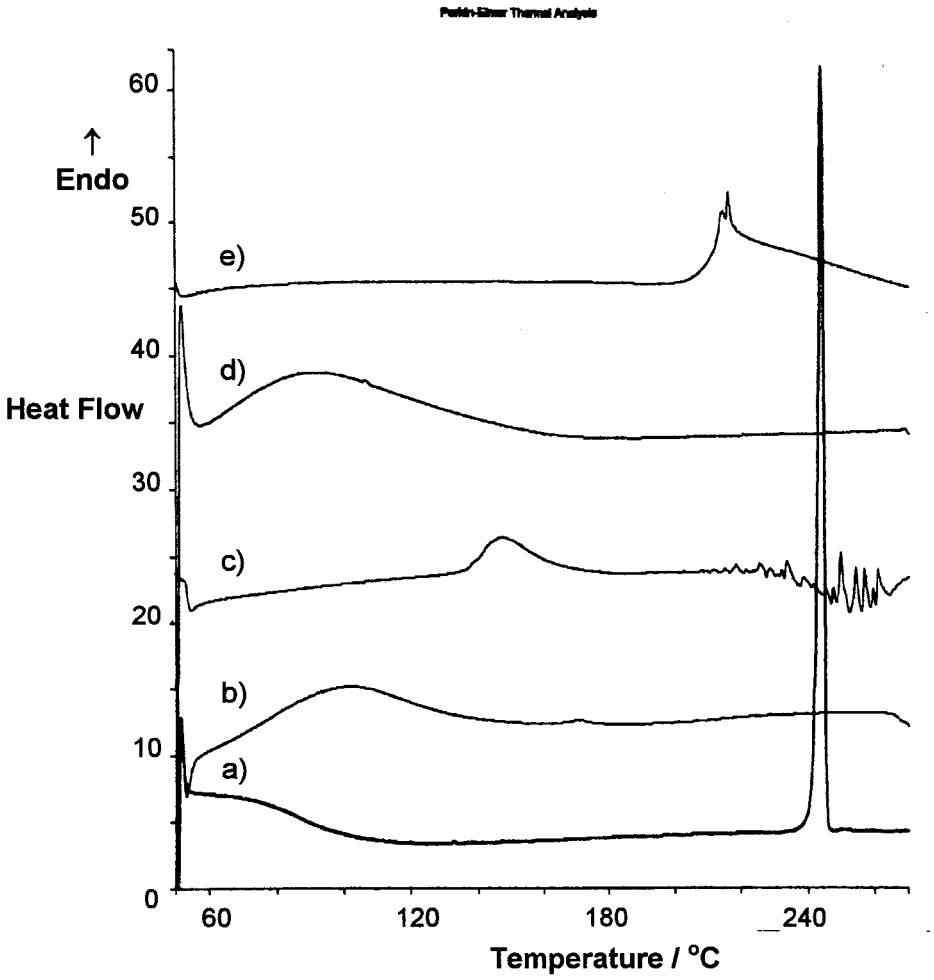


Fig. 7.6 DSC curve of a) prochlorperazine mesylate, b) Primogel, c) mesylate and Primogel, d) Starch 1500, e) mesylate and Starch 1500. The samples were heated at a rate of 10°C min^{-1} under a nitrogen atmosphere

Starch 1500 (Figure 7.6 curves d and e) showed a broad endotherm at 60 to 140°C due to the loss of adsorbed water. The drug endotherm was broadened to overlapping peaks in the mixtures.

The considerable and complex endothermic activity of Encompress (Figure 7.7, curve b) occurred below the melting point of the drug. The DSC curve of a drug-Encompress mixture (Figure 7.7 curve c) showed disappearance of the melting endotherm of the drug.

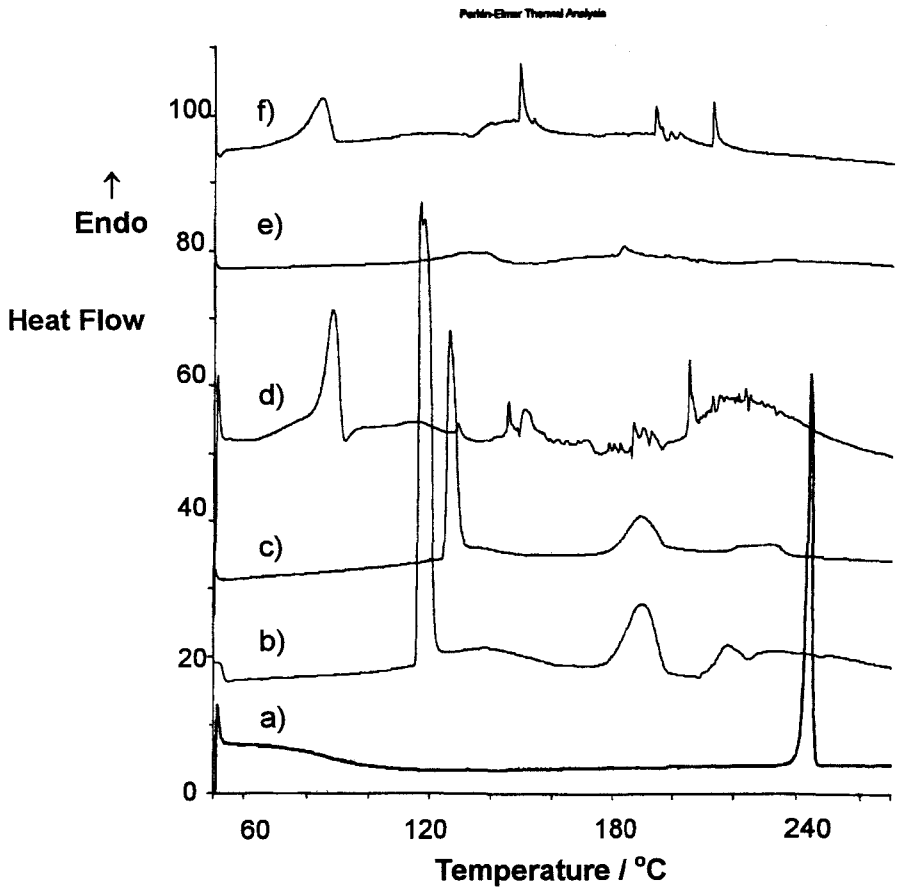


Fig. 7.7 DSC curve of a) prochlorperazine mesylate, b) Encompress, c) mesylate and Encompress, d) Emdex, e) mesylate and Emdex, f) an aged tablet of mesylate and Emdex (3 months). Samples were heated at a rate of 10°C min^{-1} under a nitrogen atmosphere

Emdex (Figure 7.7, curve d) also has multiple complex endotherms. Upon admixture with the drug, the melting endotherm of the drug is absent (Figure 7.7, curve e). Comparison of the aged Emdex – prochlorperazine mesylate tablet with a freshly prepared tablet showed the development of a deep brownish colour. The DSC trace of this aged sample (Figure 7.7, curve f) suggested that the endotherms of the Emdex excipient could be associated with water adsorption and some hydrolysis. Dextrose (Figure 7.7, curve d) is known to cause browning of tablets in the presence of amines and the addition of starch or microcrystalline cellulose to such a tablet is not recommended (Handbook of Pharmaceutical Excipients, 1986). From Literature reports, lactose, magnesium stearate, stearic acid and sodium lauryl sulfate were expected to cause incompatibilities. Magnesium stearate, particularly, is known to cause problems e.g. chlorhexidine diacetate (Pesonen, 1995), ketoprofen (Botha and Lötter, 1989), indomethacin (Venkataram *et al.*, 1995), naproxen (Botha and Lötter, 1990).

Table 7.1

Drug-excipient compatibility, where: + indicates an interaction, - indicates no interaction, +/- indicates likely interactions, -/+ indicates unlikely interactions, -* indicates no drug peak present and [&] indicates that the sample was exposed for 3 months

Sample	Compa-tibility	ΔH / J/g of fresh drug*	ΔH / J/g of aged drug*	Onset temperature / °C of melting of the drug	Colour of aged sample[&]
Lactose	+ / -	-*	-*	-*	greyish-brown
Ludipress®	+	-*		-*	yellow
Ac-Di-Sol®	+	-*	-*	-*	pale pink
Emdex®	+	-*	-*	-*	brown
Encompress®	+ / -	30.8	26.6	219	brown
Starch 1500®	+ / -	135.3	135.3	215	pinkish brown
Avicel PH 101®	- / +	25.6	116.2	236	yellow
Avicel PH 102®	- / +	65.2	80.1	237	pink
Encocel®	+ / -	31.8	81.1	237	light pink
Methocel A4M®	- / +	98.8	105.5	228	pinkish brown
Methocel A15LV®	+ / -	98.8	98.9	228	pink
Methocel E4M®	- / +	31.4	48.1	224	pink
Methocel E5®	+	41.9	57.5	223	pink
Methocel E10M CR®	- / +	87.2	55.7	223	brown
Methocel E15LV®	+ / -	17.0	81.5	225	pinkish brown
Methocel E50LV®	- / +	52.3	50.5	224	brown
Methocel K4M®	+ / -	32.0	88.3	219	pinkish brown
Methocel K4M CR®		33.6	87.5	218	brown
Methocel K15M CR®	- / +	42.4	55.2	217	pinkish brown
Methocel K100LV CR®	+ / -	39.9	89.5	217	pinkish brown
Methocel K100M CR®	- / +	33.4	82.2	219	brown
Magnesium stearate	+	-*	5.5	-*	'rusty' brown
Stearic acid	+	7.9	16.4	217	yellow brown
sodium lauryl sulfate	+	-*	-4.4	-*	rusty pink
Lubritab®	-	53.2	62.4	241	pale yellow
Explotab®	+	-*	-*	-*	deep rusty brown
Primogel®	- / +	-*	-*	-*	deep rusty brown

The results of the compatibility screening (collected in Table 7.1) could be summarised as follows:

Little or no incompatibility: Lubritab, Avicel PH 101, Avicel PH 102, Encocel, Methocel A15LV, -E4M, -E5, -E15LV, -K4M and K4M CR.

Possible incompatibility: Starch 1500, Methocel E50LV, -E5, -E10M CR, -K15M CR, -K100LV CR and K100M CR.

Probable incompatibility: Ac-Di-Sol, Emdex, Encompress, Explotab, Primogel, lactose, Ludipress, magnesium stearate, sodium lauryl sulphate and stearic acid.

In evaluating drug-excipient interactions using DSC, the following factors need to be considered:

1) DSC studies provide major information in the melting regions of the drug or the excipient, or both. Transformations occurring at these temperatures may bear no relation to those possible at room temperature.
2) If the curve of the mixture is a simple superposition of the single components, an incompatibility is highly improbable, e.g. Lubritab.
3) Where the melting peak of the active ingredient and the excipient coincide, superposition may not be clearly visible e.g. Emdex. An interaction of the drug with the excipient, however, would result in a depression of the melting point.

TLC analyses were carried out and they confirmed the various drug-excipient incompatibilities found using DSC, i.e. lactose, Ludipress, magnesium stearate, sodium lauryl sulfate, Primogel, Explotab, stearic acid and Encompress. The R_f value obtained for mesylate was found to be 0.81 (Table 7.2). A few spots were found which had different R_f values (with the parent drug present at 0.81 as well) indicating some degradation of the parent compound. These TLC plates were compared to those of the various excipients without the drug. Explotab mixtures were found to have the most degradants. Ludipress, Emdex, magnesium stearate, stearic acid and Encompress mixtures showed only one degradant each. No degradation products were found with the other excipients mixtures.

Table 7.2

The effect of different excipients on the degradation of the mesylate salt

Sample	R_f values
Prochlorperazine mesylate	0.81
lactose	0.64, 0.61
Ludipress	0.6
Emdex	0.77
magnesium stearate	0.56
sodium lauryl sulfate	0.53, 0.51, 0.43
Encompress	0.55
Primogel,	0.5, 0.41, 0.29
Explotab	0.46, 0.39, 0.28, 0.2
stearic acid	0.55

7.6 Conclusions

DSC screening has been proved by several workers (Botha and Lötter, 1989, 1990; Venkataram *et al.*, 1995; Pesonen *et al.*, 1995) to be a reliable indicator of major incompatibilities. Interpretation of the effects observed in DSC screening are not straightforward, because many types of drug-excipient interactions are possible. These include eutectic formation, solid-solid interactions (possibly complicated by solid-phase transformations), solid-liquid reactions and solid-gas reactions (particularly hydrolysis by evolved water vapour). Those excipients that undergo thermal events at temperatures below those of importance in the behaviour of the drug, are expected to produce incompatibilities through one or more of the interactions listed above.

Several excipients were found to be potentially incompatible. The behaviour observed ranged from a slight shift in the drug melting endotherm to total abolition of the melting peak such as found with sodium lauryl sulfate, magnesium stearate, Ac-Di-Sol and lactose. Some excipients such as Lubritab, Avicel PH 101, Avicel PH 102, Encocel, Methocel A15LV, -E4M, -E5, -E15LV, -K4M and K4M CR were found to be suitable.

Thus, for a specific dosage form, the combination of the drug with one or more of magnesium stearate, sodium lauryl sulfate, lactose, Ludipress, Encompress, Explotab, Primogel, Ac-Di-Sol, or Emdex should be avoided. The TLC analyses carried out confirmed the results obtained with DSC and thus DSC was found to be a reliable indicator of the major incompatibilities that could be experienced with the above excipients. Thermal analysis cannot replace chemical methods for the determination of concentration of the drug in a dosage form and does not replace the stability tests, but it is a valuable tool in the first step of formulation. If, after evaluation with DSC, drug / excipient mixtures are found to be incompatible, the excipient should preferably not be used because there are a sufficient excipients available to make it possible for only those that are unlikely to cause problems to be chosen.

CHAPTER 8. THERMAL AND PHOTO- PRODUCT IDENTIFICATION

This chapter is concerned with the identification of the degradation products obtained during the thermal and photo-stability studies of prochlorperazine mesylate and edisylate using LC-MS.

8.1 Introduction

Developing drug purity control regulations within ICH forums requires that impurities exceeding the 0.1% level be examined and identified. These impurities may result from degradation, impure starting materials, or the synthetic procedure (Erickson *et al.*, 1995). The poor stability of prochlorperazine mesylate and edisylate, especially in solution (Moore, 1977; Moore and Tamat, 1980; Pawełczyk, 1977), prompted a detailed study to identify degradants in the solid state and solution samples exposed to various photolytic and thermal conditions.

The relationship between degradants and metabolites has been discussed in Chapter 2. Although considerable information about the metabolism of chlorpromazine is available, information about many of the other drugs in this class is scant. It is becoming more important to understand the metabolic pathways of neuroleptic drugs and to measure levels of both parent drug and active metabolites (Foye *et al.*, 1995) because they are often administered for long periods.

A number of photodecomposition products of various phenothiazines obtained during irradiation under aerobic and anaerobic conditions, have been isolated and characterised (Sharples, 1981, Ljunggren and Möller, 1977; Huang and Sands,1967). These include the N-oxides, hydroxy derivatives, dimeric or polymeric products, and excimers, in addition to the sulfoxides and sulfones. Polymerisation is found to be a major process taking place under anaerobic conditions, while demethylation was found not to occur under the same conditions. Both the molecular characteristics and phototoxicity relationships of phenothiazines demonstrate that the tricyclic moiety is essential for phototoxic activity, while a Cl substituent at C_2 has also been implicated in phototoxicity studies. A number of researchers (Kofoed *et al.*, 1966; Zingales, 1968; Speaker, 1974 and De Leenheer, 1974) have described TLC systems that are useful in the separation and

identification of phenothiazines and their structurally-related compounds. The versatility of HPLC in drug assays is well known and the technique has been applied to the separation and detection of some phenothiazine derivatives such as promethazine, thioridazine and fluphenazine and its esters (Heyes and Salmon, 1978). Some phenothiazine derivatives, however, are very difficult to separate by LC methods based on adsorption, ion-pair partition, ion-exchange or reversed-phase partition processes (Li and Purdy, 1991), due to the similar polarities of the parent drug and the subsequent degradants and metabolites.

HPLC has often been used to separate, but not necessarily identify, drug enantiomers, metabolites, degradants and impurities. Mass spectrometry (MS), on the other hand, can perform separations based on molecular mass, thus the combination of HPLC and MS (LC-MS) is a powerful method for the analysis of drugs and biological substances (Mano *et al.*, 1994). LC-MS has been extensively used in the pharmaceutical industry for drug identification, metabolite profiling and identification, as well as screening and quantitation.

Because of its capability of achieving extremely low detection limits, LC-APCI MS with a heated nebuliser interface has been applied to drug quantitation (Qin *et al.*, 1994). However, only a few published reports on the application of LC-APCI MS to the structural characterisation of low-level degradants in pharmaceutical dosage forms are available (Qin *et al.*, 1994). Degradants and impurities which are similar to the drug substance, e.g. isomers, are sometimes difficult to detect when single chromatographic techniques are used. The impurities may co-elute with the main peak which may be present in a thousand-fold excess. The technique may not be able to discriminate between co-eluting compounds because closely related compounds may have identical chromophores and hence similar spectral properties (Erickson *et al.*, 1995).

LC-MS, coupled with an atmospheric pressure chemical ionisation (APCI) source, was applied to the characterisation of a low-level degradant of famotidine by Qin *et al.* (1994). Erickson *et al.* (1995) used HPLC, LC-MS and GC-MS to deduce the structure of a low-level impurity found in some batches of metoprolol tartrate because diode-array detection did not reveal the presence of the impurity since the chromophore was identical to that of metoprolol itself. In this case, peak purity determination with MS detection would be more successful although the molecular

mass of the impurity is needed in advance in order to detect it at such low concentrations (0.1%). The pharmacopoeial requirements (BP and USP) are that a compound must be between 95 and 105% purity. Degradation resulting in greater than 90% purity is usually disregarded in industry because the drugs may no longer be used in that particular form.

Stability studies on solutions of both prochlorperazine salts exposed to the various light conditions, were performed under aerobic and anaerobic conditions. During the course of the stability studies several low-level degradants were observed in the HPLC chromatograms. The presence of the buffer (KCl) prevented the isolation of pure degradants from the samples using semi-preparative HPLC. These studies on partially degraded samples allowed the use of semi-preparative HPLC to be investigated for isolation of the degradants with a view to further characterisation by MS, NMR, UV, IR and elemental analysis.

Samples exposed to irradiation were basified, extracted into chloroform, evaporated to dryness and subjected to NMR studies. These samples were also subjected to molecular exclusion (using methanol) and solid phase (gradient elution from 100% methanol to 100% water using trifluoroacetic acid) separation techniques. The various fractions were collected, chromatographed (TLC) (and the similar fractions combined), evaporated to dryness and NMR experiments conducted. The NMR data obtained from these samples resulted in poor peak resolution (broad peaks) and no useful structural information could thus be obtained.

LC-MS was thus used to identify the degradants from both solution and solid state stability studies.

8.2 Experimental

8.2.1 Reagents

All chemicals were of analytical grade. Ammonium acetate was obtained from Univar, Saarchem (Pty) Ltd.

8.2.2 Photo- and thermal stability studies

Samples used for LC-MS studies were exposed to various photolytic and thermal conditions (Table 8.1).

Table 8.1
Conditions of solution and solid state stability studies for prochlorperazine mesylate and edisylate

Conditions	Ampoules (2 mL)	Temperature / °C	Relative humidity	Irradiation period
Sunlight	Clear & amber	35 ± 2	60 ± 5 %	14 days
UV light (254 nm*)	Clear & amber	25 ± 2	60 ± 5 %	2 months
UV light (254 nm^{+})	Clear & amber	25 ± 2	60 ± 5 %	2 months
Diffuse light	Clear & amber	25 ± 2	60 ± 5 %	2 months
100°C	Clear	100 ± 2	dry heat	2 months
60°C	Clear	60 ± 2	dry heat	2 months

note:
- the conditions listed above were used for both the solid and solution state studies
* - samples were exposed 10 cm from the light source
$^{+}$ - samples were exposed 40 cm from the light source
$^{\#}$ - samples for the solid state studies were placed in petri-dishes

8.2.3 Sample preparation for liquid chromatography - mass spectrometry (LC-MS)

The dilutions for both the solid and solution state samples were prepared in a similar manner. 125 mg of each of the samples for the solid state studies were weighed and a 12.5 mg mL^{-1} stock solution was then prepared in a 80 : 20 MeOH : H_2O (30 mM ammonium acetate) solvent. The samples were then exposed to the same conditions as those for the kinetic studies carried out in Chapter 5. The stock solution was then diluted to obtain a final concentration of 0.125 mg mL^{-1}, also prepared in mobile phase for HPLC analysis.

8.2.4 Method development

A mobile phase consisting of acetonitrile : 1% aqueous KCl (85:15) was used in the analytical studies. The KCl buffer precluded the use of this mobile phase in LC-MS,

because the LC-MS interfaces developed so far do not allow the use of non-volatile buffers. Ammonium acetate and acetic acid are often used in LC-MS to aid ionisation of the analyte. Because ionisation of prochlorperazine was found to occur with ammonium acetate, the mobile phase developed was methanol with 30 mM ammonium acetate. This allowed separation and ionisation of at least 5 degradants.

The mobile phase system developed for the HPLC chromatographic method, used in the kinetic studies, was 85 : 15 (1% aqueous KCl : acetonitrile). Based on the polarity of this system and the role of KCl in retention times and peak resolution, a mobile phase of 60 : 40 methanol : 1% acetic acid (since acetic acid is required for ionisation in LC-MS studies) was chosen. This resulted in a broad drug peak with a retention time of 30 minutes. Further alteration to 70 : 30 Methanol : 1% acetic acid, gave a retention time of 23 minutes. Readjustment to 80 : 20 methanol : 1% acetic acid, resulted in a drug peak with reasonable symmetry and a retention time of 14 minutes. This allowed resolution of the degradants from the drug peak. Methanol was used because of its lower cost and greater polarity. Application of this mobile phase on the LC-MS system, however, did not result in the ionisation of all the degradants. Only the drug peak and the sulfoxide degradants were ionised. It was then decided to retain the 80 : 20 methanol : water ratio, replacing the 1% acetic acid with 30 mM ammonium acetate.

8.2.5 Final liquid chromatography- mass spectrometry (LC-MS) conditions

A Finnigan LCQ system was used for all the LC-MS studies, using a μ-Bondapak C_{18} column (4.6 x 250 mm i.d.), 10 μm, 125 Å. The mobile phase was methanol : 30 mM ammonium acetate, (80:20 v/v) at a flow rate of 1.0 mL min^{-1} with a 20 μL injection loop. The following settings, using an Atmospheric Pressure Chemical Ionisation (APCI) source, were employed to record the mass spectra, after tuning to give maximum sensitivity,: capillary temperature 138 °C, vaporiser temperature 450°C, capillary voltage 38 V, discharge voltage 5 kV, tube lens offset 19 V, discharge current 5 μA and the sheath gas was set at 20 arbitrary units.

8.3 Results and Discussion

8.3.1 Solution studies

8.3.1.1 Photostability

Between 5 and 11 unknown compounds eluted at retention times shorter than that of the parent compound, implying that the degradants are more polar than prochlorperazine. Some peaks were found to contain more than one molecular ion, implying that more than one compound was eluting from the column with the same retention times. The drug peak was found to contain only one molecular ion confirming the integrity of the drug peak and the suitability of the HPLC method. A detailed LC-MS study was thus carried out to determine the molecular masses, which would thus assist in providing structural information on degradants.

Figure 8.1 shows the total ion current chromatograms of representative exposed samples of prochlorperazine mesylate. The peak of the parent compound at approximately 14 minutes is resolved from those of the degradants which appear as 15 unknown compounds with retention times of 3.6, 3.9, 4.6, 5.4, 5.6, 6.8, 7.2, 7.4, 7.9, 8.4, 9.6, 12.2, 12.9, 13.71 and 18.5 minutes respectively. The molecular mass of prochlorperazine was confirmed to be 374, and the molecular masses , retention times and proposed structures [8] to [31] of degradants formed, are shown in Table 8.2 below.

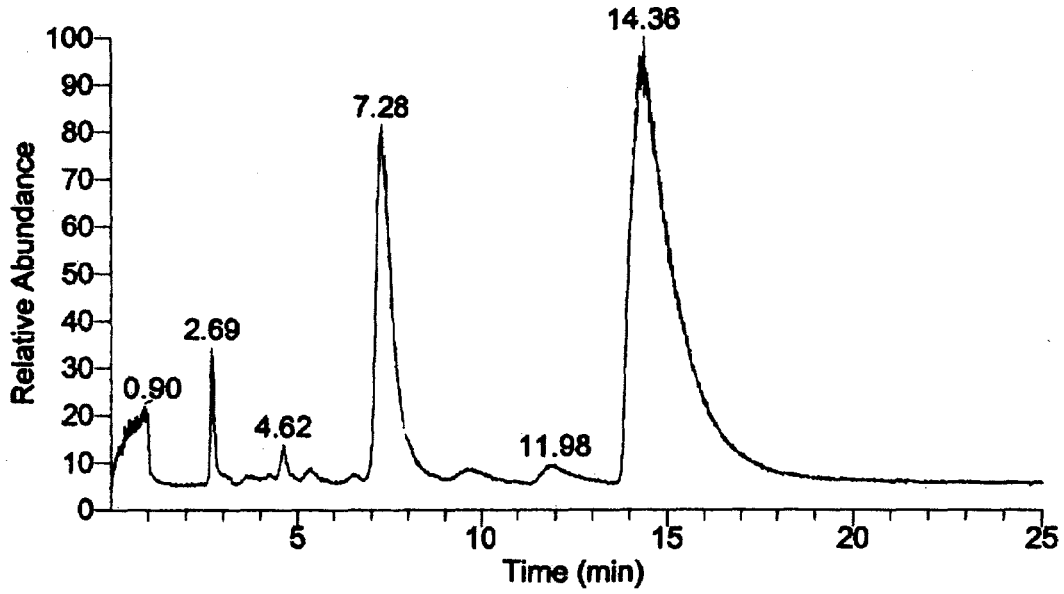


Fig. 8.1 Representative total ion current (TIC) of prochlorperazine mesylate (air) exposed to UV light at 254 nm (samples placed at a distance of 40 cm from the light source)

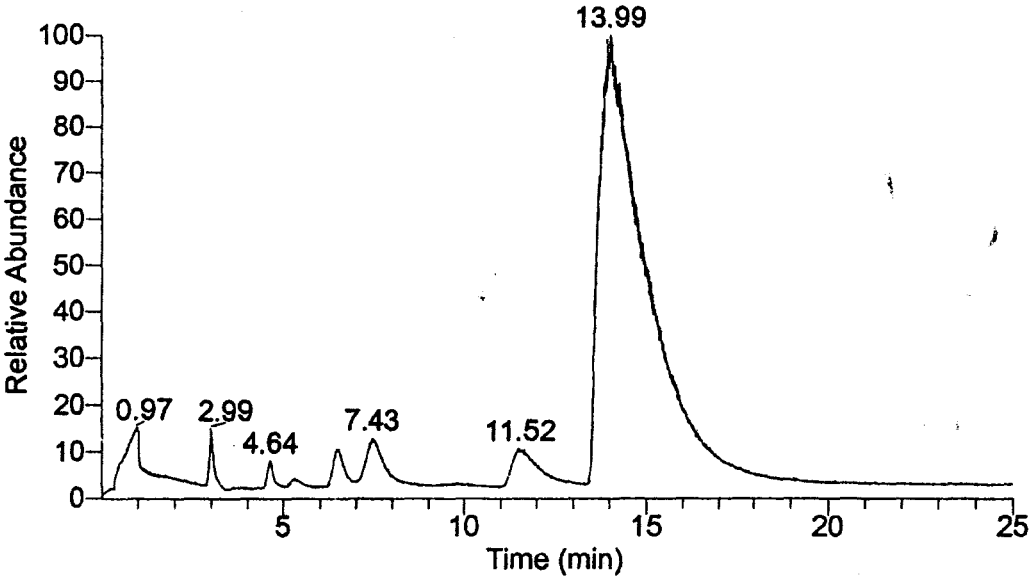


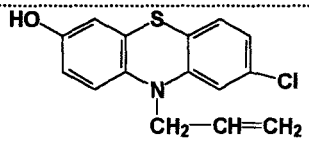
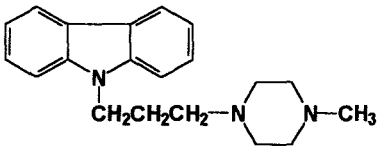
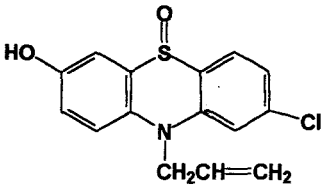
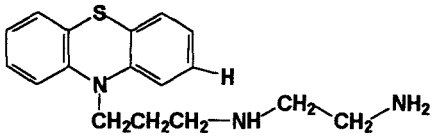
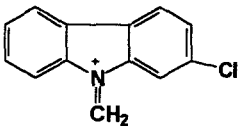
Fig. 8.2 Representative total ion current (TIC) of prochlorperazine mesylate (nitrogen) exposed to UV light at 254 nm (samples placed at a distance of 40 cm from the light source)

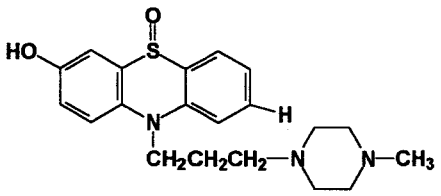
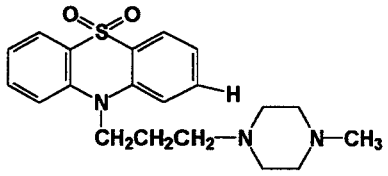
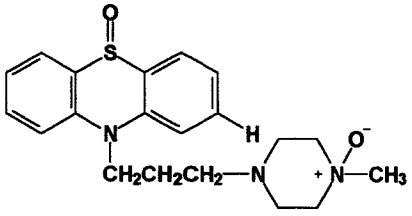
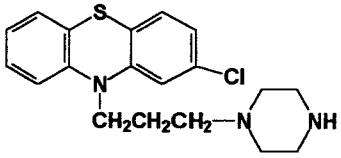
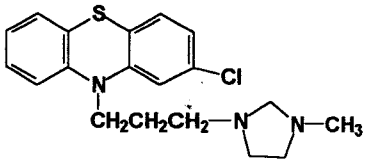
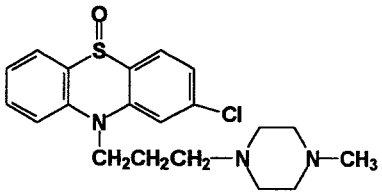
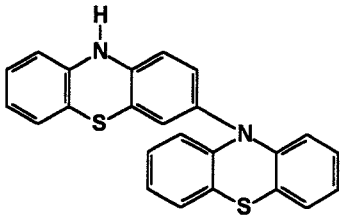
The differences between the molecular mass of prochlorperazine and those of its degradants are indicative of the substructural differences between these compounds. The structures are based on the fact that the degradants retain much of the original prochlorperazine structure [2]. Common product-ions of prochlorperazine and those of the degradants were evidence for common substructures.

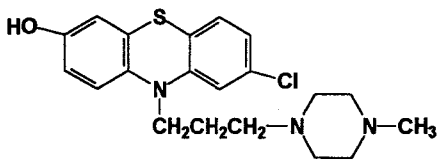
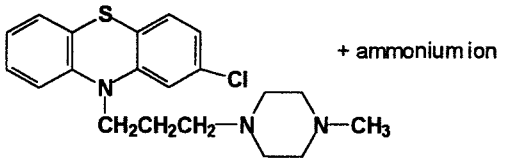
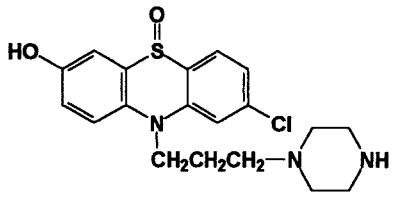
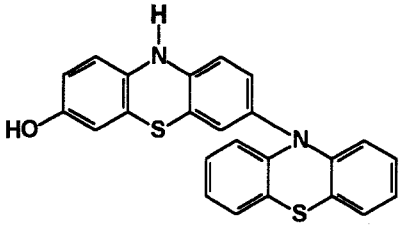
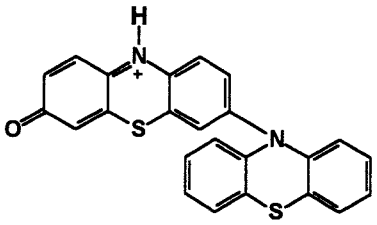
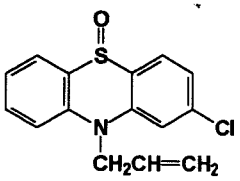
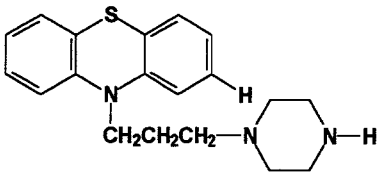
Table 8.2 gives the molecular ions found in the LC-MS studies, their retention times, the proposed structures and the literature references used.

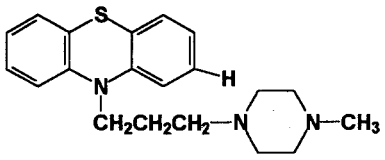
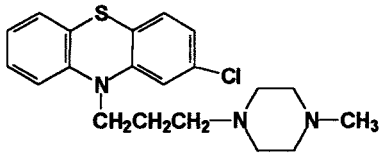
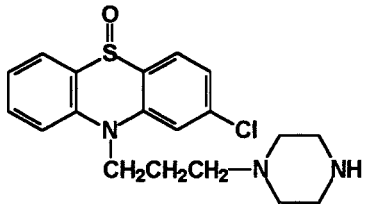
Table 8.2

Molecular ions of the degradants of prochlorperazine together with their retention times and proposed structures

Molecular Ion	Retention time (R_t)	Proposed Structures	Structure number	References
290.1	3.56		[8]	Essien *et al.*, 1975
307.9	3.92		[9]	-
		OR		
			[10]	
299.8	4.61		[11]	Breyer *et al.*, 1974
215.8	4.61		[12]	Breyer *et al.*, 1974

373.8	5.36		[13]	Kaul *et al.*, 1972
		OR		
			[14]	Foye *et al.*, 1995; Robinson, 1965
		OR		
			[15]	Foye *et al.*, 1995; Heyes and Robinson, 1985
326.3	5.61		[16]	Breyer *et al.*, 1974
359.0	6.75		[17]	Breyer *et al.*, 1974; Robinson, 1965
		OR		
			[18]	Breyer *et al.*, 1974
390.6	7.22		[19]	Foye *et al.*, 1995; Robinson, 1965
415.3	7.95		[20]	Roseboom and Perrin, 1977a

391.5	8.37		[21]	Foye *et al.*, 1995; Robinson, 1965
		OR		
			[22]	-
		OR		
			[23]	Robinson, 1965
430.9	9.14		[24]	Roseboom and Perrin, 1977a
		OR		
			[25]	Roseboom and Perrin, 1977a
290.9	9.60		[26]	Essien *et al.*, 1975
		OR		
			[27]	

340.8	12.20		[28]	Foye *et al.*, 1995
375.4	13.71		[29]	Foye *et al.*, 1995
376.0	18.45		[30]	Breyer *et al.*, 1974

Tables 8.3 to 8.8 contain the molecular ions of the degradants formed from prochlorperazine mesylate under the various light conditions.

1) Sunlight

Table 8.3 gives the molecular ions found in prochlorperazine mesylate exposed to sunlight (air and nitrogen)

Table 8.3

Relative percentage abundances of the molecular ions of the degradants found in prochlorperazine mesylate exposed to sunlight (air and nitrogen)

m/z	**Mesylate Air (clear glass ampoules)**	**Mesylate Nitrogen (clear glass ampoules)**	**Mesylate Air (amber ampoules)**	**Mesylate Nitrogen (amber ampoules)**
434.9	-	-	1.40	-
415.3	-	0.56	-	-
376.0	-	-	-	0.59
373.8	-	-	0.19	-
359.0	1.06	9.69	37.57	44.22
340.8	53.95	57.39	13.40	33.95

For the mesylate samples sealed under aerobic and anaerobic conditions in clear glass ampoules, the major degradant (*m/z* 340.8) was the dechlorinated product

[28]. In amber ampoules, the demethylated degradant, molecular ion 359.0, **[17,18]** predominates.

2) 254 nm UV light (10 cm from the irradiation source)

Table 8.4 gives the molecular ions found in prochlorperazine mesylate exposed to UV light (air and nitrogen)

Table 8.4

Relative percentage abundances of the molecular ions of the degradants found in prochlorperazine mesylate exposed to 254 nm UV light (air and nitrogen)

m/z	**Mesylate Air (clear glass ampoules)**	**Mesylate Nitrogen (clear glass ampoules)**	**Mesylate Air (amber ampoules)**	**Mesylate Nitrogen (amber ampoules)**
448.9	0.08[#]	-	-	-
435.8	-	-	-	0.77
415.3	0.46	0.34	-	-
391.5	2.03[+]	1.29	4.99	-
390.6	4.44	-	23.41	-
376.0	2.29[+]	0.43	-	-
373.8	0.24[#]	0.23	1.13	0.33
359.0	15.99	63.26	0.71	7.39
340.8	13.18	11.99	-	0.91
299.8	-	-	1.99	-
249.6	-	-	1.99	-
215.8	-	-	0.89	-

[+] note: molecular ions found under the same peak

[#] note: molecular ions found under the same peak

Clear glass ampoules

The demethylated product (*m/z* 359.0) **[17, 18]** was found to be the major degradation product (16% and 63%) in air and nitrogen, respectively, with the dechlorinated derivative (at 340.8) **[28]** present in excess of 10%. The sulfoxide (*m/z* 390.6) **[19]** is present in those samples sealed under air. Additional molecular ions were found at 391.5 (2.03%) **[21, 22, 23]**, corresponding to the sulfone, and 376.0 (2.29%) **[30]**, a product resulting from oxidation and demethylation. The

molecular ion at 391.5 (1.29%) was also present in samples sealed under nitrogen. Low-level degradants with molecular ions at 415.3 (0.46%) **[20]**, resulting from dimerisation, 373.8 (0.24%) **[13, 14, 15]** corresponding to products resulting from oxidation and hydroxylation, and 448.9 (0.08%) were also detected for those samples sealed under air. The low-level degradants for samples sealed under nitrogen included molecular ions at 415.3 (0.34%), 373.8 (0.23%) and 376.0 (0.43%).

Amber ampoules

The major photoproduct at 390.6 corresponds to sulfoxide formation (23.41%) **[19]** and a sulfone degradant with a molecular ion at 391.5 (4.99%) **[21, 22, 23]** was detected. Additional molecular ions corresponding to low-level degradants, included demethylated products (*m/z* 359.0, 0.71%) **[17, 18]**, oxidation and hydroxylated derivatives (*m/z* 373.8, 1.13%) **[13, 14, 15]**, the desulfurated and dealkylated product (*m/z* 215.8, 0.89%) **[12]**, dealkylated degradants (*m/z* 299.8, 1.99%) **[11]** and 249.6 (1.99%) were found. Samples sealed under nitrogen showed the major degradant to be the demethylated product (*m/z* 359.0, 7.39%), with low-level degradants with molecular ions at 340.8 (0.91%) corresponding to the dechlorinated derivative **[28]**, the oxidised and hydroxylated derivatives (*m/z* 373.8, 0.33%) **[13, 14, 15]** and 435.8 (0.77%).

For both sets of ampoules, sulfoxide formation (*m/z* 390.6) was found to occur only in those samples sealed in air.

3) 254 nm UV light (40 cm from the irradiation source)

Table 8.5 gives the molecular ions found in prochlorperazine mesylate exposed to UV light (air and nitrogen)

Table 8.5

Relative percentage abundances of the molecular ions of the degradants found in prochlorperazine mesylate exposed to 254 nm UV light (air and nitrogen)

m/z	**Mesylate Air (clear glass ampoules)**	**Mesylate Nitrogen (clear glass ampoules)**	**Mesylate Air (amber ampoules)**	**Mesylate Nitrogen (amber ampoules)**
435.8	-	2.44	-	-
391.5	-	-	-	1.07
390.6	1.60	2.14	7.96	-
376.0	2.46	-	-	-
373.8	0.25*	0.70	0.21	-
359.0	51.71	64.05	0.44	3.41
340.8	9.92	6.07	0.42	-
326.3	-	-	-	0.57
299.8	0.25*	0.77*	-	-
253.7	0.04*	0.77*	-	-
215.8	0.07*	0.77*	-	-

*note: molecular ions found in the same peak

Clear glass ampoules

The molecular ion with the greatest abundance is found to be 359.0 corresponding to the demethylated product **[17, 18]** in both the samples sealed under air (51.71%) and nitrogen (64.05%). A degradant with a molecular ion of 340.8 (9.92%), corresponding to dechlorination **[28]**, was found to be quite prominent, as well as the sulfoxide (*m/z* 390.6, 1.60%) **[19]** and an oxidised and demethylated product, 376.0 (2.46%) **[30]** in those samples sealed in air. Mesylate samples sealed under nitrogen displayed additional molecular ions at 390.6 (2.14%), belonging to the sulfoxide degradant **[19]**, and 435.8 (2.44%). Low-level degradants with molecular ions at 373.8, corresponding to oxidised and hydroxylated derivatives **[13, 14, 15]**, the desulfurated product (*m/z* 215.8) **[12]**, *m/z* 253.7 and the dealkylated compound (*m/z* 299.8) **[11]** were present for both the aerobic and anaerobic conditions.

Amber ampoules

The major degradant formed under these conditions was that with a molecular ion at 390.6 (7.96%) **[19]**, corresponding to the sulfoxide, for a mesylate sample sealed in

air. Additional molecular ions belonging to the demethylated product (*m/z* 359.0) **[17, 18]** and the oxidised and hydroxylated derivatives (*m/z* 373.8) **[13, 14, 15]** were formed. The major degradant formed under nitrogen was that of the demethylated product (*m/z* 359.0, 3.41%) **[17, 18]**. An additional molecular ion was found at 326.3 (0.57%) **[16]**, corresponding to a dechlorinated and demethylated degradant.

4) Diffuse light

Table 8.6 gives the molecular ions found in prochlorperazine mesylate exposed to diffuse light (air and nitrogen)

Table 8.6

Relative percentage abundances of the molecular ions of the degradants found in prochlorperazine mesylate exposed to diffuse light (air and nitrogen)

m/z	**Mesylate Air (clear glass ampoules)**	**Mesylate Nitrogen (clear glass ampoules)**	**Mesylate Air (amber ampoules)**	**Mesylate Nitrogen (amber ampoules)**
390.6	-	-	1.43	1.14
359.0	-	-	0.45	0.33
340.8	-	-	0.46	0.25

Clear glass ampoules

The samples exposed to diffuse light did not degrade enough for any molecular ions to be detected.

Amber ampoules

The major degradant formed under air was found to have a sulfoxide molecular ion at 390.6 (1.43%) **[19]**. Low-level degradants with molecular ions of 340.8 **[28]** and 359.0 **[17, 18]**, corresponding to dechlorination and demethylation respectively, were found to occur in both air and nitrogen. A molecular ion at 390.6 [19] was also present in the samples sealed under nitrogen. This may be due to reaction of the drug with dissolved oxygen in the solvent because the sample was not deaerated sufficiently.

5) *"Unpackaged" sample studies under sunlight and 254nm UV light (samples placed 10 cm from light source)*

Unpackaged samples (i.e. samples not stored in ampoules) were studied to ascertain the screening effect of glass on radiation below 310 nm. This may have implications for the degradants formed in the body. Table 8.7 gives the molecular ions found in "unpackaged" prochlorperazine mesylate and edisylate solutions under sunlight and UV light.

Table 8.7

Relative percentage abundances of the molecular ions of the degradants found in "unpackaged" prochlorperazine mesylate and edisylate solutions (sunlight and UV light at 254 nm)

	Mesylate	**Edisylate**	**Mesylate 254 nm**	**Edisylate 254 nm**
m/z	**Sunlight**	**Sunlight**	**UV light**	**UV light**
448.9	-	7.38	-	-
435.8	3.34	-	-	-
430.9	-	-	0.81	-
391.5	0.22	0.79	-	0.77
390.6	9.55	1.38	-	-
376.0	-	-	0.85	1.75
359.0	-	1.48	-	6.03
340.8	2.65	8.87	1.09	3.94
290.9	0.65	-	0.70	-
290.1	-	-	0.83	-

a) Sunlight

Mesylate

The major degradants present had molecular ions corresponding to the sulfoxide *m/z* 390.6 (9.55%) **[19]**, the dechlorinated derivative resulting from photo-reduction (*m/z* 340.8, 2.65%) **[28],** and 435.8 (3.34%). Molecular ions belonging to the sulfone (*m/z* 391.5, 0.22%) **[21, 22, 23]** and the oxidised and dealkylated product or the dechlorinated derivatives (*m/z* 290.9, 0.65%) **[26, 27]** were present at low-levels.

Edisylate

Edisylate was found to degrade faster than mesylate in the kinetic studies conducted. The major degradants found in edisylate samples were those with molecular ions resulting from dechlorination (*m/z* 340.8, 8.87%) **[28]** and 448.9 (7.38%). Additional molecular ions were found at 359.0 (1.48%) corresponding to demethylation **[17, 18]** and to sulfoxidation (*m/z* 390.6, 1.38%) **[19]**. A low-level degradant with a molecular ion at 391.5 (0.79%) corresponding to sulfone formation **[21, 22, 23]** was also found.

b) 254 nm UV light (10 cm from irradiation source)

Mesylate

A compound with a molecular ion at 340.8 (1.09%) belonging to the dechlorinated product **[28]** was found to be the major degradant. Additional molecular ions corresponding to a dechlorinated or oxidised dealkylated degradant (*m/z* 290.9, 0.70%) **[26, 27]**, a hydroxylated derivative (*m/z* 290.1, 0.83%) **[8]**, oxidised dimers (*m/z* 430.9, 0.81%) **[24, 25]** and 376.0 (0.85%) **[30]** were present as low-level degradants.

Edisylate

Molecular ions at 359.0 (6.03%) **[17, 18]** and 340.8 (3.94%) **[28]**, belonging to the demethylated and dechlorinated products respectively, were found to have the greatest abundance. Additional molecular ions corresponding to low-level degradants were found at 391.5 (0.77%) **[21, 22, 23]** and 376.0 (1.76%) **[30]**.

6) "Unpackaged" sample studies under UV light (samples placed 40 cm from light source) and diffuse light

Table 8.8 gives the molecular ions found in "unpackaged" prochlorperazine mesylate and edisylate solutions under UV light and diffuse light

Table 8.8

Relative percentage abundances of the molecular ions of the degradants found in "unpackaged" prochlorperazine mesylate and edisylate solutions (UV light at 254 nm and diffuse light)

m/z	**Mesylate 254 nm UV light**	**Edisylate 254 nm UV light**	**Mesylate Diffuse**	**Edisylate Diffuse**
448.9	-	-	-	0.43
391.5	1.37	0.69	0.25	0.84
390.6	-	-	35.92	45.58
376.0	1.17	-	-	-
373.8	0.26	-	-	-
359.0	5.87	6.65	-	0.76
340.8	1.83	3.73	0.75	2.18
290.9	1.06	0.23	0.17	-

c) 254 nm UV light (40 cm from irradiation source)

The major degradant found in both mesylate and edisylate solutions corresponded to a demethylated derivative (molecular ion 359.0) **[17, 18]** with abundances of 5.87% and 6.65%, respectively. Additional peaks were observed for the dechlorinated derivative at 340.8 **[28]**, the oxidised derivative at 391.5 **[21, 22, 23]** and the dechlorinated and demethylated derivatives at 290.9 **[26, 27]**. Molecular ions corresponding to low-level degradants in the mesylate sample were found to be the oxidised derivatives at 373.8 **[13, 14, 15]** and 376.0 **[30]**.

d) Diffuse light

A molecular ion belonging to the sulfoxide, observed at 390.6 **[19]**, was found to be the major degradant for both mesylate and edisylate. Low-level degradants with molecular ions at 340.8 corresponding to the dechlorinated product **[28]** and the oxidised products at 391.5 **[21, 22, 23]**, were also found. Additional molecular ions were observed at 290.9 belonging to the dechlorinated and demethylated derivatives **[26, 27]** and 448.9 for mesylate and edisylate.

8.3.1.2 Thermal stability

Samples for the thermal stability studies were subjected to the conditions described previously in Table 8.1. The relative abundances of the molecular ions found in the thermal stability studies are given in Table 8.9.

Table 8.9

Relative percentage abundances of the molecular ions of the degradants found in prochlorperazine mesylate after heating at 100°C in clear ampoules (air and nitrogen)

m/z	Mesylate Air	Mesylate Nitrogen
390.6	72.52	5.55
372.0	1.07*	0.78*
359.0	2.40	0.42
340.8	1.07*	0.42*
307.9	0.52	-
292.0	0.35	-

*note: molecular ions found in the same peak

The sulfoxide **[19]** (*m/z* 390.6) was found to be the major degradant (abundance of 77.52%) in those mesylate samples sealed in air. More sulfoxide degradants were formed at 100°C than at 60°C, as expected. Low-level degradants were found at 340.8 **[28]**, 359.0 **[17, 18]**, 373.8 **[13, 15]**, 307.9 **[9]** and 292.0.

A relatively small amount of sulfoxide **[19]** formation (5.55%) occurred in those samples sealed under nitrogen (probably due to a lack of insufficient purging of the samples with nitrogen). Additional molecular ions indicating the presence of low-level degradants were found at 340.8 (dechlorination) **[28]**, 359.0 (demethylation) **[17, 18]** and 373.8 (oxidation) **[13, 14, 15]**.

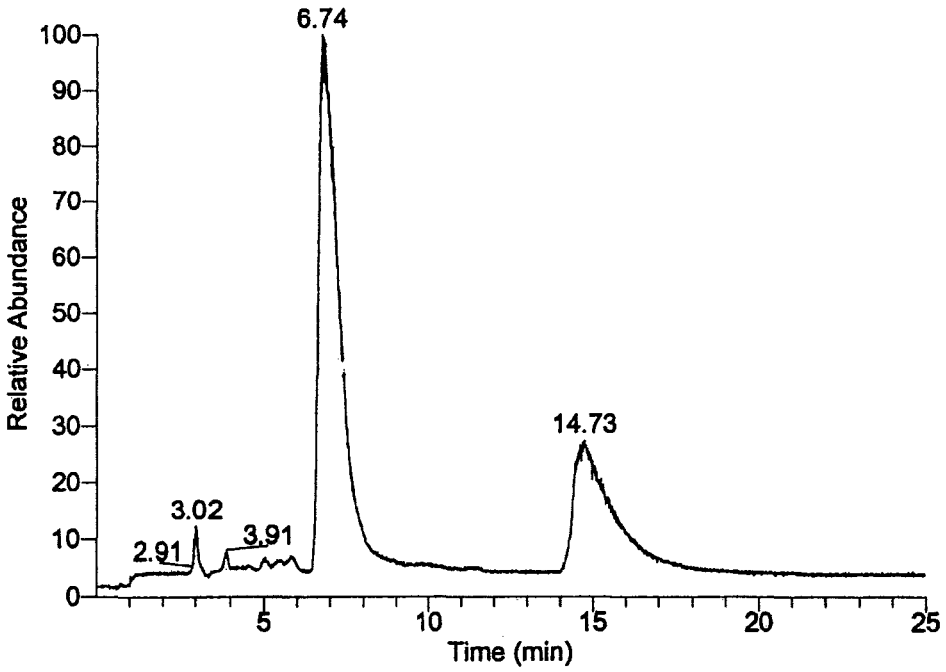


Fig. 8.3 Representative TIC chromatogram for prochlorperazine mesylate at 100°C under air

8.3.1.3 Photoproduct identification and proposed degradation pathways

The pathways proposed for degradation of prochlorperazine are given in Figures 8.4a and 8.4b.

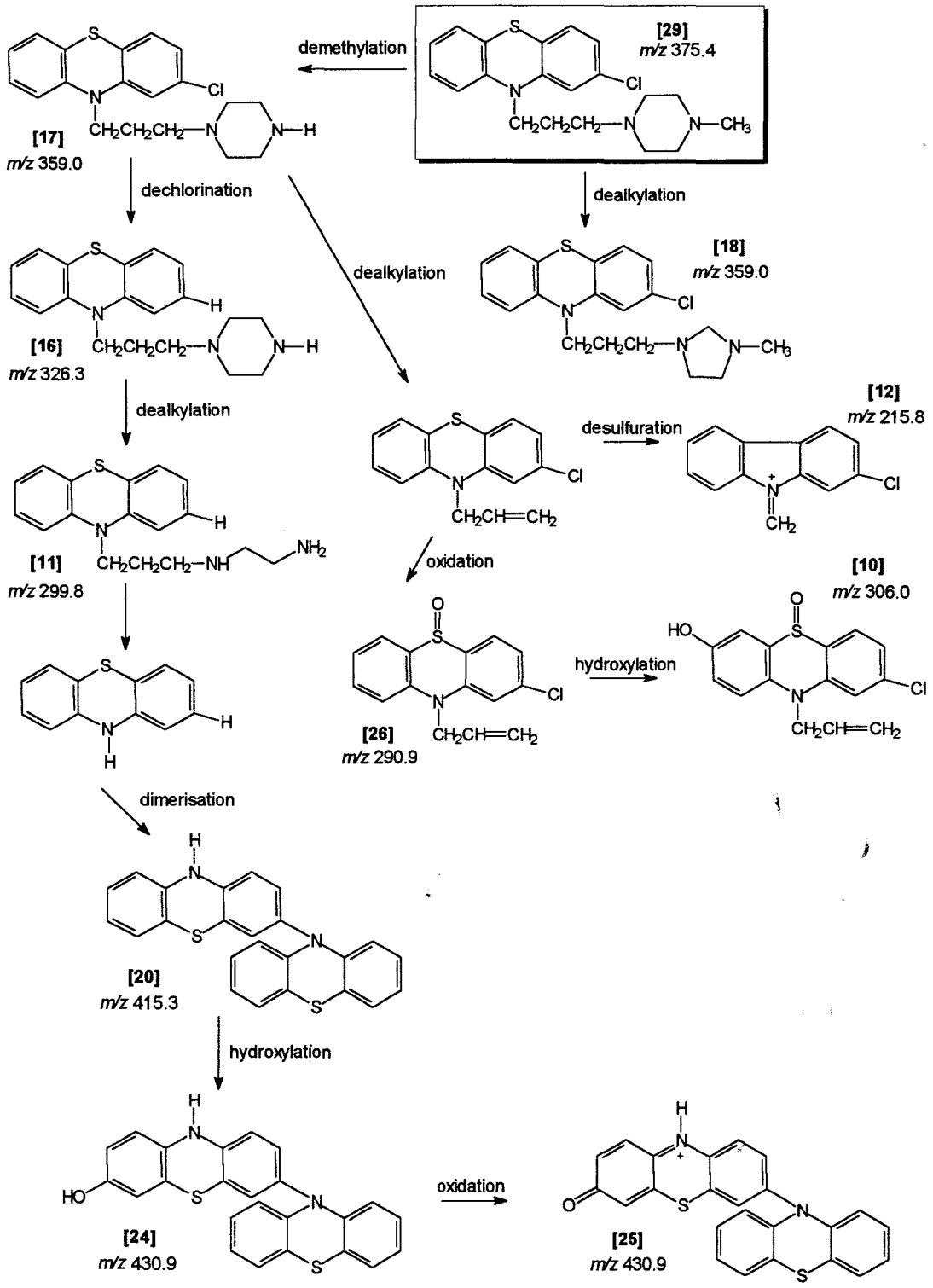


Fig. 8.4a Proposed degradation pathways for prochlorperazine

The degradation pathway is continued in Figure 8.5b following the discussion of the figure above.

Degradant **[16]**, formed by the elimination of the chlorine atom, is proposed to be a photoreduced product, the result of photodechlorination and the addition of hydrogen (Breyer, 1974). Photodechlorination is known to be a common procedure for chloroaromatic compounds. Degradant **[15]** was formed after demethylation **[17]** of the parent drug **[29]** which was then followed by dealkylation to form degradant **[11]**, accounting for the loss of 26.6 daltons. Cleavage of the side chain at the ring nitrogen is proposed to lead to dimerisation of the phenothiazine ring system to form degradant **[20]**. Hydroxylation of this dimer **[24]** followed by further oxidation would result in a degradant with a molecular mass of 430.9 **[25]**, a difference of 16 daltons. Formation of these dimers was observed primarily in those samples sealed under nitrogen as reported in the literature (Huang and Sands, 1967). Exceptions to this include mesylate samples sealed in air and exposed to UV irradiation (10 cm from the light source), as well as a sample exposed to sunlight in an amber ampoule, where the dimers were present as low-level degradants (*m/z* 448.9 and 434.9, respectively). These degradants could also be due to adducts formed between the mobile phase used and the various degradants formed.

Partial cleavage of the side chain, followed by oxidation of the demethylated parent drug **[16]**, may be responsible for the formation of degradant **[26]** which accounts for the difference of 68.1 daltons. Further cleavage of the methylene bridge of the remaining side chain, followed by desulfurisation, led to the formation of a degradant **[12]** which has a molecular ion of 215.8. Hydroxylation of degradant **[26]** in position 7 of the phenothiazine ring system gave product **[10]**. Fragmentation processes involving the methylene bridge were found to be a common occurrence (in those phenothiazines that have a methylene bridge) by Melikian *et al.* (1977). Fragmentation produced weak ions that corresponded in mass to the substituted phenothiazin-10-yl nucleus and to its methylene homologue.

Dealkylation of the piperazine ring system of the parent drug **[29]** is proposed in the formation of degradant **[18]** (loss of 35 daltons), although the mechanism leading to this is uncertain. Loss of a chlorine atom, would also account for the loss of 35 daltons and seems to be a more likely explanation. Literature reports indicate that

the methyl derivatives, prochlorperazine and trifluoperazine, are more susceptible to *N*-dealkylation than the β-hydroxyethyl derivatives (trifluoperazine). Breyer *et al.* (1974) studied the four structurally-related phenothiazines and showed that the piperazine ring can be partially degraded to produce an ethylenediamine structure with ring cleavage occurring to a considerable extent. The methyl or the hydroxyethyl group is retained on the terminal nitrogen atom. The piperazine ring cleavage was assumed to proceed via two consecutive *N*-dealkylation reactions (including products **[11]**, **[26]**, **[12]** and **[10]**).

Further degradation pathways proposed for prochlorperazine are continued in Fig. 8.4b.

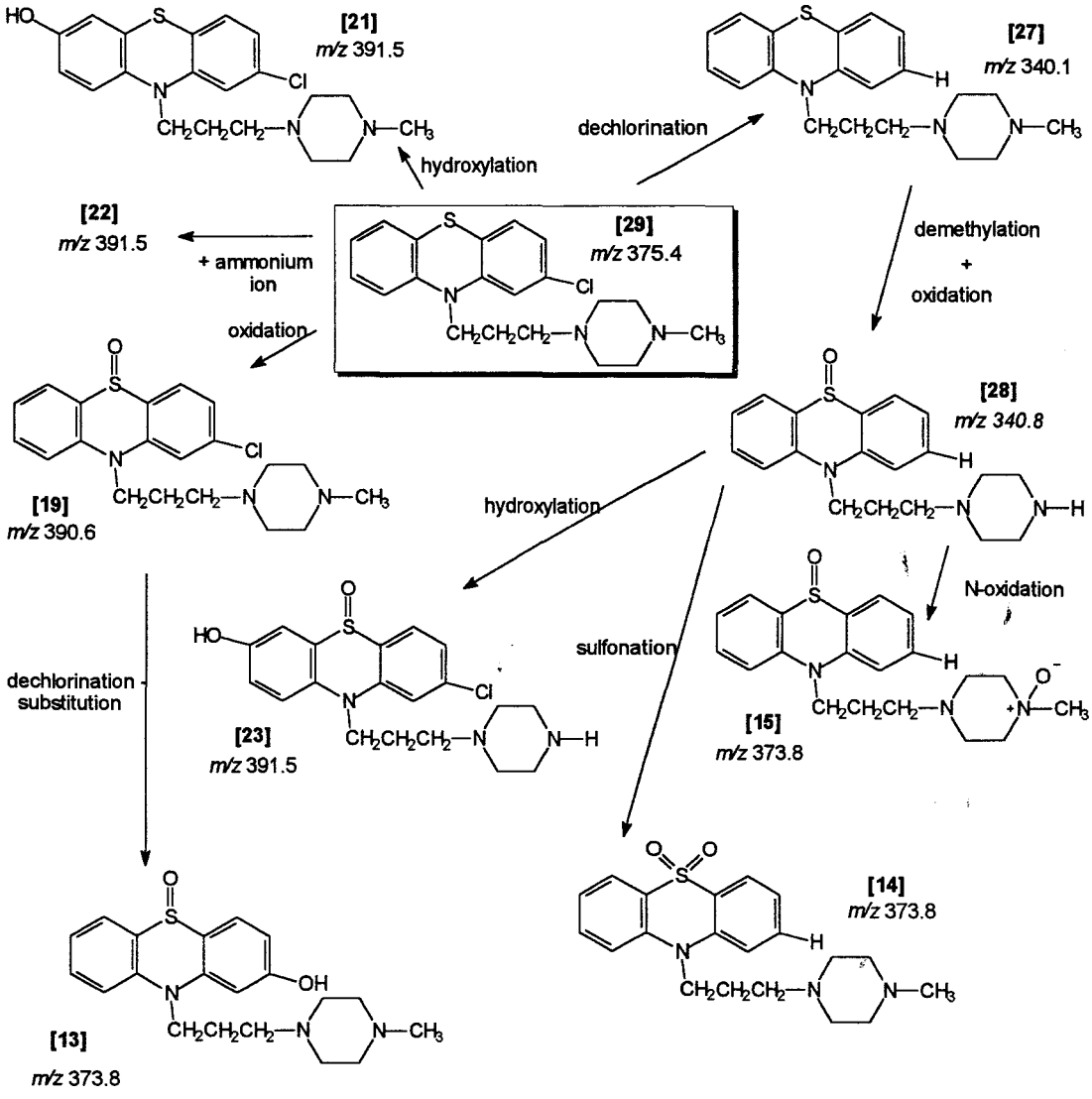


Fig. 8.4b Further proposed degradation pathways for prochlorperazine (continued from Fig 8.4a)

Photoreduction and photosubstitution of the chloro-derivatives are anticipated during irradiation of solutions of the derivatives, owing to the photolabile nature of chlorine. Following dechlorination of the parent drug, oxidation may result in sulfoxidation of **[27]** to give degradant **[28]**. Further oxidation leading to sulfone formation, or *N*-oxidation, results in the formation of products **[14]** and **[15]**, respectively. Hydroxylation of **[28]** leads to the formation of product **[23]**.

Hydroxylation of the parent drug **[29]** results in the formation of **[21]** (*m/z* 391.5). A molecular ion at 391.5 could also be the result of an ammonium adduct with the parent drug to form **[22]** (difference of 18 daltons).

Oxidation of the parent drug **[29]** results in the formation of the degradant **[19]** with a molecular ion at 390.6. This is the sulfoxide **[19]** and is one of the most common degradants formed in phenothiazines. Further oxidation of the sulfoxide results in the formation of the sulfone **[14]**, which is also a common degradant. Dechlorination of this sulfoxide and subsequent photosubstitution forms **[13]** with *m/z* 373.8.

Attempts were made at determining the degradation mechanism from the LC-MS spectra patterns obtained of the degradants. However, for molecular ions at *m/z* 249.6, 430.9, 435.8 and 448.9 present in the solution studies, there is some degree of uncertainty with respect to the structures of the degradants. The product ion at 430.9 may be due to a solvent addition product (MeOH), in conjunction with the sodium adduct, which would give an additional 55 daltons to the molecular mass of prochlorperazine. The other product ions may be the result of dimers, because of the high molecular masses, but at this stage no conclusive evidence is available in support of this.

8.3.2 Solid state studies

Table 8.10 gives the molecular ions found in the solid state studies using LC-MS, their retention times, the proposed structures and supporting literature references. The structures are proposed on the basis of the molecular masses obtained and results reported in the literature.

The solid state samples heated at 60°C and 100°C showed the formation of degradants with molecular ions at *m/z* 307.3 **[9]**, 371.4 **[13]** and 429.0 **[24, 25]**. The corresponding proposed structures are shown in Table 8.2. The proposed degradation pathways for these structures have already been given in section 8.3.1.3. Oxidation of the parent drug **[29]**, following dechlorination, at the ring sulfur atom and the piperazine nitrogen atom, resulted in the formation of degradant **[15]**. Sulfoxidation as well as hydroxylation, following dechlorination of the parent drug, may have resulted in the formation of degradant **[13]**. Sulfone formation **[13]** could also be responsible for the presence of the degradant with a *m/z* at 371.4. Dealkylation followed by cleavage of the side chain at the ring nitrogen of the parent drug **[29]** is proposed to lead to dimerisation of the phenothiazine ring system to form degradant **[20]**. Hydroxylation of this dimer **[24]** followed by further oxidation would lead to the formation of degradant **[25]**. Cleavage of the side chain followed by sulfoxidation and hydroxylation may have been responsible for the formation of degradant **[10]**. Desulfuration of the parent drug could also be responsible for the presence of the molecular ion at 307.3 **[9]**.

The degradants formed in the solid state samples were found to the same as those present in solution.

8.4 Conclusions

LC-MS is a useful tool for rapidly obtaining detailed structural information on low-level degradants observed in HPLC analyses of samples. Furthermore, chromatographic resolution of co-eluting or unresolved components is not essential to obtain product ion data for structural analysis, due to the mass-resolving capability of mass spectrometry. This strategy has a significant implication for industry, because impurities at greater than the 0.1% levels need to be examined and identified.

The TIC chromatograms obtained from LC-MS illustrate essentially the same resolution and retention times for degradants as observed in the analytical LC analyses.

Phenothiazine antipsychotic agents invariably possess at least two readily oxidisable heteroatoms; one or more sulfur atoms and one or more aliphatic tertiary amine groups (Jaworski *et al.*, 1993). The principle degradation products of the phenothiazines have been reported to be the corresponding sulfoxides (Abdel-Moety *et al.*,1996). Sulfoxidation resulting in 5-sulfoxide (ring sulfoxide) metabolites is a common biotransformation pathway in all phenothiazines. Phenothiazine antipsychotic agents are extensively metabolised by various routes, which include aromatic hydroxylation, ring S-oxidation, *N*-dealkylation, and *N*-oxidation (Aravagiri *et al.*, 1985) as found to occur in the present degradation studies.

The distribution of π electrons in the drug may lead to the formation of many radical forms, such as at the S atom, at the N_{10} atom, and between the S and N atoms on the phenothiazine ring, as a result of the resonances. The formation of the radical on the S atom is expected under the influence of brief UV irradiation (Abdel-Moety, 1996). Sharples (1980) stated that, in general, 2-substituted phenothiazines photodecompose to yield the corresponding sulfoxide. The 2-chloro derivatives, however, can also lose the 2-substituent through free radical formation:

$$ArCl \xrightarrow{UV} Ar\bullet + Cl\bullet \rightarrow ArH + HCl$$

Sulfonation, hydroxylation, dealkylation, dehalogenation and *N*-dealkylation are amongst the degradation pathways observed in the photostudies conducted. In the "unpackaged' sample studies, the sulfoxide (and the subsequent sulfone) were formed. Sulfoxidation is evident throughout the solution studies, except for those samples sealed under nitrogen, due to the lack of oxygen. Under these conditions, dimerisation was found to occur, whereas little or no dimerisation took place in samples sealed under air. Sulfoxidation was found to be the major degradation pathway in the thermal stability studies of both salts. These results correspond with the routes of degradation which have been documented in *in vitro* studies (Pawełczyk et al., 1975).

The photostability studies of prochlorperazine seems to indicate a less-complex pattern of degradation for the solid state than for aqueous solutions. The degradants present in the solid state samples were found to be mainly the result of oxidation, hydroxylation, desulfuration, dimerisation and dealkylation.

CHAPTER 9. CONCLUSIONS

Propyl piperazine-substituted phenothiazines are an important group of neuroleptic drugs possessing increased potency over chlorpromazine. In addition to the susceptibility to oxidation (Huang and Sands,1967; Ljunggren and Möller, 1977; Sharples, 1981; Moore and Tamat, 1980), the chlorine substituent has been implicated in the photoinstability of phenothiazines (Moore, 1977). This was confirmed by Pawełczyk *et al.*, in photodegradation studies (Pawełczyk and Marciniec, 1977 a; Pawełczyk and Marciniec, 1977 b). The presence of a 2-chloro substituent in prochlorperazine and the ICH requirements for identification of degradants provided a motivation to investigate the photo- and thermal stability of prochlorperazine mesylate and edisylate.

The HPLC method, developed to analyse the products in the photo- and thermal stability studies, was validated to ensure that the performance and reliability of the method met the requirements of the analytical function. Although a complete validation includes degradants and synthetic precursors, the validation in this study satisfied the linearity, accuracy and precision, selectivity, LOD and LOQ and the ruggedness parameters. The method was validated in respect of the drug and its major degradant, the sulfoxide. Photodiode array, as well as mass spectrometry ensured the integrity of the drug peak when applying the method to the determination of the kinetics of degradation of the drug. The selectivity was confirmed under light, heat, oxidative, acid and base conditions, indicating that the method is specific for prochlorperazine. Correlation coefficients of > 0.999 for the linearity and precision studies were obtained, which is within acceptable limits for linearity. The lowest amounts of analyte which were reproducibly quantitated for duplicate injections is 1.25 $\mu g\ mL^{-1}$, while the lowest amount of analyte which could be detected above baseline noise is 0.13 $\mu g\ mL^{-1}$. It was therefore concluded that the analytical method for prochlorperazine could be applied with reasonable assurance of the reliability of the procedure.

Potassium ferrioxalate was used as a chemical actinometer to allow for variations in light intensity. The actinometric studies indicated that the light sources used for the studies were fairly stable over time. The quantum yield data indicated that deactivation processes are more important than chain reactions in the degradation of prochlorperazine.

The main oxidative product of the phenothiazines is the sulfoxide (Felmeister and Schaubman, 1969 b). The synthesis of the 5-sulfoxide to provide standards to aid in their identification and characterisation in Chapters 5 and 8 was thus important. Although, various synthetic methods were considered, an adapted version of the Owens *et al.* (1989) method was used and the sulfoxides formed (for both edisylate and mesylate salts) were then characterised using NMR, IR, UV, TLC, MS, DSC, mp and HPLC.

The photolytic degradation studies showed that samples in aqueous solution sealed under nitrogen degraded faster than those sealed under air. This suggests that the oxygen present in the air acts as a triplet-state quencher. That the oxygen molecules scavenge the free-radicals formed on irradiation of the drug is confirmed by enhanced degradation of the drug in the absence of oxygen. The photolytic studies also showed that the use of amber ampoules retarded the degradation. The demethylated product [17, 18] was found to be the major photolytic degradant in clear glass ampoules (64.05%), while dechlorination [28] (9.92%) and sulfoxidation products [19] (1.6%) were also detected in significant amounts. Results from the amber ampoules studies indicated that the sulfoxide [19] was present as the major degradant (7.96%), while the demethylated product [17, 18] was present in 3.41% abundance. As would be expected, the fastest degradation occurred under sunlight, while the least degradation occurred under diffuse light.

The thermal stability studies indicated that prochlorperazine is quite stable in solution up to 60°C, degrading at temperatures exceeding 100°C. Samples sealed under nitrogen were found to be quite stable even at elevated temperatures with sulfoxide formation being the main degradation route.

A decrease in pH for the degraded solutions of prochlorperazine, implying the liberation of HCl, was observed. The liberation of HCl has been suggested as the possible cause of photosensitivity and skin discolouration observed in patients receiving chlorpromazine.

The thermal and photostability studies conducted on prochlorperazine in the solid state indicated the drugs were more stable to both heat and light than in aqueous solution, but still considerably unstable. Small quantities of degradants were found

in the samples exposed for 6 months indicating the greater stability of prochlorperazine in the solid state.

DSC screening has been used by many researchers as an indicator of major incompatibilities. Several excipients were found to be potentially incompatible, for example, sodium lauryl sulfate, magnesium stearate, Ac-Di-Sol and lactose. Some excipients such as Lubritab, Avicel PH 101, Avicel PH 102, Encocel, Methocel A15LV, -E4M, -E5, -E15LV, -K4M, AND -K4M CR were found to be suitable for use in prochlorperazine tablets. TLC analyses carried out confirmed the results obtained with DSC and, thus, DSC was found to be reliable indicator of the major incompatibilities that could be experienced.

Developing drug purity control regulations within ICH forums requires that impurities above the 0.1% level be examined and identified. A detailed study to identify degradants in the solid state and solution samples, exposed to various photolytic and thermal conditions, was thus done. An inability to adjust the mobile phase to exclude non-volatile buffer components (e.g. KCl) and maintain adequate separation, precluded the use of semi-preparative HPLC. Solid-phase extraction with gradient elution also proved unsuccessful due to the broad NMR signals obtained. LC-MS is a useful tool for rapidly obtaining structural information on low-level degradants observed in HPLC analyses and has been well documented in structural elucidation (Erickson *et al.*, 1995; Qin, *et al.*, 1994; Chen *et al.*, 1991; Mano, *et al.*, 1994). Thus it was applied to examining the degradants formed in the solution and solid state thermal and photostability studies.

Photodecomposition pathways such as sulfonation, hydroxylation, dealkylation, dehalogenation, dimerisation and *N*-dealkylation were observed. These products have previously been isolated and identified from solutions irradiated under aerobic and anaerobic conditions (Sharples, 1981, Ljunggren and Möller, 1977, Huang and Sands, 1967). Sulfoxidation is evident in all of the solution studies conducted, except for those samples sealed under nitrogen, where dimerisation was found to occur. Sulfoxide formation was found to be the major degradation pathway in the thermal stability studies conducted in air as has been documented previously in studies of metabolite formation (Pawełczyk *et al.*, 1975). In the solution study, dechlorination, with subsequent photoreduction and demethylation, occurred in

varying amounts under all light conditions. The sulfoxide and 2-hydroxy derivative also occurred in significant amounts.

The photostability studies of prochlorperazine in the solid state indicate a less complex pattern for degradation than for aqueous solutions, indicating the drug is more stable in the solid state than in solution. The degradants present in the solid state samples were found to be mainly the result of oxidation [13], hydroxylation [24, 25], desulfuration [9], dimerisation and dealkylation.

This study of the rates of degradation and the nature of the degradants has been successful in providing understanding of the photolytic and thermal degradation pathways of prochlorperazine.

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