A PREFORMULATION STUDY OF PYRIDOXAL HYDROCHLORIDE FOR SOLID DOSAGE FORM DESIGN AND DEVELOPMENT

by

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ABSTRACT

In this dissertation physicochemical properties of the B_6 vitamer, pyridoxal hydrochloride (PL HCl), are investigated with the aim of generating the necessary profile for the rational development of a stable, safe and effective formulation containing this drug. Recent research suggests that administration of PL HCl may be particularly effective in raising the depleted intracellular pyridoxal phosphate levels found in many asthmatics treated with theophylline. The solubility characteristics of PL HCl suggest that its absorption and bioavailability should not be problematic. An investigation of the solid state properties of PL HCl, using thermal analysis combined with infra-red spectrophotometry indicated the existence of at least two polymorphs. Subsequent studies demonstrated that polymorphic transformations may also be induced at moisture levels of 57-68% relative humidity. The moisture sorption isotherm derived for PL HCl by Karl Fischer titration also provided evidence of two polymorphs of markedly different hygroscopicity. Atmospheric moisture was also shown to be a highly significant destabilizing factor with respect to chemical stability. In a further study a Plackett-Burman factorial design was used to investigate PL-excipient compatibility. Colloidal silicone dioxide, celluloses and magnesium stearate were found to have significant stabilizing effects, whilst lactose, starch and mannitol

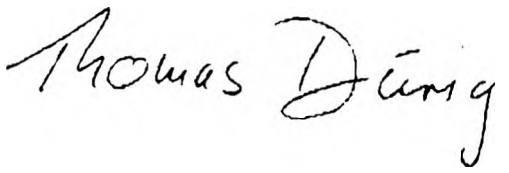
were found to be incompatible. These results were also confirmed by a study employing differential scanning calorimetry and conventional "one factor at a time" isothermal stress testing. Many of the formulation problems posed by PL HCl can be related to the hemiacetal group and its corresponding aldehyde. These make PL HCl an inherently unstable and reactive drug molecule. A further contributing factor is the hygroscopicity of the compound. Taking the above findings into consideration, with careful selection of formulation components, manufacturing procedures and storage conditions, a stable and effective drug delivery system containing PL HCl can be envisaged.

DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted for the degree of Master of Pharmacy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other university.

Thomas Durig

The eighth day of August, 1991.



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Durig, T. and Fassihi, A.R., The influence of atmospheric moisture on the solid state stability of pyridoxal hydrochloride. Presented at the twelfth Annual Congress of the Academy of Pharmaceutical Sciences, Potchefstroom, April 1991.

E Sa, C. and Durig, T., Simultaneous determination of theophylline, pyridoxal and pyridoxine by paired-ion, reverse phase high performance liquid chromatography. Presented at the twelfth Annual Congress of the Academy of Pharmaceutical Sciences, Potchefstroom, April 1991.

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CHAPTER ONE

1 INTRODUCTION

Preformulation research encompasses the pharmaceutical and analytical investigations which precede and support the development and design of new dosage forms. Preformulation can thus be regarded as the first learning phase about a new drug candidate which has been selected (on the basis of pharmacological and toxicological studies), for development into a marketable pharmaceutical product. Such products should be stable, possess good bioavailability and be patient acceptable. The principle activity during this developmental phase is the generation of a detailed profile of pharmaceutically relevant and useful physicochemical properties of the drug substance and its combination with other bioactive substances, selected solvents, excipients and packaging components (Motola and Agharkar, 1984).

There is no definitive approach to preformulation. A number of reviews outlining various preformulation methodologies and interpretations have been published (Wells, 1989a; Motola and Agharkar, 1984; Fiese and

Hagen, 1986; Nyqvist, 1986; Boatman and Johnson, 1981). However, certain fundamental aspects are common to all preformulation studies. Preformulation studies are undertaken during the early stages of product development and often only small quantities of bulk drug (milligrams rather than grams) are available for experimentation (Wells, 1989b). Furthermore, due to the competitive nature of the pharmaceutical industry and the high costs involved in research, time is also limited. Crucial data must therefore be generated within months in order to (a) highlight the need for further modification to the drug substance (eg. salt formation or crystal modifications) to eliminate undesirable physicochemical properties and (b) to rapidly facilitate progress toward clinical studies. It is for this reason that the determination of the intrinsic solubility and pk_a are mandatory for any new compound (Wells, 1989b). These fundamental properties immediately indicate the need and possibility of making more soluble salts of the drug to eliminate solubility related bioavailability problems.

The dual restrictions of limited time and initial drug quantity make it essential that investigators be pragmatic and only generate data which is of immediate relevance (eg. detailed mechanistic and kinetic studies should be reserved for later stages of the development program). In addition, experiments must always be designed to obtain maximum useful information with

limited bulk. To achieve this, interrelationships between various physicochemical properties can be made use of. By determining a number of fundamental physicochemical properties a number of others can be derived (Wells, 1989b). Various physicochemical properties which are commonly examined in preformulation and their interrelationships are shown in figure 1.1.

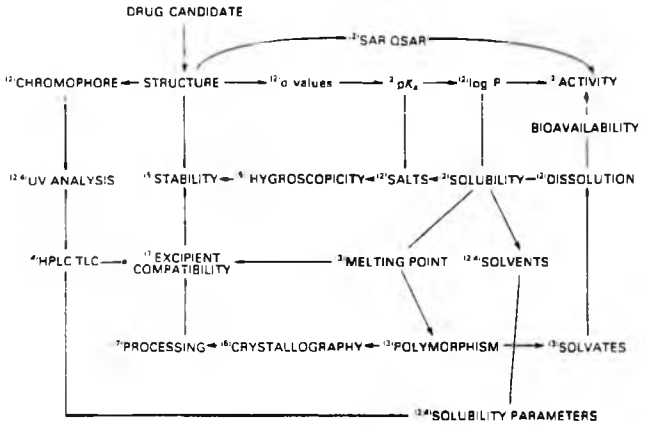
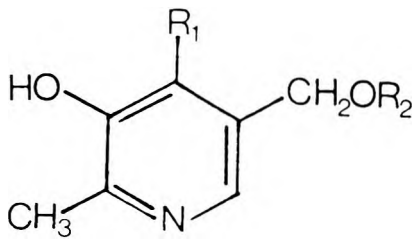


Figure 1.1 The interrelationship of drug properties examined in preformulation

Furthermore, wherever possible theory and prediction should be applied to reduce experimentation or to channel experimentation into more specific directions. It is also often possible to limit experimentation by making use of existing data on the compound. Sources include reports from the organic research chemists involved in the synthesis of new substances and reports on pharmacological, toxicological and biochemical studies on the new compound. Useful data may also be obtained from literature describing analogues of the compound which may already be in use.

In preformulation each drug candidate must therefore be considered individually and the amount of pre-existing data and the types of dosage forms likely to be employed must be taken into account before the scope and design of the preformulation program is decided upon.

In this dissertation a preformulation study of pyridoxal hydrochloride is presented. Pyridoxal hydrochloride (PL HCl) is the hydrochloride salt of pyridoxal (PL), one of six B_6 vitamers found in nature (Figure 1.2).



R_1	R_2	
CH_2OH	H	Pyridoxine(PN)
CH_2OH	PO_3^{2-}	Pyridoxine-5-phosphate(PNP)
CH_2NH_2	H	Pyridoxamine(PM)
CH_2NH_2	PO_3^{2-}	Pyridoxamine-5-phosphate(PMP)
CHO	H	Pyridoxal(PL)
CHO	PO_3^{2-}	Pyridoxal-5-phosphate(PLP)

Figure 1.2 The six B_6 vitamers

The term vitamin B_6 refers to all 3-hydroxy-2-methyl pyridine derivatives which mimic the biological activity of pyridoxine (PN). However pyridoxal-5-phosphate (PLP) is the only active coenzyme form of vitamin B_6. The other vitamers therefore undergo biotransformation to PLP in order to exert their biological effect.

PLP participates in at least 100 different enzymatic

reactions (Sauberlich, 1985). PLP is an essential coenzyme in amino acid metabolism and participates in the deamination, decarboxylation, transamination, racemization and transsulphuration of amino acids. In brain metabolism PLP acts as the codecarboxylase to specific amino acid decarboxylases involved in the production of neurogenic amines and neurotransmitters (eg. serotonin, dopamine and noradrenaline) from amino acids such as tryptophan, tyrosine and phenylalanine (Table 1.1) (Marks, 1975).

Table 1.1 Biologically important amines derived from amino acids or amino acid derivatives under action of decarboxylase and PLP

Amino Acid	Amine
Histidine	Histamine
5-Hydroxytryptophan	5-Hydroxytryptamine(serotonin)
Aspartic acid	β-Alanine
Glutamic acid	γ-aminobutyric acid (GABA)
Phenylalanine	Noradrenaline
Tyrosine	Adrenaline
	Tyramine
	Dopamine
Cysteic acid	Taurine

Further biochemical processes which require the presence of PLP include the biosynthesis of coenzyme A, the conversion of linoleic acid to arachidonic acid in the metabolism of essential fatty acids, the incorporation of iron during haemoglobin synthesis and porphyrin biosynthesis (Marks, 1975; Ebadi, 1986).

Furthermore PLP takes part in the metabolism of a number of other vitamins. Vitamin B_6 deficiency results in

reduced absorption and storage of vitamin B_{12} and increased vitamin C excretion. Through its involvement in tryptophan and kynurenine metabolism, PLP is also involved in nicotinic acid (vitamin B_3) biosynthesis which is disrupted in vitamin B_6 deficiency (Marks, 1975; Ebadi, 1986).

Due to its important physiological functions and its widespread involvement in metabolism throughout the body, vitamin B_6 has also been implicated in the aetiology and therapy of a number of disease states and physiological conditions. The therapeutic use and pharmacology of vitamin B_6 has been comprehensively reviewed by a number of authors (Reynolds and Leklem, 1985; Lui and Lumeng, 1986). This topic is only briefly discussed here with emphasis on some of the more recent findings concerning the therapeutic use of vitamin B_6. Apart from nutritionally induced vitamin B_6 deficiency, reports of the successful treatment of a whole range of seemingly unrelated diseases with vitamin B_6 are available. These include asthma (Reynolds and Natta, 1985), liver disease (Lui and Lumeng, 1986), autistic syndrome (Rimland et al, 1978), premenstrual tension (William et al, 1985) and gestational diabetes (Bennik and Scheurs, 1975). Furthermore, a series of genetic diseases which do not cause vitamin B_6 deficiency, but which respond to B_6 therapy is known. These include homocystinuria, gyrate atrophy of the choroid and retina, hereditary sideroblastic anaemia, infantile

convulsion, primary cystathioninuria, xanthuremic aciduria and primary hyperoxaluria (Lui and Lumeng, 1986). Vitamin B_6 deficiency has also been found to be prevalent in pregnancy and lactation. Finally, vitamin B_6 deficiency can also be acquired from the use of certain drugs. Increased vitamin B_6 requirements are frequently found in alcoholics and women using oral contraceptive agents. Other drugs known to interact with vitamin B_6 are isoniazid, cycloserine, penicillamine, hydralazine and levodopa. In the case of levodopa, vitamin B_6 acts as an antagonist, completely anulling the beneficial effects of levodopa in Parkinsons disease. However, serious vitamin B_6 deficiency does not usually occur and vitamin B_6 is therefore contra-indicated in levodopa therapy (Lui and Lumeng, 1986).

More recently, signs of vitamin B_6 deficiency first observed in some asthmatics by Collip and co-workers (1973) and later by Reynolds and Matta (1985), have been observed and reported by Delport et al (1988). These workers demonstrated significantly decreased plasma PLP levels in asthmatics. In their study they also observed that 82% of patients regularly received theophylline therapy. Administration of theophylline to a group of 17 volunteers also resulted in large reductions in plasma PLP, however plasma PL was not affected. In a placebo controlled, double blind follow up study theophylline induced depressed plasma PLP levels were again demonstrated. It was also shown that theophylline

is a potent non-competitive inhibitor of PL kinase (EC 2.7.1.35).

PL kinase is a key enzyme in vitamin B_6 metabolism and is widely distributed in mammalian tissues (McCormick et al, 1961). Extracellular (plasma) PL is the major source of PLP (the active coenzyme) for extrahepatic tissues. Extracellular PL is transported across the plasma membrane and subsequently phosphorylated by pyridoxal kinase (Figure 1.3) (Mehanso and Henderson, 1980; Anderson et al, 1971; McCormick et al, 1961). In contrast, plasma PLP can not serve as a direct source of intracellular PLP as it is predominantly albumin bound and is more polar than PL. It therefore can not be transferred across plasma membranes (Anderson et al, 1971; Lumeng and Li, 1980). Plasma PLP can thus only serve as a vitamin B_6 source to extrahepatic tissues after dephosphorylation to PL. Although PN (the most commonly found form of vitamin B_6) can cross plasma membranes and can be phosphorylated by PL kinase, it too can not act as a direct source of intracellular PLP as conversion of PNP to PLP requires pyridoxine-5-phosphate oxidase enzyme which is absent in most extrahepatic cells (Lumeng et al, 1985). Most extrahepatic tissues are therefore dependent on adequate levels of plasma pyridoxal and pyridoxal kinase activity to ensure adequate intracellular PLP levels.

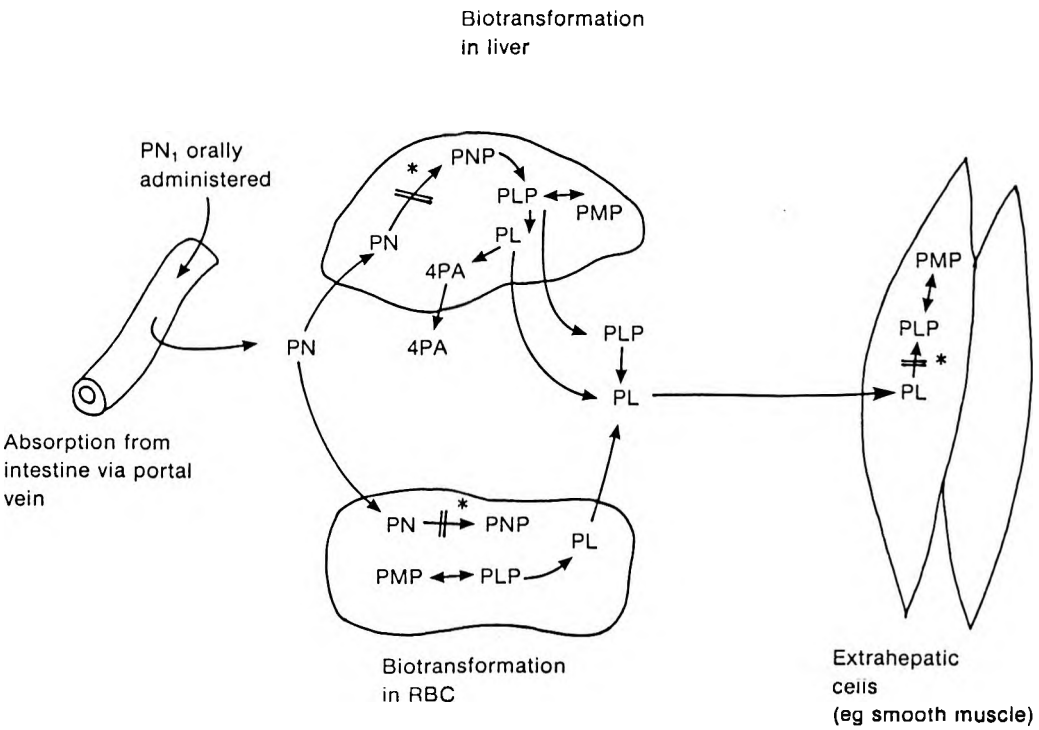


Figure 1.3 Pathway for conversion and transport of vitamin B_6 compounds in plasma after pyridoxine (PN) ingestion. * Denotes points where theophylline inhibits vitamin B_6 metabolism. Key: PL-pyridoxal; PM-pyridoxamine PNP, PLP, PMP-phosphorylated derivatives of PN, PL and PM respectively; 4PA-4 pyridoxic acid; RBC red blood cell;

Due to the widespread involvement of PLP in human metabolism, theophylline induced inhibition of PL kinase could have serious and far reaching consequences for asthmatics receiving theophylline treatment. Ubbink et al (1989) have shown that after short term theophylline treatment, the deranged PLP metabolism can be restored to normality by vitamin B_6 supplementation (10 mg PN daily). In previous studies clinical improvement in asthmatic patients was also observed after vitamin B_6 supplementation (Reynolds and Natta, 1985; Collip et al, 1975).

It is therefore apparent that vitamin B_6 supplementation may be of considerable value in asthmatics receiving

theophylline therapy. Furthermore the use of pyridoxal rather than pyridoxine should be considered. Currently PN HCl is the only form of vitamin B_6 approved for clinical use. This can be attributed to the superior stability profile of PN HCl when compared to the other B_6 vitamers and the lack of evidence supporting the need for any other B_6 compound in clinical practice (Lumeng, 1986). However as it is now clear that adequate intracellular PLP is dependant on adequate circulating PL levels, it is obvious that administration of PL is advantageous as this would be directly available to the circulation and the extrahepatic tissues. PL could thus rapidly counteract the imbalance in intracellular PLP which may be present in theophylline treated asthmatics and which may also prevail in many other vitamin B_6 dependent conditions. On the other hand, as previously outlined, PN can not be directly converted to PLP by extrahepatic tissues. Utilisation of PN thus depends on adequate vitamin B_6 metabolism in the liver (Figure 1.3). This is particularly problematic in patients receiving theophylline therapy as phosphorylation of PN by PL kinase is one of the critical steps in this process. As theophylline is now known to inhibit PL kinase it can therefore also be expected that hepatic conversion of PN to PL is not as efficient as in normal patients. This would result in a lower circulating PLP pool and ultimately lower levels of extracellular PL. Finally, although PL has been clearly shown to be more unstable than PN, this should not be viewed as an insurmountable

obstacle as with modern formulation technology, accurate analytical instruments and a better understanding of incompatibility and stability programs many unstable drugs can be stabilised and incorporated in specific drug formulations.

As a first step in the development of a solid dosage form containing pyridoxal, possibly in combination with theophylline or other multivitamins, a preformulation study of PL HCl was undertaken. For economic and practical reasons the hydrochloride salt of PL was selected as this is currently the only commercially available form of PL. It was therefore decided that synthesis and use of other salts of PL would only be considered if the physicochemical properties of PL HCl were found to be totally unsuitable for the purposes of producing a stable, solid dosage form.

The aim of this preformulation study is to generate the necessary physicochemical data to enable the rational formulation design of stable, safe and effective solid dosage forms containing PL HCl. In order to achieve this the study was subdivided into the following four sections:

(1) analytical preformulation,
(2) elucidation of the solid state properties of PL HCl,
(3) solubility analysis of PL HCl and
(4) stability analysis of PL HCl.

CHAPTER TWO

2 METHODS FOR THE ANALYSIS OF PYRIDOXAL HCL

2.1 INTRODUCTION

The first task that the preformulation scientist faces is the development of suitable analytical methods. In this process use must be made of any relevant information which may have accumulated during the synthesis and basic chemical research on the compound. A literature search for references to the compound or to better known analogues must also be conducted. As both material and time is usually limited during this stage, the methods should be relatively simple, thus not requiring lengthy development. Naturally the analytical methods should also be sensitive thus only requiring small samples. Analytical preformulation can be seen to serve two purposes: The immediate and obvious purpose is the development of methods that enable the elucidation of pharmaceutically relevant physicochemical properties. Secondly, the methods developed and the information gathered in the process may form the basis for analytical methods used in later, more detailed formulation studies and for quality control of the final dosage form. Valuable analytical methods routinely used

in preformulation studies include ultra violet (UV) spectroscopy, thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and thermal analysis. Others include infra-red (IR) spectroscopy, x-ray diffraction techniques, Karl Fischer titration and other titrimetric techniques.

Establishing a UV spectrophotometric method is often the first step in preformulation. For most drugs UV spectroscopy can be used to make sensitive, quantitative determinations of the pure drug in solution (Wells,1988c). This method can then be applied to the study of vital solubility characteristics such as intrinsic solubility, pH solubility profiles, pk_a values and partition coefficients.

TLC is frequently used as a semi-quantitative stability indicating method. It is generally regarded as a simple and reliable method for separating drug from impurities, degradation products and excipients. TLC therefore frequently finds application in the initial solid state stability analysis and in excipient compatibility studies. TLC is especially useful in excipient compatibility studies , where numerous drug-excipient combinations have to be tested. With TLC up to 15 samples can be analysed simultaneously allowing considerable savings of time and solvents when compared to HPLC.

HPLC is currently acknowledged as the most versatile and powerful technique in pharmaceutical analysis and is the method of choice for stability studies (Wells, 1988d). HPLC has advantages over traditional techniques such as spectrophotometry, titrimetry and TLC in that it is both highly specific and quantitative. Furthermore the technique lends itself well to automation. The disadvantages are the relatively high hardware costs as well as the high running and maintenance costs (columns, solvents and other disposables). In the absence of automation HPLC can also be very time consuming (Boehlert, 1984). HPLC should therefore be reserved for applications where simpler quantitative techniques such as UV spectroscopy are not specific enough, or where the semi-quantitative approach offered by TLC is inadequate.

Also valuable in preformulation are the thermal analytical techniques. Various thermal techniques are available. These include differential scanning calorimetry (DSC), thermal gravimetric analysis (TGA) and hot stage microscopy (HSM). Other thermal techniques, less frequently used in preformulation studies include thermomechanical analysis and evolved gas detection. Thermal analysis is generally rapid and requires only a few mg of drug, yet techniques such as DSC, TGA and HSM can yield a wide range of qualitative data. DSC can be applied to accurately determine the purity of the drug candidate and can be used together with TGA and HSM to rapidly generate a physical profile

of the drug substance (eg melting point, extent of crystallinity, the presence of amorphous forms, polymorphs and solvates). DSC is also increasingly used in drug excipient compatibility screening (Giron, 1986; Giron-Forest, 1984; Wollmann and Braun, 1983). Unlike traditional compatibility screening tests, no prolonged storage of samples under isothermal stress conditions is required. However, interpretation of the thermograms can be difficult and confirmation of stable drug-excipient mixtures is therefore usually obtained by isothermal stress testing and subsequent TLC or HPLC analysis (van Dooren, 1983).

Karl Fischer titration is an analytical method used for the determination of water. It is mentioned here as moisture content and moisture sorption are of central importance for both physical and chemical drug stability and no preformulation study can be completed without considering the effect that atmospheric moisture has on the drug. Moisture content and moisture sorption profiles are also frequently determined gravimetrically. However, gravimetric techniques do not account for residual organic solvents which may be displaced by adsorbed moisture (Nyqvist, 1986). Furthermore with modern automated equipment quantities of water as low as 10 ug (Mettler instrumentation specifications) can be accurately and rapidly determined by Karl Fischer titration.

The five above mentioned techniques were extensively used during the preformulation investigation of PL HCL. The development of assays using these techniques is described in detail in this chapter. Where necessary, assay validation was carried out using standard statistical procedures as described by Cavenaghi et al (1987). IR spectroscopy and scanning electron microscopy (SEM) were also used. However, these did not require any specialized method development and are therefore not discussed here.

2.2 UV SPECTROPHOTOMETRIC ANALYSIS OF PYRIDOXAL HCL

2.2.1 OVERVIEW OF EXISTING METHODS

In aqueous solution PL undergoes a number of ionic transitions with changing pH. Consequently UV light absorption and the spectral properties of PL are also highly pH dependant (Morosov, 1986) (see section 4.3.2). Any method for the spectrophotometric determination of PL must therefore specify the pH at which absorption measurements are made. At pH 2 the absorption maximum for PL in aqueous solution is 286 nm. This maximum can be conveniently used to quantitatively assay PL in solution (Strohecker and Henning, 1965). However, this simple method is only suitable for the determination of pure PL solutions with no UV absorbing impurities and is generally not suitable for the determination of PL in the presence of other vitamins and drugs. Better selectivity can be achieved by making use of reagents

which react with specific functional groups on the PL molecule. The newly formed complex can then be measured spectrophotometrically. Two such methods are the method of Hochberg et al (1944) (reaction with dichloroquinone chlorimide) and the method of Hrdy and Urbanova (1957) (reaction with p-diethylaminoaniline).

These methods are sufficiently selective to allow the determination of pyridoxine (PN) in the presence of closely related PL . They have also been successfully used to determine PN in multivitamin preparations. These methods could be modified to enable the determination of PL rather than PN. However both methods have serious disadvantages. The method of Hochberg et al (1944) has poor colour stability and all measurements have to be made within 60-80 seconds (optimally 80 seconds) of reagent addition. The method of Hrdy and Urbanova (1957) on the other hand requires extraction of the PN complex into benzene before measurement.

An alternative method was therefore developed based on a procedure recently reported for PN determination (Nirmalchandar et al, 1987). The method utilizes the coupling reaction of 4-aminoantipyrine with phenolic type compounds in the presence of a strong oxidizing agent. This reaction has long been exploited for the spectrophotometric determination of phenolics in industrial effluent (Mohler and Jacob 1957). Similar assays have also been reported for phenylephrine and piroxicam (Parasrampuria and Das Gupta, 1990).

2.2.2 METHODOLOGY

2.2.2.1 MATERIALS AND APPARATUS

The following analytical grade reagents were used: Pyridoxal HCl (Fluka, Buchs), pyridoxine HCl (BASF, Ludwigshafen), 4-aminoantipyrine (Fluka, Buchs), ammonium persulfate (BDH, Poole), hydrochloric acid (BDH, Poole). Double distilled water (Milli Q System, Millipore, Bedford MA) was used throughout. The following excipients were mixed with PL HCl to simulate analytical samples: Avicel PH 101 (FMC Corp,Philadeladelphia PA) , corn starch (Holpro, Johannesburg), anhydrous lactose (Sheffield,Norwhich NY) and magnesium stearate B.P.. A Phillips/Pye Unicam (Cambridge) PU8700 UV/visible spectrophotometer with 1 cm quartz cells was used for the absorbance measurements.

2.2.2.2 PROCEDURE

A PL stock solution was prepared by dissolving approximately 10 mg of PL HCl (accurately weighed) in 100 ml of distilled water. A series of PL standards ranging between 10 and 30 mg/l was prepared in 10 ml volumetric flasks by taking appropriate aliquots of stock solution, adding 1ml of a 0.4% aminoantipyrine and a 1% ammonium persulfate solution respectively and making up to volume with distilled water. This procedure was also used to prepare analytical samples consisting of pure PL in solution. Analytical samples consisting of

PL-excipient mixtures were added to 100 ml volumetric flasks, made up to volume with water and shaken for 10 minutes. An aliquot of this solution (3 ml) was then passed through a 0.45 µm filter membrane (Millex HV, Millipore, Bedford MA). The procedure described for the preparation of standard solutions was then followed. All analytical sample solutions were adjusted to pH 2.5 with dilute hydrochloric acid. Standard and sample solutions were allowed to stand for 20 minutes after reagent addition. Absorbance was then measured at 408 nm against a reagent blank. The linearity, detection limit, precision and accuracy of the method were assessed. Selectivity was evaluated by the addition of various water soluble vitamins including pyridoxine. All solutions were prepared in red light and stored in closed bottles. The vitamin solutions were overlayed with nitrogen gas and stored in a refrigerator. Reagent solutions and the PL stock solution were prepared daily.

2.2.3 RESULTS AND DISCUSSION

The absorption spectrum has a broad maximum at 408 nm (Figure 2.1). In the pH range of 2.45-2.60 absorption was found to be constant, but time dependent. Maximum absorbance was reached 20 minutes after the reaction was initiated and remained constant for a further 15 minutes (Figures 2.2 and 2.3).

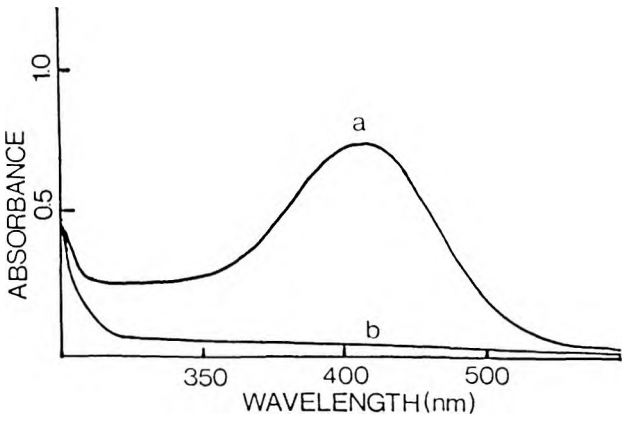


Figure 2.1 Absorbance spectra of a) reagent blank and b) PL HCl (21 mg/l).

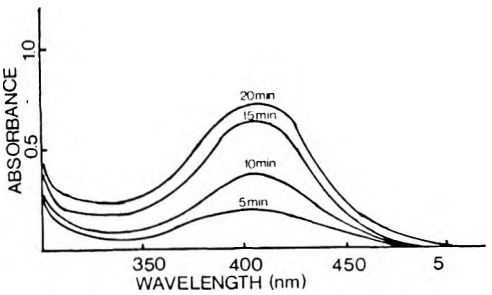


Figure 2.2 Changes in the absorption spectrum with time .

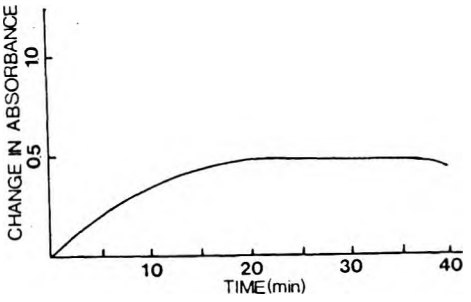


Figure 2.3 Rate of change in absorbance at 408 nm (PL HCl 21 mg/l).

A typical calibration curve making use of 5 PL standards of different concentration yielded the following equation :

Absorbance = 0.0278 Concentration + 0.0085

A regression coefficient of 0.9997 was determined, thus indicating good linearity and adherence to the Beer-Lambert law (refer to Appendix Ia for calibration data). A detection limit of 0.33 mg/l was calculated (Appendix

Ib). Precision was assessed by analysing 5 sample replicates from an analytical sample containing 10.7 mg/l PL HCl. The results are summarized in Table 2.1.

Table 2.1 Determination of precision

Sample replicate	A	B	C	D	E
Concentration (mg/l)	10.8	10.9	10.8	10.7	10.7

A mean of 10.78 mg/l with a relative standard deviation of 0.78% and a standard error of 0.037 was determined. A confidence interval of 100-101.5% at the 90% confidence level was calculated for the assay.

To determine accuracy, samples containing various amounts of PL HCl were assayed (Table 2.2).

Table 2.2 Determination of accuracy

Sample	A	B	C	D
True Concentration (mg/l)	10.7	6.5	13.5	22.3
Measured Concentration (mg/l)	10.8	6.6	13.3	22.5

An average relative bias of 0.47% was calculated showing that the method tends to slightly over estimate the PL concentration. However this was not found to be significant as determined by the student-t test at the 95% level of confidence.

No interference was observed when pyridoxine,

nicotinamide or thiamine (up to 10mg/l respectively) were present in the sample solution. Samples extracted from mixtures containing common tableting excipients (microcrystalline cellulose, starch, lactose and magnesium stearate up to 50 mg/l respectively) also did not show any increased absorption. However, the presence of riboflavin, folic acid and ascorbic acid tended to increase absorbance values. It is remarkable that no interference from pyridoxine was observed. In the procedures reported by Nirmalchandar et al(1987) and Hochberg et al (1944) interference from PN was eliminated by the addition of boric acid. Boric acid complexes with the primary alcohol groups of PN thus preventing the interaction of PN with the coupling agent. Boric acid addition does not appear to be necessary in the current procedure. A possible reason for this is the relatively low pH (2.5) used in this method. The method of Nirmalchandar et al(1987) specifies a pH of 8 for the coupling reaction of PN with 4-aminoantipyrine.

In summary, the above method has been shown to be suitably sensitive, accurate and precise for the determination of PL in solution and also in the presence of excipients.

2.3 THIN LAYER CHROMATOGRAPHIC ANALYSIS OF PYRIDOXAL HCL

2.3.1 BACKGROUND

TLC is a popular technique for the analysis of vitamin B_6. Indications of this are the detailed reviews on the TLC analysis of vitamin B_6 published by several authors (Bolliger, 1965; Bolliger and Konig, 1969; Ahrens and Kortnyk, 1970; Coburn, 1986). Both cellulose and silica gel layers have been used, but it is clear from the reviews that silica gel stationary phases are best suited for the analysis of vitamin B_6.

A common problem in the TLC analysis of PL HCl is the instability of PL in aliphatic alcohols. In acidic and neutral solutions PL exists as an hemiacetal and readily reacts with alcohols such as methanol and ethanol, forming PL methyl or ethyl acetal respectively (Nurnberg, 1961; Kortnyk and Singh, 1963) (Figure 2.4). The common practice of using alcohol as a rapidly evaporating sample application solvent thus has to be avoided, as the chromatograms usually show multiple zones corresponding to the hemiacetal, the full acetal and/or the free aldehyde (Nurnberg, 1961).

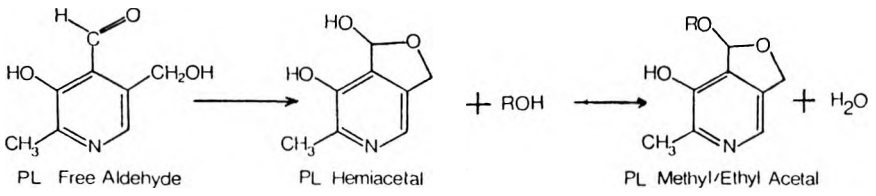


Figure 2.4 Hemiacetal and full acetal formation in PL.

By using water as the sample solvent this problem can be avoided , although sample application becomes more difficult. However, acetal formation may also occur during chromatography. Many mobile phases described for the chromatography of B_6, including PL, contain substantial amounts of either methanol, ethanol, propanol, n-butanol, tert-butanol, iso amyl alcohol or combinations of these (Ahrens and Kortnyk, 1970; Coburn, 1986). Acetal formation may be overcome by prechromatographic derivatization of PL HCl. This can be achieved with carbonyl reagents such as phenylhydrazine, hydroxylamine and semicarbazide (Ahrens and Kortnyk, 1970). Alternatively PL can be completely converted to its methyl acetal by refluxing in methanol for 1 hour (Nurnberg, 1961) . However, for the analysis of numerous small samples(< 2 ml), refluxing in methanol is time consuming and impractical. Furthermore the reliability of these derivatization reactions in complex biological and pharmaceutical samples has been questioned (Coburn, 1986).

Eleven solvent systems previously reported to yield successful separations of PL on silica gel were therefore investigated with the aim of finding a stability indicating TLC method that meets the following requirements:

i) Suitability for the separation and identification of PL and breakdown products.

ii) Overcome the need for prechromatographic

derivatization

iii) Suitability for detection by UV light.

2.3.2 METHODOLOGY

2.3.2.1 MATERIALS AND EQUIPMENT

Only analytical grade solvents (listed in table 2.3) and double distilled water (Milli Q system, Millipore, Bedford M.A.) were used. PL HCl (Fluka, Buchs) was used to prepare the PL standards. For the excipient mixtures PL HCl (approximately 10 mg) was mixed with 50 mg of Avicel 101 (FMC Corp., Philadelphia PA), Ludipress (BASF, Ludwigshafen) or mannitol (Riedel de Haen, Seelze). Samples were applied to silica gel plates (HF 60, 20x20, E Merck, Darmstadt) with a microlitre syringe (Hamilton, Bonaduz). Standard rectangular glass tanks with lids (N-tank, Camag, Muttenz) lined with absorbent paper were used for development. A photographic stand with UV light source (Reprostar, Camag, Muttenz) and a 35 mm camera (Minolta XGE) were used to observe and photograph the chromatoplates.

2.3.2.2 PROCEDURE

PL standards were prepared by dissolving 10 mg PL HCl in 2 ml of water. To obtain samples containing breakdown products, PL HCl (10 mg) and PL HCl-excipient mixtures in open vials were stored in a hygrostat containing a saturated NaCl solution (aproximately 75% relative humidity (RH)) at 55°C for 2 weeks. Distilled water (2

ml) was then added to the PL samples. PL-excipient mixture samples were prepared by adding 5 ml distilled water, centrifuging for 5 minutes and filtering the supernatant through a 0.45 µm membrane (Millex HV, Millipore, Bedford M.A.). 1 µl of each standard solution and 2.5 µl of excipient sample solutions were applied to the origin of a silica gel chromatoplate. Before use the plate was prewashed in a chloroform : methanol (1:1) mixture, dried at 70°C for 30 minutes and then allowed to equilibrate in laboratory air. Development was effected in mobile phase saturated glass tanks. The mobile phases which were evaluated are listed in Table 2.3. Solvent strength parameters (E_o) were calculated for the mobile phases according to the values provided by Snyder (Wells, 1988e) (Appendix Ic). The solvent migration distance was limited to 12 cm for all systems. After development the plates were air dried and briefly exposed to diethylamine vapour. The chromatograms were then observed and photographed under UV light (256 and 366 nm).

2.3.2.3 RESULTS AND DISCUSSION

Of the 11 solvent systems investigated, only one system (methanol : chloroform, 25 : 75) (Ahrens and Kortnyk, 1970) fulfilled all the criteria (Table 2.3). The other systems either showed inadequate resolution or additional zones, probably due to acetal formation. The methanol : chloroform (25 : 75) system yielded compact dark blue/black zones with R_f values of 0.46-0.50 when

Table 2.3 Evaluation of various solvent systems for the analysis of PL HCl

Mobile Phase	Eo	Resolution	R_f	Time (mins	Degradation Products	References
A	0.54	good resolution	0.46	45	3 distinct zones	Ahrens and Kortnyk (1970)
B	-	marked tailing zone is poorly visible	0.54	50	broad tail is visible at 366 nm	Bolliger (1965)
C	0.59	good resolution but multiple zones due to acetal formation	0.47	65	broad tail is visible	Puech et al,(1981)
D	0.56	marked tailing	0.32	60	poorly visible	Nuernberg (1961)
E	0.57	marked tailing	0.31	50	poorly visible	Coburn (1986)
F	0.40	no elution	0	60	-	Coburn (1986)
G	-	elution as 2 distinct zones slight tailing	0.47	180	good resolution	Coburn (1986)
H	-	elution as 2 zones	0.57	150	good resolution	Coburn (1986)
I	0.59	only partial resolution	0.25	45	-	Dement'eva et al. (1968)
J	-	marked tailing	0.8 0.71	60	Broad tail is visible	Mc Coy et al,(1979)
K	0.58	elution as multiple zones	0.4	45	good resolution	experimental system *

Key: A chloroform: methanol (75:25); **B** 25% ammonia: water (1:139) **C** chloroform: 2-propanol: methanol: water (58:30:10:2); **D** acetone followed by acetone: dioxane: 25% ammonia (45:45:10); **E** acetone followed by acetone: ethanol (95: 5); **F** chloroform: ethanol (99:1) **G** 1-butanol: 1M acetic acid (5:1); **H** water: acetone: tert-butanol: acetic acid (20:35:40:5); **I** ethyl acetate: acetone: 25% ammonia (20:10:1.5); **J** Isoamyl alcohol: acetone: water: diethylamine (24: 18:6:8) followed by 2-butanol: ethanol: 25% ammonia: isoamyl alcohol: diethylamine (20:6:7:7:2); **K** chloroform: ethanol: acetic acid: water (54:27:9:4); E_o solvent strength parameter; R_f relative migration distance.

viewed at 256 nm. At 366 nm PL is visible as a bright blue/yellow zone. Decomposition products are visible as three distinct zones with a long tail behind the PL zone (Figure 2.5). Decomposition products were also visible in the chromatograms of the PL-excipient mixtures subjected to accelerated ageing (Figure 2.6), indicating the methods suitability for use in excipient compatibility testing. Pyridoxine (PN) was also readily separated by this method (R_f 0.37-0.41) and could thus be used as an internal standard. The method is also suitable as a rapid identity test to distinguish between PL and PN.

The study of the various mobile phases indicated that acetone based mobile phases were generally not suitable. It appears that good resolution can be achieved with solvent systems containing a substantial amount of alcohol with E_o values between 0.5 - 0.6 . However, the ratio of chloroform to alcohol and the absence of any strong acids is critical to avoid acetal formation.
The observation that a simple mobile phase of chloroform and methanol (75 : 25) prevented acetal formation is consistent with findings made during Karl Fischer titration of aldehydes. It has been found that when titrating aldehydes in methanol, acetal formation can be suppressed by substituting a large portion of the methanol (at least 50%) with chloroform (Imfanger and Schauwecker, 1984).

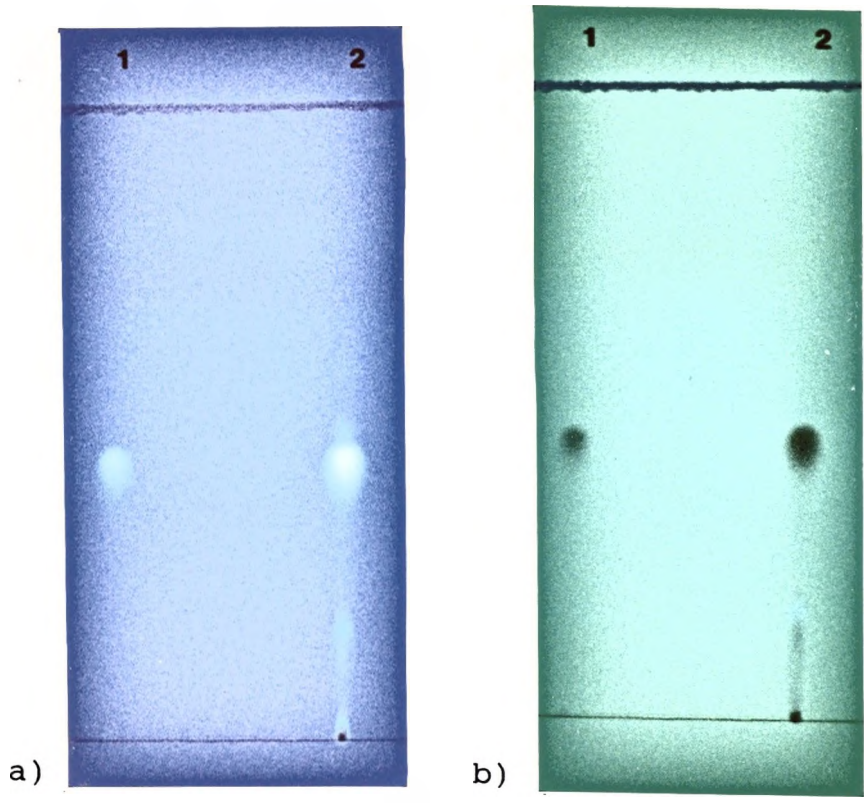


Figure 2.5 Chromatogram of PL standard (1) and degraded PL (2) viewed at (a)366 nm and (b) 254 nm.

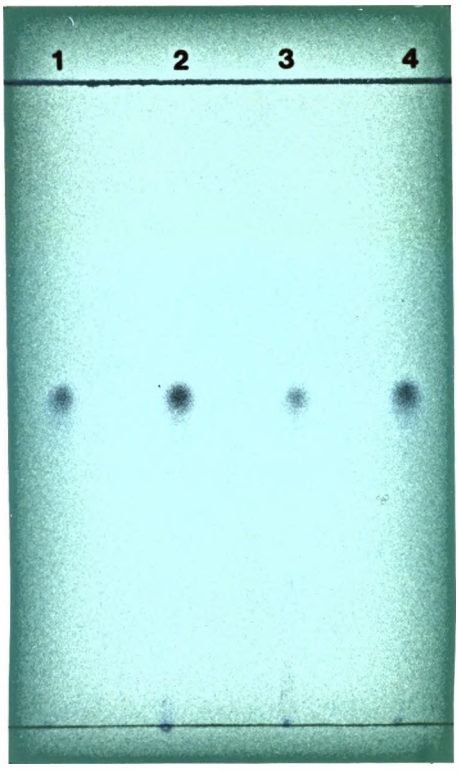


Figure 2.6 Chromatogram of PL standard (1), PL stored with Ludipress (2), mannitol (3) and Avicel (4).

This study once again indicates that the reactivity of drugs containing aldehyde and hemiacetal functional groups can give rise not only to stability- but also to analytical problems. The solvent system first reported by Ahrens and Kortnyk (1970) provides a good separation on a standard silica gel stationary phase and can be used as a simple inexpensive stability screening test.

2.4 HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF PYRIDOXAL HCl

2.4.1 OVERVIEW OF EXISTING METHODS

The HPLC methods available for the analysis of vitamin B_6 either make use of ion exchange- or reverse phase chromatography. Very successful and sensitive anion and cation exchange methods have been reported (Vanderslice et al, 1981; Coburn and Mahuren, 1983; Lui et al,1985). However, these methods require sophisticated equipment and procedures such as column switching, the use of ternary solvents and post column derivatization. Such methods, although very sensitive, are not ideally suited for preformulation studies and for routine analysis of pharmaceutical dosage forms.

Reverse phase chromatography, on the other hand, is a simpler and more versatile technique. A number of procedures have been reported using this mode of separation (Morita and Mizuno, 1980; Lim et al, 1980; O'Reilly et al, 1980; Pierotti et al, 1984; Tryfiates

and Sattsangi, 1982). Some of these methods do not completely separate PL and PN, but this can easily be remedied with ion pairing reagents such as sodium octane sulfonate (Tryfiates and Sattsangi 1982). These methods are generally not sensitive enough for B_6 vitamer plasma analysis as UV detection is used. However, this is adequate for the analysis of pharmaceutical samples. Sensitivity can be further increased by using fluorimetric detection and derivatization (Gregory and Feldstein, 1985; Ubbink et al, 1985; Hollins and Henderson, 1986; McChrisely et al, 1988).

For the preformulation study of PL HCl a simple, quantitative stability indicating method for the analysis of PL HCL in pharmaceutical samples was required. For this purpose a reverse phase, ion pairing method based on the procedure reported by Tryfiates and Sattsangi (1982) was developed.

2.4.2 METHODOLOGY

2.4.2.1 MATERIALS AND EQUIPMENT

The following analytical or HPLC grade materials were used: PL HCl (Fluka, Buchs), PN HCl 99.7% pure (Roche, Isando), 2-propanol (Merck, Darmstadt), triethylamine (Merck, Darmstadt), acetic acid (Merck, Darmstadt),PIC B7 and PIC B8 (sodium heptane- and sodium octane sulfonate) (Waters Associates, Milford, MA.). Double distilled water (Milli Q system, Millipore, Bedford, MA)

was used throughout. The following excipients were used to simulate analytical samples: Methyl cellulose 25 cp USP (Fluka, Buchs), Eudragit RSPM (Rohm Pharma, Weiterstadt), mannitol (Riedel de Haen, Seelze) and magnesium stearate (Fluka, Buchs).

Analyses were carried out on a System Gold (Beckman, San Ramon, CA.) liquid chromatograph equipped with an Altex (Beckman) 210A injector valve with a 20 µl loop, a model 126 programmable solvent delivery module and a model 168 diode array detector. The system was fitted with a Beckman Ultrasphere ODS (particle size 5 µm, 4.6 mm id., 15 cm) analytical column. Model P1000 and P5000 digital micropipettes (Pipetman, Gilson, Villiers-le-bel) were used to make the necessary dilutions.

2.4.2.2 PROCEDURE

To prepare the internal standard approximately 10 mg of PN HCl was accurately weighed and dissolved in 100 ml of water. A stock solution of PL HCl was prepared by dissolving approximately 10 mg of PL HCl, accurately weighed, in 100 ml of distilled water. PL HCl standards ranging from 0.05 to 0.0001 mg/ml were prepared in 10 ml volumetric flasks by taking appropriate aliquots of stock solution, adding 1 ml of internal standard solution and making up to volume with distilled water. Analytical samples consisted either of PL HCl powder or PL HCl-excipient mixtures exposed to various environmental conditions. These samples were added to

100 ml volumetric flasks, made up to volume with water and shaken for 10 minutes. An aliquot (3 ml) of this solution was passed through a 0.45 µm filter membrane (Millex HV, Millipore, Bedford, MA.). The procedure described for the preparation of PL HCl solutions was then followed. Standards and samples were refiltered through a 0.45 µm membrane before injection onto the column. All solutions were prepared in red light and stored in closed bottles, overlayed with nitrogen, in a refrigerator. PL HCl standards were prepared on a daily basis. Acidic and neutral PN solutions show good stability if protected from light (Shephard and Labradarios, 1986). The internal standard was therefore prepared on a weekly basis.

The mobile phase (A) was composed of 10% 2-propanol and a buffer consisting of 0.09% acetic acid, 0.1% triethylamine and a mixture of sodium heptane sulfonate and sodium octane sulfonate (0.004M). Two additional mobile phases (B and C) were prepared for purposes of comparison. Solvent B consisted of 10% 2-propanol and 0.09% acetic acid as described by Tryfiates and Sattsangi (1982). Mobile phase C was identical to mobile phase A except that no triethylamine was added. All solvents were filtered through a 0.22 µm filter (Type GV, Millipore, Bedford, MA.) and degassed before use. A solvent flow of 1.0 ml/min and a pressure of approximately 1.1 MPa was maintained. Prior to use, the column was equilibrated by passing 15 ml of solvent

through the system. The eluent was monitored at 288 nm and 313 nm for comparative purposes and the detector was programmed to analyse peaks using the purity scan mode.

The procedure was compared to the original method of Tryfiates and Sattsangi (1982) and the specificity, linearity, precision and accuracy of the method was assessed according to the guidelines of Cavenaghi et al (1987) and Boehlert (1984). For this purpose samples consisting of PL HCl (approximately 10 mg accurately weighed), methyl cellulose (50 mg), Eudragit RSPM (50 mg) and mannitol (50 mg) were used.

2.4.3 RESULTS AND DISCUSSION

The chromatograms obtained after injection of the PL standard and sample solutions are shown in Figure 2.7.

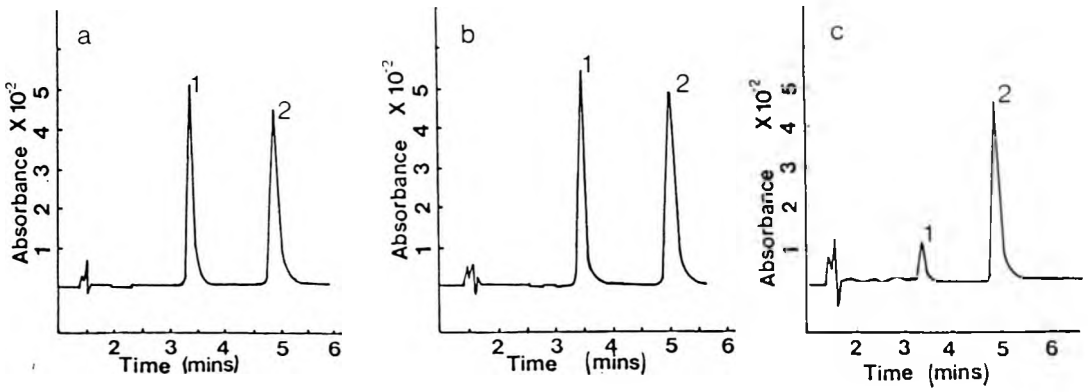


FIGURE 2.7 Chromatograms of: a) PL standard, 1 PL (3.39 mins), 2 PN (4.91 mins); b) PL sample, 1 PL (3.46 mins), 2 PN (5.01 mins); c) PL sample with decomposition products, 1 PL (3.38 mins), 2 PN (4.93 mins).

The peaks were identified by their retention times and peak purity was confirmed by scanning and comparing the UV spectra of the leading slopes, the apices and the

trailing slopes of the peaks (Figure 2.8). Correlation coefficients (r) for the normalized scans were better than 0.9999, indicating peak purity.

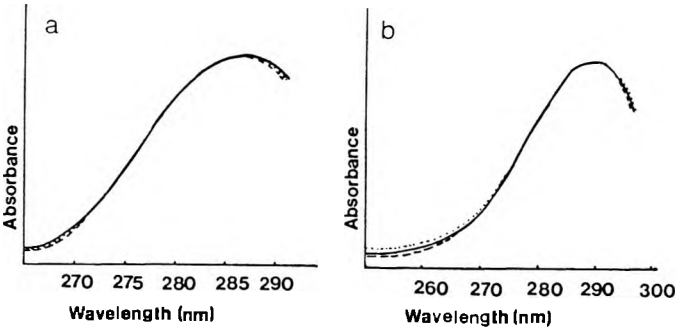


Figure 2.8 UV spectra of the leading edges, the apices and the trailing edges of the peaks. a) PL; b) PN.

Figure 2.7c shows the separation of PL from its decomposition products. As far as could be ascertained the pathway of PL HCl decomposition is not documented in the literature. A possible route of decomposition is discussed in chapter 5. However, the decomposition products were not identified. Part of the problem was that the decomposition products did not elute as well defined peaks. Furthermore some of the products seemed to elute at the solvent front. Stability was therefore monitored by measuring the decrease in the PL peak and using the purity scan mode detector to confirm the integrity of the PL and PN peaks.

The method was found to be linear over the concentration

range of 0.0005 to 0.1 mg/ml. Seven concentration levels were used and three injections were made per concentration level. The equation for a typical calibration curve is $y = 0.010824x + 0.000241$, where x is the ratio of the PL and PN peak areas and y refers to the PL concentration. A regression coefficient of $r = 0.9995$ was determined. No detector response was obtained for the lowest concentration standard (0.0001 mg/ml). The sensitivity limit therefore lies between 0.0001 and 0.0005 mg/ml. The relevant calibration data can be found in Appendix Id.

Precision was assessed from the analysis of 5 sample replicates from an analytical sample containing 10.4 mg/ml PL HCl. The results are tabulated below (Table 2.4).

Table 2.4 Precision analysis

Sample replicate	A	B	C	D	E
Concentration (mg/ml) 10^{-2}	10.1	10.4	10.1	10.3	10.3

A mean of 0.01022 mg/ml with a standard error of 0.00006 was determined. From this the 90% confidence interval for the assay was calculated to be 97.2%-99.7% .

To determine accuracy, five PL-excipient mixtures, each containing 10.0 mg PL HCl, were assayed (Table 2.5). A mean of 0.01002 mg/ml was determined. From this a

relative bias of 0.2% was calculated. This was not significant as determined by the students t-test (95% confidence level). Recovery from the powder mixtures is therefore essentially complete and the method is accurate.

Table 2.5 Accuracy determination

Sample	A	B	C	D	E
Concentration (mg/ml) 10^{-2}	10.2	10.0	9.8	10.2	9.9

The original method of Tryfiates and Sattsangi (1982) requires that after 5 minutes elution time a solvent switch is made to solvent B which is free of counter ions and that elution be monitored at 313 nm. Their method did not employ triethylamine in the mobile phase. Monitoring elution at 288 nm clearly resulted in increased sensitivity (Figure 2.9).

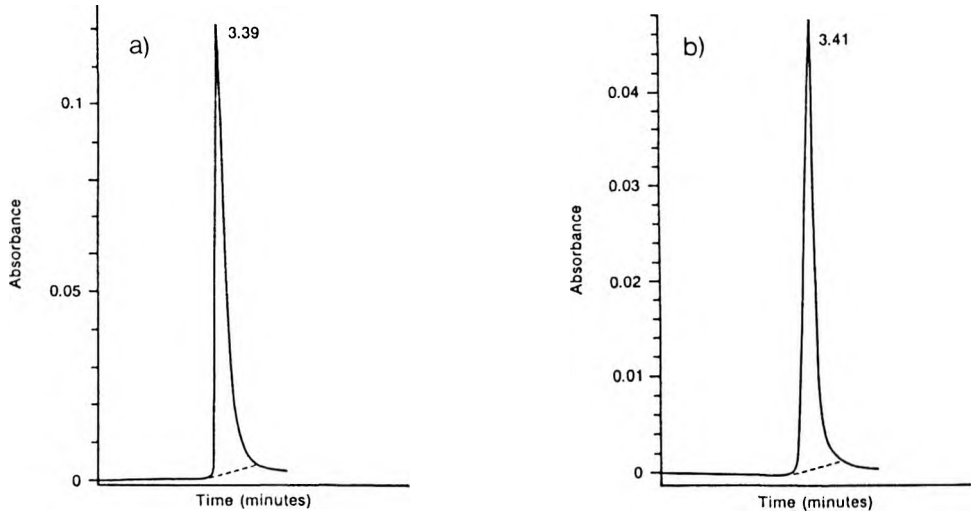


Figure 2.9 Detector response for PL sample at a) 288 nm and b) 313 nm.

This is not surprising as 288nm coincides with the UV absorption maxima of PL and PN in the mobile phase (Figure 2.10).

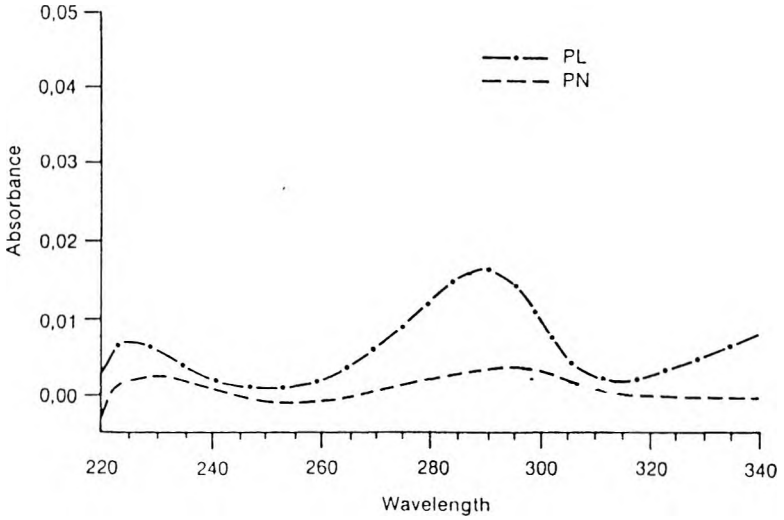


Figure 2.10 UV spectra of PL and PN in the mobile phase.

Switching solvents at 5 mins. elution time was found to be unnecessary. This resulted in a considerable time saving as the need to re-equilibrate the column with the first solvent before each new run was eliminated. Addition of a small amount of triethylamine (0.1%) produced narrower, sharper peaks and reduced retention times by approximately 1 minute.

2.4.4 CONCLUSION

Compared to the procedure of Tryfiates and Sattsangi (1982), the adapted method utilizes a simpler mobile phase and does not require lengthy column re-equilibration before each run . The method also has a shorter elution time. Thus a considerable saving in time and solvents was achieved. An improvement was also made

to the sensitivity of the method. The accuracy, precision and selectivity of the procedure were found to be suitable. Ideally a stability indicating method should enable the quantitation and identification of both the drug and the decomposition products. It was not possible to identify and quantitate the decomposition products. However, PL and PN peak purity can be ascertained with the aid of the diode array detector. Therefore it is felt that the method is a suitable stability indicating assay for preformulation purposes.

2.5 DETERMINATION OF WATER CONTENT IN PYRIDOXAL HCl BY KARL FISCHER ANALYSIS

2.5.1 THEORETICAL BACKGROUND

The Karl Fischer titration is based on the reaction of water with iodine in the presence of sulfur dioxide and a suitable base. The process can be summarized by the following equation:

$$H_2O + I_2 + SO_2 + CH_3OH + 3RN \longrightarrow [RNH]SO_4CH_3 + 2[RNH]I$$

RN = Base

During an accurate titration, the end point is usually reached within two minutes. To avoid a drifting end point samples have to be prepared so that the water is freely available and interfering side reactions must be suppressed. Of particular relevance to the moisture determination in PL HCl is the reaction of aldehydes with the non aqueous solvent (ie. methanol) resulting in acetal formation :

$$RCO + CH_3OH \longrightarrow RCOCH_3 + H_2O$$

In some aldehydes the rate of reaction is quick enough to interfere with the titration. However, this reaction can be readily suppressed by partially substituting the methanol with chloroform (Infanger and Schauwecker, 1984). An additional factor which may have to be considered during method development is the reaction pH: the Karl Fischer reaction only proceeds smoothly in the range between pH 4 and 7.

2.5.2 METHODOLOGY

2.5.2.1 MATERIALS AND EQUIPMENT

A Karl Fischer titrant containing iodine, sulfur dioxide and pyridine (Riedel de Haen, Seelze) was used. A mixture of specially dried methanol (Carlo Erba, Milan) and chloroform (BDH, Poole) was used as titration solvent. The moisture determinations were made with a DL18 Karl Fischer titrator (Mettler, Greifensee).

2.5.2.2 PROCEDURE

Methanol and chloroform (20 ml of each) were introduced into the titration vessel and pretitrated to eliminate any traces of water. The apparatus was then calibrated with 50 mg of water (accurately weighed). Accurately weighed samples (approximately 500 mg) of PL HCl (Fluka, Buchs) were then introduced into the titration vessel. 90 seconds stirring time was allowed before initiating the titration. Accuracy and precision were determined by

analysing samples of pure water and samples of PL HCl (Fluka, Buchs) respectively.

2.5.3 RESULTS AND DISCUSSION

It was found that a stir time of 60-90 seconds before titration was necessary to ensure that water was freely available. Reproducible end points were then reached within 2 minutes. However, in PL HCl samples with low moisture contents (0.1-0.5%) the end point was reached considerably earlier. It therefore appears that the use of chloroform as an auxilliary solvent was effective in suppressing acetal formation. The method was deemed accurate, provided water is freely available. A relative bias of -0.0005% was determined (Table 2.6).

Table 2.6 Determination of accuracy. Analysis of pure water samples

Sample	% Water content
1	99.87
2	100.10
3	99.89
mean	99.95
bias	0.05

Using the students t-test the bias was found not to be significant at the 95% confidence level.Precision was also deemed suitable. Analysis of 3 standard samples of PL HCl provided a mean of 0.218% with a standard error of 0.0046. A 95% confidence interval of 0.198-0.238% was calculated (Table 2.7).

Table 2.7 Determination of precision. Analysis of PL HCl samples.

Sample	% Water content
1	0.227
2	0.212
3	0.214
mean	0.218
standard deviation	0.0079
standard error	0.0046

The method is therefore suitable for the determination of equilibrium moisture contents in PL HCl.

2.6 THERMAL ANALYSIS OF PYRIDOXAL HCl

2.6.1 THEORETICAL BACKGROUND

The thermal analytical techniques used in this study were DSC and TGA. These two techniques are often used in conjunction and appear to be the major thermal techniques used in pharmaceutical analysis (Giron, 1986). With DSC, thermal energy changes occurring in a sample which is subjected to a controlled temperature change, can be measured quantitatively. This allows the monitoring of a wide range of temperature induced exothermic and endothermic physical and chemical phenomena. Phenomena which can be detected as exothermic peaks on a DSC thermogram include adsorption, crystalline transitions, crystallization (including devitrification), oxidation reactions, decomposition reactions, redox reactions and solid state reactions. However, decomposition reactions, redox reactions, solid

state reactions and chemical reactions in general may also be endothermic. Events which are specifically endothermic include desorption, fusion, vapourization, sublimation, glass transitions, reduction reactions, dehydration and desolvation (Dodd and Tonge, 1987a).

The energies measured in DSC are small and experimental conditions are therefore a major factor determining the actual response recorded by the instrument. Experimental conditions which have to be considered include sample mass, sample geometry, heating rate and the ambient sample atmosphere (Giron-Forest, 1984). Generally larger sample masses result in poorer resolution of the thermogram (Giron-Forest, 1984). In large samples there is a significant temperature gradient and volatile products may not escape as easily. Typical sample masses for DSC are therefore usually in the region of 1-10 mg (Dodd and Tonge, 1987b). Sample geometry may also significantly affect resolution and reproducibility. Powders should be uniform, finely subdivided and packed into the pan as a thin layer, thus making maximum thermal contact with the bottom of the pan and avoiding large temperature gradients. A low heating rate (<10°C/min) is important for accurate quantitative measurement and for the resolution of thermal events which occur within a few degrees of each other (Dodd and Tonge, 1987b; Giron-Forest, 1984). The ambient sample atmosphere can also significantly affect the nature of the observation. To prevent oxidation

reactions, it is common to continuously purge the DSC cell with nitrogen. This then makes the hermetic sealing of pans unnecessary. Such conditions may assist in observing evaporation and desolvation phenomena. On the other hand, degradation and oxidation reactions can also be suppressed by hermetically sealing sample pans. Experiments can then be conducted in a static air atmosphere (Mettler Instrumentation mannual).

Instrumental factors over which the analyst usually has little control include the type of thermocouples and the construction of the platform on which the thermocouples are seated (glass and porcelain sensors tend to be more sensitive than the more durable, traditional metal sensors). A further factor which varies with each type of instrument is the construction of the sample pan.

In TGA mass changes in a sample subjected to a controlled temperature change are monitored. This allows the detection of any temperature dependent phenomena which result in mass changes, eg chemical reactions, adsorption, desorption, desolvation and evaporation. Many of the factors affecting DSC also significantly affect the observations made by TGA: For optimum resolution a small sample (a few mg) packed in a thin layer and heated at a slow heating rate(< 10°C) is required. In addition the ambient atmosphere profoundly affects TGA response. TGA sample containers have to be

open in order to allow mass transfer(eg. loss of volatile substances). If oxidation reactions are to be avoided, purging with nitrogen gas is essential. Conversely if oxidation is to be studied, purging with oxygen or compressed air should be considered.

As part of preformulation investigations simple thermal methods have been developed to study physical changes in PL HCl and in particular the occurrance of polymorphism, solvate formation (pseudopolymorphism) and amorphous states. In addition DSC has been utilized in studying drug excipient interactions.

2.6.2 METHODOLOGY

2.6.2.1 MATERIALS AND EQUIPMENT

PL HCl (Fluka, Buchs) was used throughout this experiment. DSC was carried out in standard aluminium pans on a Mettler (Greifensee) TA3000 system equipped with a DSC 20 cell containing a glass sensor. TGA was performed in open platinum pans on a Du Pont 9900 system.

2.6.2.2 PROCEDURE

The DSC system was calibrated against a pure indium standard (99.999% pure, mp 156.6°C), as well as against pure lead and zinc standards. PL HCl samples (2-5 mg) were accurately weighed into aluminium pans and slightly tamped to form thin layers. The pans were then

hermetically sealed and heated from 30°C to 200°C at rates of 20, 10 and 7°C/min. A sealed, empty aluminium pan was used as a reference. For purposes of comparison samples were then also analysed in an atmosphere of flowing nitrogen (20 ml/min). Here the aluminium sample pan lids were perforated to allow free circulation of nitrogen throughout the system. For TGA, samples (2-5 mg) were packed thinly into the open pans and analysed in an atmosphere of flowing nitrogen (20 ml/min) at the most suitable heating rate.

2.6.3 RESULTS AND DISCUSSION

Figure 2.11 shows the DSC thermograms obtained at different heating rates in a static air atmosphere (hermetic seal). PL HCl has two endothermic peaks separated by an asymmetric, concavely sloping exotherm occurring above 170°C. A change in heating rate does not obscure any of the essential features of the curve. However, at increased heating rates there is a clear trend towards higher peak temperatures and areas (enthalpies). These are therefore procedural peak temperatures and enthalpies rather than actual temperatures and enthalpies of transition. However, the values obtained at lower heating rates tend to reflect the true values. This a general trend observed for DSC (Dodd and Tonge, 1987b).

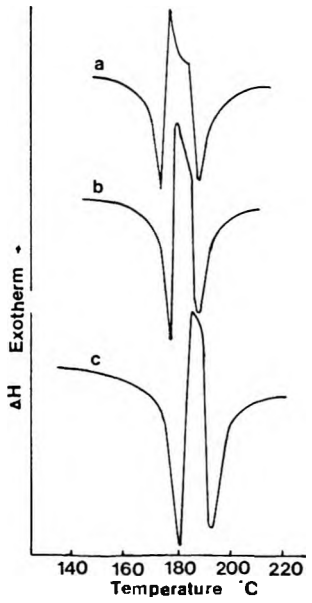


Figure 2.11 DSC thermograms of PL HCl heated at a) 7°C/min; b) 10°C/min; c)20°C/min.

The thermogram observed for PL HCl is typical of a polymorphic transition. Three types of DSC curves which may indicate polymorphic transitions have been identified (Giron, 1986; Giron-Forest, 1984) (Figure 2.12).

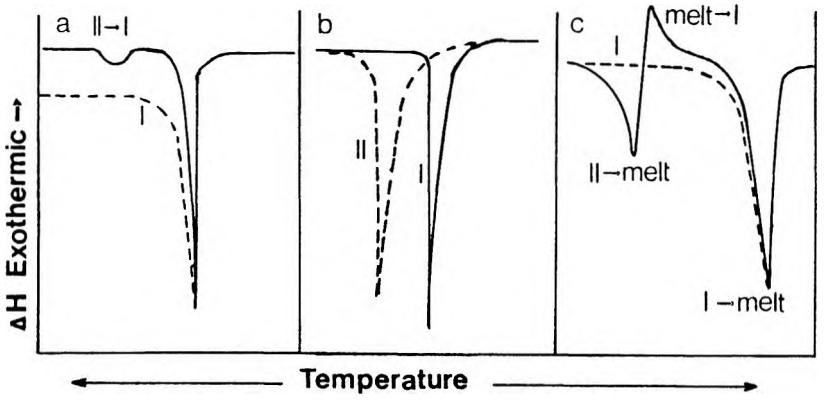


Figure 2.12 Manifestations of polymorphism. a) solid-solid transition before melting; b) melting of each polymorph without transition to the more stable form; c) melting of the metastable form and re-crystallization of the stable form (Giron-Forest, 1984)

In all graphs the stable polymorph is denoted as "I" and

the metastable forms are denoted by higher roman numerals (eg. II).

PL HCl clearly resembles the curve in Figure 2.12 c). This phenomenon involves melting of metastable form II and recrystallization of form I from the melt. Such curves have also been observed for the polymorphic transitions of sulfamethoxypyridazine (Maury et al, 1985), carbamazepine (Krahn and Mielck, 1987) and a dibenzoxapine component (Gibbs et al, 1976). Further evidence of polymorphism in PL HCl is discussed in section 3.3.3.3.2).

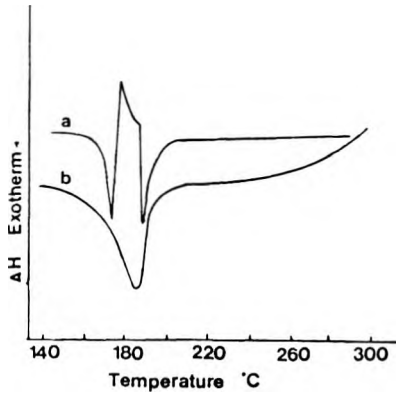


Figure 2.13 a) Typical thermogram of PL HCl hermetically sealed in static air atmosphere. b) Typical thermogram of PL HCl in flowing nitrogen (7°C/min heating rate in both cases).

Based on these studies a heating rate of 7°C/min was chosen for all future experiments as this gave good and reproducible resolutions of the various thermal events occurring in PL HCl (Figure 2.13a). Lower heating rates were not investigated as these were considered impractical.

In an open pan under flowing nitrogen the thermogram has just one broad exothermic melting peak at approximately 182°C(Figure 2.13b). Onset of melting occurs at approximately the same temperature as in the static air atmosphere (155°C), but it appears that the exothermic recrystallization phase is suppressed in a flowing nitrogen atmosphere, resulting in a single broad endothermic peak. A further significant feature of the curve is the upward drift occurring at 220°C. This is indicative of sample decomposition and is probably the result of pyrrolytic decomposition occurring in the molten phase. This is not observed in the static air atmosphere as degradation and oxidation reactions are suppressed in the closed system formed by the hermetically sealed pan (Mettler Instrumentation Mannual).

The TGA and DTGA (derivative thermogravimetric analysis) traces of PL HCl obtained at 7°C/min in flowing nitrogen are shown in Figure 2.14.

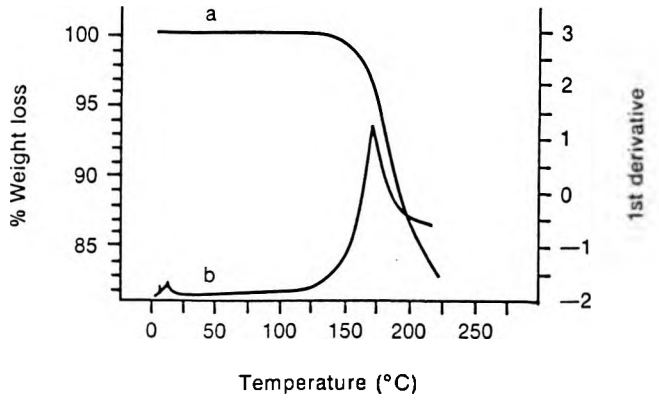


Figure 2.14 a) TGA and b) DTGA traces of PL HCl (7°C/min, flowing nitrogen)

As expected, these show a weight loss due to pyrrolytic

decomposition occurring with the onset of melting. However, no other processes resulting in significant weight changes are evident.

It is therefore apparent that in DSC analysis a static air atmosphere in hermetically sealed pans provides a more detailed picture of the physical changes which occur in PL HCl upon heating. However the thermal lability of PL HCl is not adequately reflected in this system. DSC analysis with a heating rate of 7°C/min in a static air atmosphere was therefore combined with TGA (7°C/min, flowing nitrogen 20 ml/min) to investigate any crystal transformations and solvates in PL HCl (see section 3.3.3.3.2). However, to investigate excipient compatibility DSC analysis with nitrogen purging was used (chapter 5). This procedure provides a more accurate reflection of the stability characteristics of PL HCl and also provides simpler thermograms of PL HCl. This is important as the thermograms of drug-excipient mixtures are often difficult to interpret due to the occurrance of many overlapping or interfering thermal features (van Dooren, 1983).

CHAPTER THREE

3 THE SOLID STATE PROPERTIES OF PYRIDOXAL HCl

3.1 INTRODUCTION

Drugs are most frequently administered as solid dosage forms. According to a recent survey (Wells, 1988b), tablets and capsules accounted for 58.8% of all dosage form types manufactured in the UK. Reasons for the popularity of the solid dosage form include the fact that it can be administered orally with a minimum of discomfort and inconvenience to the patient, thus improving compliance. Tablets and capsules can also be manufactured with relative ease and at low cost when compared to such dosage forms as sterile parenterals. In addition, they provide accurate dosing and tend to have good stability characteristics, enabling uncomplicated long term storage.

The investigation of solid state drug properties is therefore an important aspect of most preformulation studies. Solid state properties are defined here to

include the molecular structure of the solid compound, its stability profiles, the crystal properties and other bulk characteristics (eg. surface morphology, hygroscopicity, particle size, bulk density and compressibility).

The most basic characteristic of any drug substance is the molecular structure, as it affects all other physicochemical properties of the drug. The solid state characterization of PL HCl was therefore initiated with an investigation of the molecular structure. This was followed by a bulk characterization consisting of a study of the crystal properties, particle characteristics and hygroscopicity .

3.2 THE MOLECULAR STRUCTURE OF PYRIDOXAL HCl IN THE SOLID STATE

3.2.1 BACKGROUND

Rabinowitz and Snell (1948) demonstrated that vitamin B_6 is widely distributed in 3 forms in nature, namely pyridoxine (PN), pyridoxal (PL) and pyridoxamine (PM). The view is widely held that these 3 vitamers differ structurally mainly in the nature of their 4- position functional groups, which may be an alcohol, aldehyde or amine moiety (Figure 1.2). However,in the case of PL this is not strictly correct. A fundamental characteristic of the aldehydes is their ability to react with alcohols, yielding acetals in the process. In

PL this reaction can occur internally between the 4-formyl group and the neighbouring 5-methyl hydroxy group resulting in the formation of an internal hemiacetal (Figure 3.1) (Nurnberg, 1961).

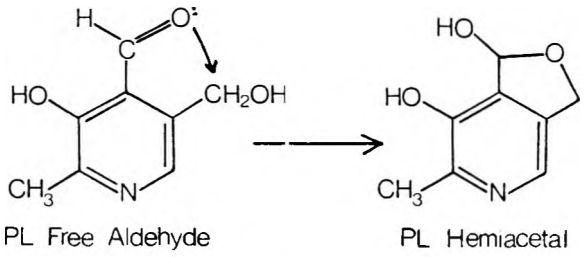


Figure 3.1 PL hemiacetal formation

Early studies of the structure of PL base using IR-, NMR- and UV spectroscopy showed that PL base exists as an internal hemiacetal in neutral and acidic solution, as well as in the solid state (Heinert and Martell, 1959; Kortnyk and Singh, 1963; Metzler and Snell, 1954). More recently X-ray diffraction techniques have been used to establish the solid state structure of PL base (Rao et al, 1982). These studies showed that PL exists exclusively in the hemiacetal conformation as proposed by previous workers.

It is obvious that this structure differs markedly from that of the free aldehyde. Such differences in structure result in significant differences in physicochemical properties. However, only one author (Kortnyk, 1986) has provided data indicating that PL HCl (the salt) could also exist as an internal hemiacetal. To confirm the molecular structure of the compound used in this preformulation study, PL HCl was analysed by IR

spectroscopy. IR spectroscopy is a particularly suitable method of analysis, as carbonyl functional groups show very characteristic and prominent peaks at approximately 1700 cm^{-1} which are seldomly obscured (Morrison and Boyd, 1984). If present, the carbonyl group should therefore be easily identifiable. Similarly the "C-O-C" ether linkage of the hemiacetal yields very characteristic peaks.

3.2.2 METHODOLOGY

Pyridoxal HCl (Fluka, Buchs) and potassium bromide (Uvistat,E Merck, Darmstadt) were used. The potassium bromide was dried at 50°C for two hours before use. Potassium bromide/PL HCl pellets were compressed on a hydraulic press (Beckmann, San Ramon, CA). Infrared absorption spectra (4000-4600 cm^{-1}) were obtained with a Nicolet (Madison,WI) FDX IR spectrophotometer connected to a 5DX data processor.

3.2.3 RESULTS

The infra red spectrum of PL HCl is shown in Figure 3.2. Table 3.1 provides a summary of the absorption bands and a comparison with the data reported by Kortnyk (1986).

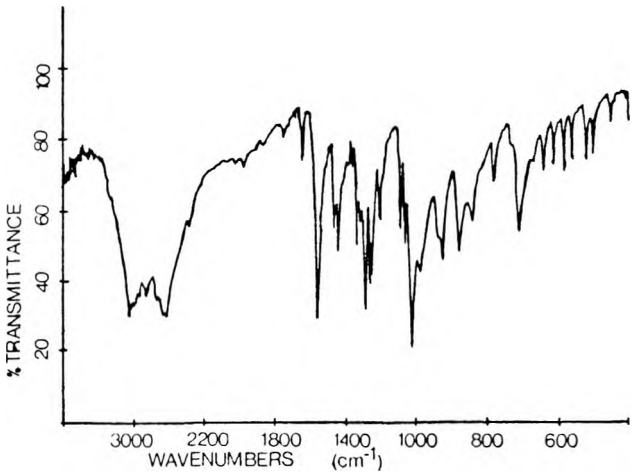


Figure 3.2 Infra-red spectrum of PL HCl (KBr pellet).

Table 3.1 Characteristic infra-red absorption peaks and bands of PL HCl

Frequency of peaks and bands (cm^{-1})	Assignment	Frequency reported by Kortnyk (1986) (cm^{-1})
1018	C-O stretch	1000
1058 1083 1246 1261	hemiacetal ring, C-O-C stretch	several peaks at 1050 and 1250
1445 1466 1556	pyridine ring vibrations	1430 1500 1570
1639-1643	ring vibrations representing $C=N^{+}H$ (HCl peak)	1630-16660
2600-3032	phenolic hydroxyl group hydrogen bounded to pyridine vitrogen	2500-2900

3.2.4 DISCUSSION

If PL HCl were present in the free aldehyde form, a prominent peak in the region 1675-2000cm^{-1} would be expected. No such peak is evident. The only peak to come into consideration is the medium intensity band at 1639-1643cm^{-1}. However, this is outside the normal range for "aldehyde peaks" and correlates well with Kortnyks assignment of the "hydrochloride peak" (due to ring vibrations representing C=N^{+}H) . In addition, the acetal is confirmed by the characteristic high intensity peaks at approximately 1250, 1050 and 1000cm^{-1} .

The spectrum correlates well with the description and interpretation of the IR spectrum provided by Kortnyk (1986). The spectrum shows the characteristic signs of a hemiacetal. On the other hand there is no evidence of a carbonyl group. A molecular structure similar to that reported for PL base (Rao et al, 1982) appears to be appropriate for PL HCl (Figure 3.3).

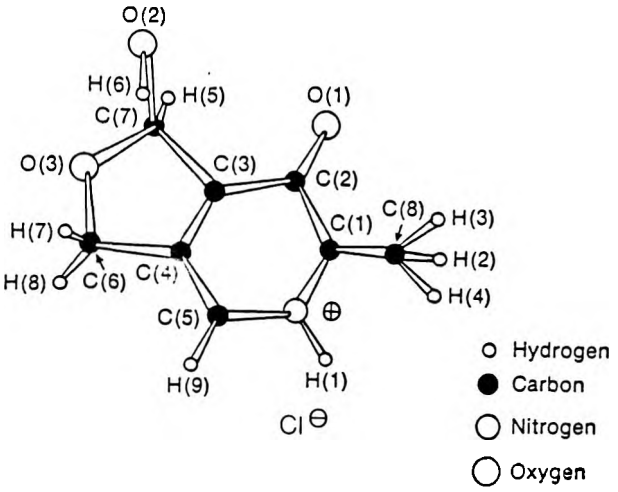


Figure 3.3 Proposed structure of PL HCl. Adapted from Rao et al (1982).

Similar to PL base, (Heinert and Martell,1958; Rao et al, 1982) , there are also clear indications of intermolecular hydrogen bonding between the phenolic hydroxyl group and the pyridine nitrogen of adjacent molecules in PL HCl (Table 3.1) . The similarity may be due to the fact that in both PL base and PL HCl the pyridine nitrogen is protonated. Although not evident from the IR scans, it is likely that the phenolic hydroxyl group is also involved in intermolecular hydrogen bonding with the hydroxyl group of the hemiacetal ring as is the case for PL base (Rao et al, 1982) .

3.3 BULK CHARACTERIZATION OF PYRIDOXAL HCl

3.3.1 OVERVIEW

Characterization of bulk properties such as particle size, bulk density, flowability, crystallinity and hygroscopicity is essential as bulk lots of drug often vary in these properties resulting in significant changes in stability, bioavailability and manufacturability (Fiese and Hagen, 1986) . Especially important for tableting and encapsulation are flowability, bulk density and compressibility. However, the average dose of vitamin B_6 per tablet seldomly exceeds 50mg (Bauernfeind and Miller, 1978). This represents only a small proportion of the bulk of a tablet. The bulk density, flowability and

compressibility of PL HCl were therefore not investigated as it is unlikely that small quantities of PL HCl would significantly affect the tableting process and properties of the formulation or the final product.

3.3.2 FINE PARTICLE CHARACTERIZATION

3.3.2.1 THEORY

Surface area controlled processes and physicochemical properties such as dissolution, adsorption and chemical reactivity are directly affected by particle size distribution and shape. An example is the poorly soluble drug, griseofulvin, which shows increased bioavailability when administered in a finely subdivded state (Gibaldi, 1984). Particle size distribution is also important for formulation homogeneity and dose uniformity. This applies especially to drugs administered in low doses. Generally finer materials interdisperse more readily and more randomly (Wadke et al, 1989).

However, size reduction may also result in disadvantages such as a larger surface area for chemical interaction with oxygen, moisture, light and excipients. An increased surface area also results in increased hygroscopicity and electrostatic agglomeration (Wadke et al, 1989).

Numerous methods are available for particle sizing.

These include light microscopy, electron microscopy, conductivity measurements (Coulter Counter) and laser light scattering measurements (Royco Particle counter). The particle size distribution and surface morphology of PL HCl were investigated by scanning electron microscopy (SEM). Particle shapes are discussed later (see 3.3.3.3.1 Crystal Habit).

3.3.2.2 METHODOLOGY

PL HCl (Fluka Ag, Buchs) was dried at reduced pressure over phosphorus pentoxide (SARchem, Krugersdorp) for 24 hours. The samples were then sputter coated with colloidal graphite before fine coating with a gold/platinum film. Photomicrographs were obtained with a Jeol (Tokyo) JSM-840 scanning electron microscope at 15KV accelerating voltage. Particle sizes were measured on a series of 9 electron photomicrographs using Ferets diameter as an indicator of gross external dimension (Figure 3.4) (Wells, 1989f).

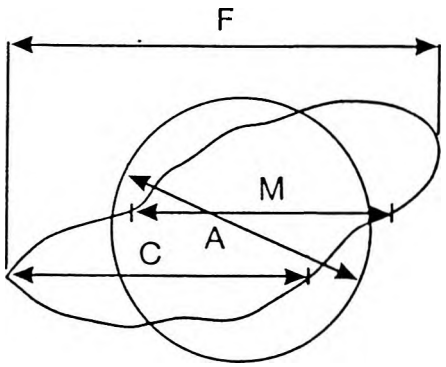


Figure 3.4 Various statistical particle diameters for the assessment of irregular particles. A, projected area diameter; C, maximum horizontal cord; F, Ferets diameter; M, Martins diameter. (F>C>M) Adapted from Wells (1989f).

Particle size data was analyzed by means of a frequency distribution histogram and a log normal cumulative distribution curve. The geometric mean size (M) and the geometric standard deviation (σ_g) were determined from the cumulative distribution curve. M can be read from the graph at 50% oversize and σ_g can be calculated as follows:

$$\sigma_g = \frac{\text{size at 16\% (oversize)}}{\text{size at 50\% (oversize)}} \quad \text{(Wells, 1989f)}$$

3.3.2.3 RESUTS AND DISCUSSION

Typical scanning electron micrographs of PL HCl are shown in figure 3.5. At low power magnification the marked agglomeration of the particles is clearly visible. In general, particle surfaces are smooth with occasional fissures in evidence.

Size analysis reveals a large proportion of smaller particles with sizes varying from 3 to 60 µm (Table 3.2 and Figure 3.6). The skewed distribution was normalized by plotting particle size on a log scale. This yielded a typical sigmoidal cumulative distribution curve (Figure 3.7). The system thus follows the general model for particulate distribution as proposed by Hatch and Choate (1929).

A)

B)

C)

Figure 3.5 Scanning electron photomicrographs of PL HCl. A) x 500 magnification, B) x 950 magnification, C) x 2700 magnification.

Table 3.2 Particle size analysis by SEM

Particle size (µm)	Particle count	Cumulative count	Cumulative percentage
>40	9	9	6.1
30 - 40	12	21	14.2
20 - 30	21	42	28.4
10 - 20	55	97	65.5
5 - 10	43	140	94.6
0 - 5	8	148	100.0

Using the cumulative distribution curve, the geometric mean particle size was estimated as 15.8 μm and a value of 1.78 was calculated for the geometric standard deviation (σ_g). A wide particle size distribution typically gives values >2 for σ_g, whereas tight particle size distributions yield values for σ_g in the region of 1.5 (Wells, 1989f).

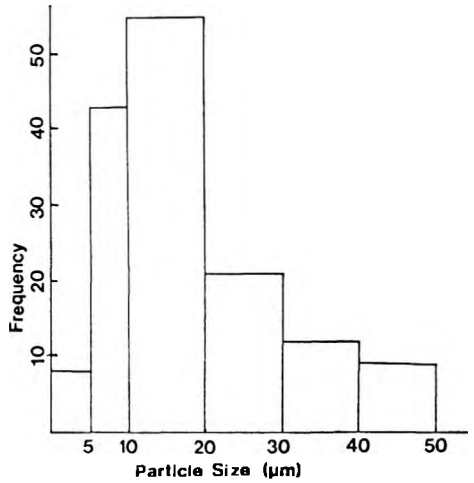


Figure 3.6 Frequency distribution histogram of PL HCl particle size.

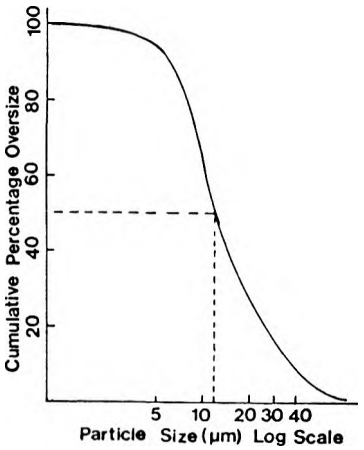


Figure 3.7 Cumulative distribution curve of PL HCl particle size.

Generally the experimental samples of PL HCl show a

particle size distribution typical of most particulate systems with a relatively low σ_g. Of the particles analysed, 66% fall into the range of 5 - 20 µm as measured by their Ferets diameter. Size uniformity could be increased further by milling. However, the present distribution can be regarded as suitably tight for a low potency drug administered in low doses.

3.3.3. THE CRYSTAL PROPERTIES OF PYRDOXAL HCl

3.3.3.1 THEORETICAL BACKGROUND

Similar to the molecular structure and fine particle characteristics, the crystalline characteristics of a drug may significantly influence the solubility and bioavailability and other important physicochemical properties . Important crystal properties relevant to pharmaceuticals have been outlined by Halebian (1975) (Figure 3.8).

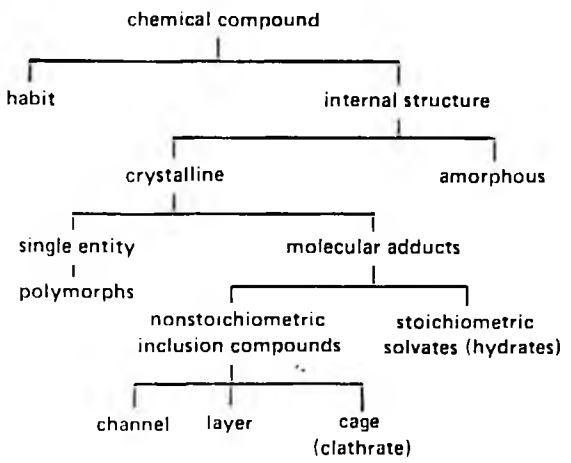


Figure 3.8 Outline of important crystal characteristics.

Two major properties highlighted by Halebian are the crystal habit (the external appearance of the crystal) and the internal structure (the molecular arrangement within the crystal). In crystalline solids the molecular arrangement is repetitive and shows long range order, while in amorphous solids no such repetitive order exists.

Many crystalline drugs also have more than one internal structure. These variations in crystal structure are known as polymorphs. At any one temperature only one crystal form will be stable, the other forms being metastable. Crystal variations known as solvates can also be produced by inclusion of solvent molecules in the crystal lattice.

Rao and his co-workers (Rao et al, 1982) have studied the crystal structure of PL base in detail. They found trigonal, prism shaped crystals and by X-ray diffraction were able to determine the exact intermolecular arrangement in PL. However, these results can not be directly applied to PL HCl as chemical changes such as salt formation usually result in altered internal structures and crystal habits (Fiese and Hagen, 1986). Furthermore, it appears that polymorphic behaviour and solvate formation in PL HCl have not been studied previously. A brief study of the crystal habit and of possible variations in the internal structure of PL HCl was therefore undertaken.

3.3.3.2 METHODOLOGY

3.3.3.2.1 MATERIAL AND EQUIPMENT

Analytical grade PL HCl (Fluka Ag, Buchs), acetone (E Merck, Darmstadt), chloroform (BDH, Poole) and methanol (BDH, Poole) were used. Double distilled water was obtained from a Milli-Q purifying apparatus (Millipore, Bedford M.A.). DSC was carried out in hermetically sealed standard aluminium pans on a Mettler (Greifensee) TA3000 system equipped with a DSC 20 cell. Thermogravimetric analyses were performed in platinum pans on a Du Pont 9900 system. Silica gel HF60 plates (E Merck, Darmstadt) were used for thin layer chromatography.

3.3.3.2.2 PROCEDURE

The crystal habits of PL HCl were investigated with SEM as previously described (section 3.3.2.2). In order to investigate the existence of metastable and solvated forms, DSC analysis was carried out as described in chapter 2 (2.6.2) on PL HCl samples recrystallized from acetone, chloroform and water. Aliphatic alcohols (eg methanol and ethanol) are commonly used for recrystallization. However, these could not be used for PL HCl as they react with the hemiacetal resulting in the formation of methyl and ethyl acetals (Nurnberg, 1961). In addition PL HCl was heated past the onset of melting (175°C) and then cooled before reheating by DSC. PL HCl was tested for decomposition during this

treatment by using the previously detailed TLC procedure (section 2.3.2).

3.3.3.3 RESULTS AND DISCUSSION

3.3.3.3.1 CRYSTAL HABIT

PL HCl (Fluka AG, Buchs) exists as a mixture of large tabular or platy forms (approximately 60um diameter) and smaller anhedral prismatic forms (10-15um in diameter) (Figure 3.7). The smaller prismatic forms appear to have no clearly defined edges and plane faces. This large variation in size and shape may be due to impeded crystal growth during recrystallization (Halebian,1975). Alternatively the smaller particles may simply represent debris, formed when the larger plates were fractured. A further possibility is that the different crystal habits are a manifestation of polymorphism. This aspect is discussed in more detail below.

3.3.3.3.2 POLYMORPHISM AND SOLVATE FORMATION

The DSC thermogram of PL HCl shows two endothermic peaks at ca. 170°C and 185°C separated by an exothermic peak at 175°C (Figure 3.9a). An additional sample of PL HCl was heated to 175°C, the approximate exothermic peak temperature, and then solidified by cooling. When this sample was reheated only one peak corresponding to the second endothermic peak of the initial thermogram (Figure 3.9a) appeared (Figure 3.9b). As discussed previously (section 2.6.1) such thermograms are

characteristic of monotropic polymorphic transformations involving the melting of the metastable form II (endothermic process) and crystallization of the stable form I from the melt (exothermic process) (Giron-Forest, 1984). The second endothermic peak denotes the melting of form I. TLC analysis of samples heated to 175°C in hermetically sealed pans indicated that no decomposition had occurred. Decomposition reactions and oxidation could thus be discounted as causes of the exothermic peak.

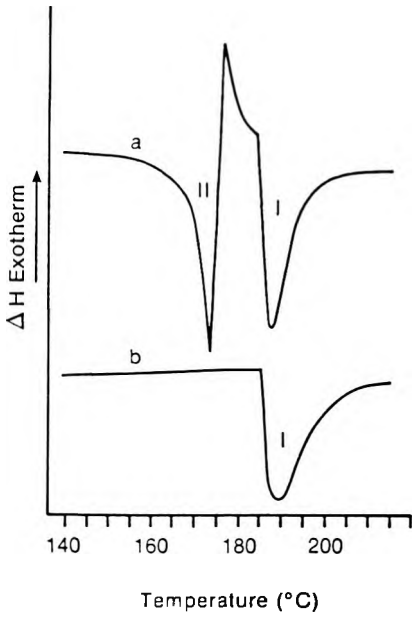


Figure 3.9 DSC thermogram of a) PL HCl as supplied and b) PL HCl after heating and cooling

This is also borne out by the fact that the baseline remained stable after the second endotherm, indicating that the hermetic seal was sucessful in suppressing any oxidation or pyrrolysis. The presence of pseudopolymorphism was ruled out as no weight loss

indicative of desolvation occurred before melting as illustrated by TGA analysis (see chapter 2, Figure 2.14). The rapid weight loss coinciding with melting can be attributed to pyrrolytic decomposition accompanying the melting process. This is in agreement with previously published reports (Budavari et al, 1989).

Slight differences between the two forms were also observed by IR spectroscopy. Figure 3.10 shows the differences between PL HCl (as supplied by Fluka) and PL HCl after melting and resolidification by cooling. However, it could not be ascertained wether PL HCl exists only as form II or as a mixture of both polymorphs in its untreated state.

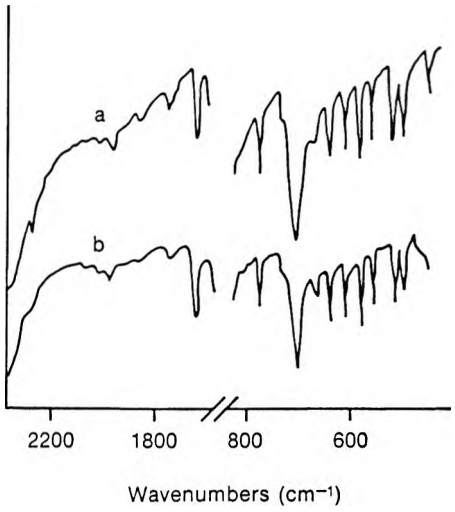


Figure 3.10 Differences in the IR spectra of a) PL HCl as supplied and b) PL HCl after heating and cooling.

The cooling and reheating studies failed to reveal any amorphous states of PL HCl. Amorphous compounds can often be produced by melting and then rapidly cooling

the crystalline solids. The DSC curve of an amorphous solid shows a characteristic jump in the baseline be cause of the sudden change in heat capacity (cp) at the glass transition temperature (Tg) and an anomalous endothermic peak (heat capacity maximum) (Figure 3.11) (Fukuoka et al, 1986).

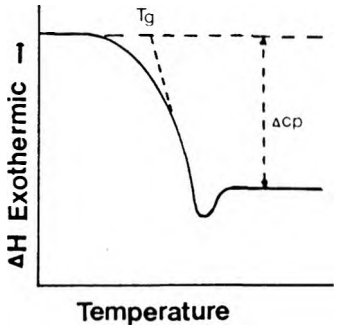


Figure 3.11 Characteristic features of the DSC curve of an amorphous solid. Adapted from Fukuoka et al (1986)

The presence of solvates was investigated by TGA and DSC. TGA has to be performed in open containers and PL decomposition is therefore expected to occur with the onset of melting (Budavari et al, 1989). However, solvates should still be detectable as desolvation is expected to occur below the melting point. The TGA and DTGA traces of standard PL HCl and PL HCl recrystallized from water, acetone and chloroform clearly showed weight loss due to pyrrolytic decomposition occurring with the onset of melting (Figure 3.12). However, no significant weight loss was observed prior to melting, indicating the absence of any solvates. DSC thermograms of the recrystallized samples show only slight changes in peak shape and area when compared to the original untreated

sample (Figure 3.13). The lack of desolvation peaks confirms the absence of solvates. It is also apparent from these DSC curves that it was not possible to produce only stable form I crystals by recrystallization.

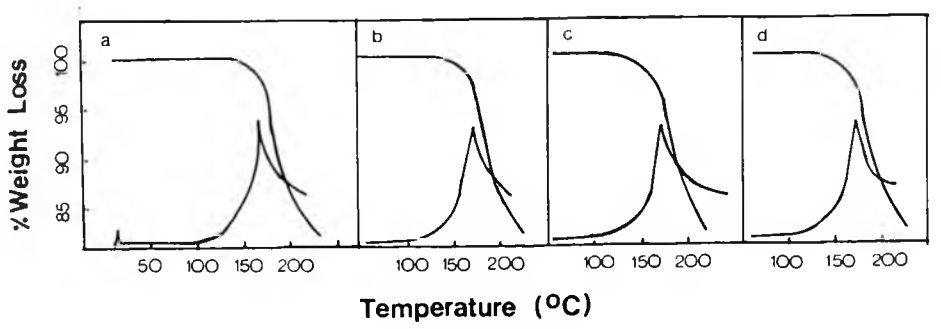


Figure 3.12 TGA and DTGA curves for PL HCl recrystallized from a) acetone, b) water, c) chloroform and d) untreated PL HCl

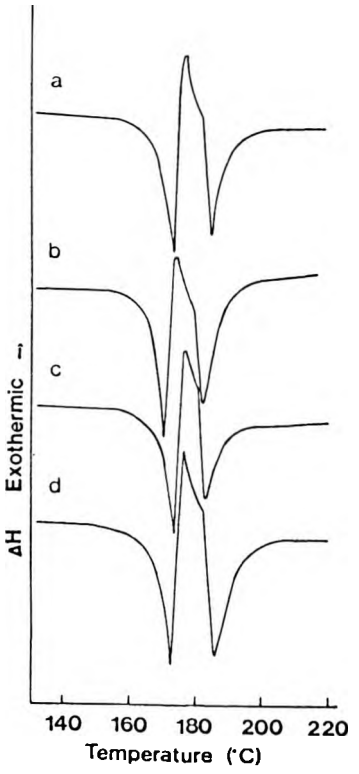


Figure 3.13 DSC thermograms of PL HCl recrystallized from a) acetone, b) water, c) chloroform and d) untreated PL HCl

3.3.3.4 CONCLUSION

Thermal analysis strongly indicates that at least two polymorphic forms of PL HCl exist. It appears that the equilibrium between the polymorphs is monotropic. When PL HCl was recrystallized from chloroform, acetone and water no solvates were formed. PL HCl is a very water soluble compound (chapter 4) and has a rapid dissolution rate. A significant difference in bioavailability between the two polymorphs is therefore unlikely. For this reason no attempt was made to separately measure the intrinsic solubilities of the two crystal forms. The influence of environmental factors such as moisture and temperature on polymorphic behaviour will be considered in section 3.3.4.

3.3.4 THE HYGROSCOPICITY OF PYRIDOXAL HCl

3.3.4.1 INTRODUCTION

The term "Hygroscopicity" has no accepted definition as it encompasses both thermodynamic and kinetic components (Van Campen et al, 1980). A distinction can therefore be made between equilibrium hygroscopicity and dynamic hygroscopicity. Equilibrium hygroscopicity relates to the total amount of moisture adsorbed by a compound if allowed to equilibrate at a specific relative humidity (RH), whereas dynamic hygroscopicity refers to the rate at which a compound tends to sorb moisture from its surroundings (Umprayn and Mendes, 1987).

The study of the hygroscopic nature of a solid drug is an essential part of every preformulation investigation, as solids containing residual water exhibit significant changes in many physicochemical properties relative to their dry state. These properties include chemical stability, crystal structure, powder flow, compactability, lubricity, dissolution rate and polymer film permeability (Zografi, 1988). Specifically in the case of chemical stability, moisture is considered to be the most deleterious environmental factor (Monkhouse, 1984). This is exemplified by numerous reports of the detrimental effect of moisture on the chemical stability of drugs (Okamura et al, 1980; Yoshioka et al, 1979; Lee et al, 1965; Hasegawa et al, 1975; Carstensen et al, 1969).

A solid can be classified according to its hygroscopicity by determining the critical relative humidity (i.e. the RH below which moisture adsorption does not occur) or the hygroscopic point (i.e. the RH at which the solid can take up 1% water in 24 hours) (Umprayn and Mendes, 1987). Callahan et al, (1986) have proposed an alternative classification which recognises 4 degrees of hygroscopicity:

Class 1, Non hygroscopic: Essentially no moisture increases occur below 90% RH. The increase in moisture content after storage for one week above 90% RH is less than 20%.

Class 2, Slightly hygroscopic: Essentially no

moisture increases occur below 80% RH. The increase in moisture content after storage for one week above 80% RH is less than 40%.

Class 3, Moderately hygroscopic: Moisture content does not increase, more than 5% after storage at relative humidities below 60%. The increase in moisture content after storage for one week above 80% R.H. is less than 50%.

Class 4, Very hygroscopic: moisture content increases may occur at relative humidities as low as 40%. The increase in moisture content after storage for one week above 90% RH may exceed 30%.

An indication of a drugs equilibrium hygroscopicity is also given by the moisture adsorption isotherm. An adsorption isotherm not only yields information about the total amount of moisture taken up by the drug if allowed to equilibrate at various RH's, but also often provides an indication of the mechanism of moisture uptake and how the moisture is accommodated within the solid.

Generally water is adsorbed onto solid surfaces by physical- or van der Waals adsorption. Experimentally obtained physical adsorption isotherms can be grouped into 5 categories (Figure 3.14) (Glasstone and Lewis,1976). Type I isotherms are observed when a tightly held unimolecular layer of adsorbate is formed. Once all "active" sites on the solid surface have been covered, a limit in adsorption is reached. This type of

isotherm is also observed for chemisorption. For Types II and III the formation of several layers of adsorbate has been postulated. In Type II adsorption, additional layers of water are adsorbed once the tightly held monolayer has been formed (indicated by the point of inflection). This occurs in nonporous amorphous or partially amorphous solids, eg. starch and cellulose (Zografi and Kontny,1986, Zografi et al.1984).

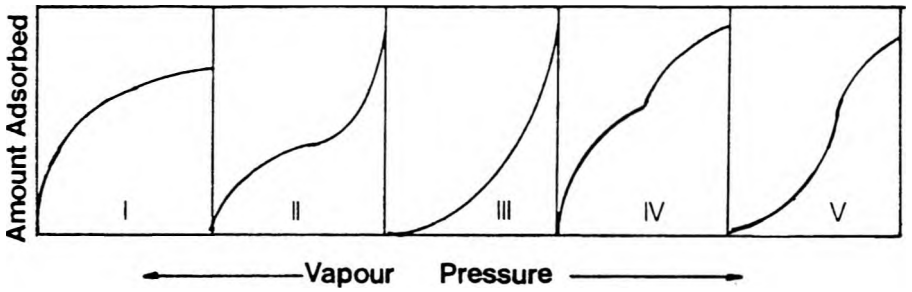


Figure 3.14 The five types of physical adsorption isotherms

Type III adsorption tends to occur in crystalline, nonporous solids (Umprayn and Mendes, 1987), eg aspirin (Mitrevej and Hollenbeck, 1983). Type IV and V adsorption isotherms are characteristic of porous solids. In Type IV adsorption a monolayer is initially formed (indicated by the first point of inflection) followed by continued multilayer adsorption and condensation in the capillaries and pores (indicated by the second point of inflection).

Type I,II and III isotherms can be described by the BET (Brumauer, Emmet and Teller) equation (Glasstone and

Lewis,1976). This equation is based on the assumption that water associated with a solid exists in two thermodynamic states. These are: tightly bound water (monolayer water which is not available for chemical interaction and surface dissolution) and solvent-like, "free" water (bulk water which is readily available for dissolution of the solid and for chemical reactions) (Zografi,1988; Monkhouse,1984). An extension of this concept is the GAB (Guggenheim, Anderson and de Boer) equation which proposes a third thermodynamic state of water, namely intermediate, loosely bound water (Zografi,1988):

$$W = \frac{W_m c_b k \frac{p}{po}}{(1 - k\frac{p}{po})(1 - k\frac{p}{po} + c_b k\frac{p}{po})}$$

Where W is the weight of water adsorbed per unit weight of dry solid at relative pressure p/po, W_m is the weight of water adsorbed corresponding to monolayer coverage and c_b and k are equilibrium constants. Compared to the BET equation, this equation has been found to produce a superior fit for Type II adsorption isotherms (Zografi and Kontny, 1986; Zografi et al,1984).

The concept of rigidly categorising adsorbed water into several thermodynamic states, with bound water having little effect on the chemical degradation or physical properties of the solid has recently been challenged by

Ahlneck and Zografi (1990). These authors have noted that the degree and strength of hydrogen bonding between tightly held water and the solid is variable (also Zografi, 1988) and can lead to "bound" water being quite mobile within the solid system. They have also pointed out that in many cases small amounts of adsorbed moisture (insufficient for multilayer formation) are able to promote chemical degradation and physical changes within the solid. This would not be possible if only bulk water were capable of causing physical and chemical changes. Instead it is proposed, (based on experimental evidence), that adsorbed water acts primarily as a plasticizer by preferentially dissolving into regions of molecular disorder (eg crystal defects, imperfections and amorphous regions). Molecules in regions of disorder are in an activated state because of increased molecular mobility and because more reactive chemical groups are exposed. This accounts for the greater ability to take up water. Once sufficient water is taken up, the system becomes plasticized. The resulting increased molecular mobility may then lead to greater chemical reactivity and to physical processes such as recrystallization.

The presence of water also appears to be highly detrimental to the stability of most vitamins. This manifests itself in the numerous stability problems experienced with liquid formulations (Buhler, 1988). To investigate the effect of atmospheric moisture on PL HCl

the equilibrium hygroscopicity of PL HCl was studied. A complete moisture adsorption isotherm was derived. Simultaneously physical changes were monitored with the aid of SEM and thermal analysis. The influence of atmospheric moisture on chemical stability is discussed in chapter 5.

3.3.4.2 METHODOLOGY

3.3.4.2.1 MATERIALS AND EQUIPMENT

Pyridoxal HCl was obtained from Fluka AG (Buchs). Sodium dichromate, sodium chloride and phosphorus pentoxide were obtained from SAR chem (Krugersdorp). Lithium chloride, potassium acetate, magnesium chloride, sodium bromide, copper chloride, lithium sulfate and potassium sulfate were obtained from E Merck, (Darmstadt). All materials were analytical grade. Double distilled water (Milli Q system, Millipore, Bedford M.A.) was used throughout. Moisture determinations were made with a Mettler (Greifensee) DL18 Karl Fischer Titrator. DSC was carried out on a Mettler (Greifense) TA 3000 system (DSC20 cell) with hermetically sealed standard aluminium pans. TGA was carried out on a Du Pont TG 9900 system with open platinum pans. A Jeol (Tokyo) JSM 840 scanning electron microscope was used to observe and study surface characteristics and morphology of the particles. Saturated salt solutions were kept at constant temperature in a Memmert (Schwarzbach) constant temperature cabinet.

3.3.4.2.2 PROCEDURE

PL HCl was dried at reduced pressure over phosphorus pentoxide for 24 hours. Nine different controlled humidity environments were obtained by equilibrating various saturated salt solutions at 25°C. The salt solutions and humidities reported by Nyquist (1983) were used for this purpose (Table 3.3). Where possible salt solutions were selected that showed little variation in humidity with temperature changes. The solutions used have been reported to maintain constant humidity for five years.

Table 3.3 Relative humidities produced by the saturated salt solutions used. Mean values with standard errors (n=10)

Salt	Temperature °C		
	20	25	30
LiCl	11.3 (0.065)	11.3 (0.104)	11.3 (0.096)
CH_3COOK	22.5 (0.192)	21.6 (0.095)	21.5 (0.192)
$MgCl_2$	33.1 (0.198)	32.8 (0.086)	32.4 (0.253)
Na_2CrO_7	54.8 (0.093)	54.4 (0.165)	54.0 (0.120)
NaBr	59.5 (0.101)	57.5 (0.177)	56.0 (0.161)
$CuCl_2$	67.7 (0.143)	68.0 (0.178)	69.0 (0.093)
NaCl	75.5 (0.089)	75.3 (0.062)	75.1 (0.134)
$LiSO_4$	88.2 (0.248)	87.8 (0.209)	87.5 (0.292)
k_2SO_4	97.6 (0.430)	97.3 (0.401)	97.0 (0.512)

Adapted from Nygvist,1983.

Approximately 200ml of each saturated solution with an excess of solid phase was placed in a 1 litre glass dessicator and allowed to equilibrate at 25°C for 48 hours. Three dried samples of PL HCl (approximately 500mg) in open vials were then placed in each hygrostat for 12 days (pilot studies showed no significant difference in moisture content between samples

equilibrated for 12 and 15 days). Percentage moisture content was then measured by Karl Fischer titration as described earlier (section 2.5). Thermal analysis and SEM was carried out as described previously (sections 2.6 and 3.3.2.2) on samples stored at 11% RH and 75% RH at 25°C and 40°C for two weeks.

3.3.4.3 RESULTS AND DISCUSSION

An examination of the equilibrium moisture contents (Table 3.4) shows that above 90% RH PL HCl is deliquescent. At 97.3% RH half the sample weight is contributed by water and the sample can be seen to be dissolving in the surrounding liquid. Another striking feature is the discontinuity in the adsorption isotherm (Figure 3.15) in the region of 60 - 68% RH.

Table 3.4 Results of moisture content determination.

Relative Humidity (%)	Average Moisture Content (n=3) (mg/g)	Standard Error
11.3	1.9	0.0123
21.6	2.7	0.0039
32.8	2.9	0.0064
54.4	3.7	0.0056
57.5	3.9	0.0042
68.0	2.4	0.0053
75.3	9.3	0.0269
80.9	15.8	0.0249
97.3	517.2	0.3629

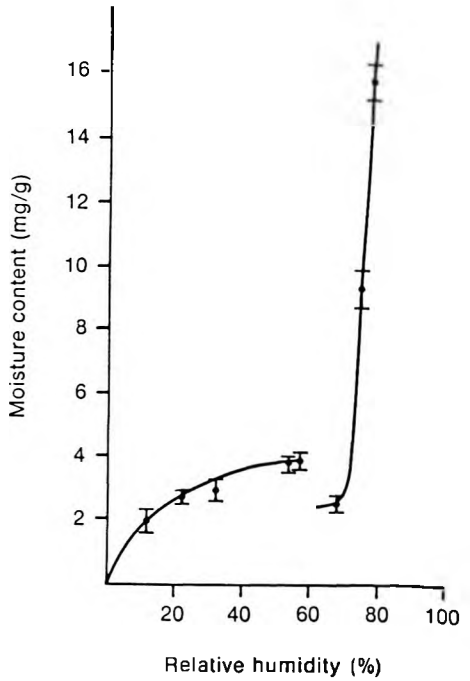


Figure 3.15 Moisture adsorption isotherm for PL HCl (25°C) with confidence intervals ($p>0.05$, $n=3$)

Moisture isotherms of this nature have previously been reported for amorphous systems which transform into energetically more stable, less hygroscopic crystalline systems when exposed to moisture (Umprayn and Mendes, 1987; Makower and Dye, 1956) (Figure 3.16).

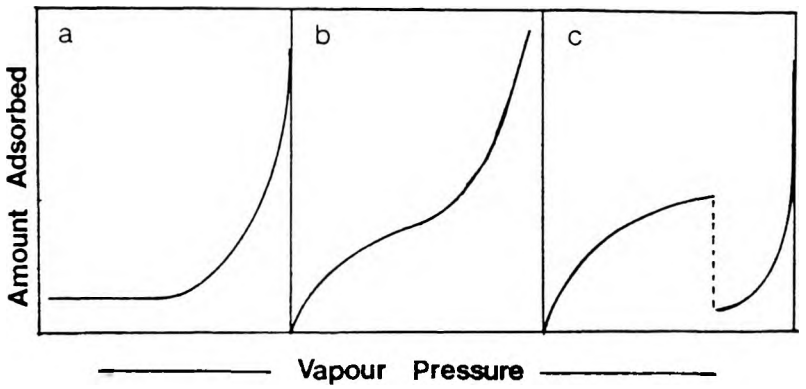


Figure 3.16 Typical moisture adsorption isotherms of a) an amorphous system (eg sucrose) undergoing transition to a crystalline system, b) an amorphous or partially amorphous system and c) a crystalline system

Although hygroscopicity is highly order dependant (Huttenrauch, 1977), even small changes in crystal structure can lead to significant differences in hygroscopicity. This has been exemplified by the marked differences in hygroscopicity of the polymorphs of indomethacin (Imaizumi et al, 1980) and chlortetracycline (Miyazaki et al, 1974). It should be noted that metastable polymorphs have energetically unstable molecular arrangements and can therefore be regarded as activated systems relative to their stable form. Consequently metastable polymorphs can be expected to be more hygroscopic.

In the present study a moisture induced transition from the predominantly metastable state (II) to a more stable, less hygroscopic form is evident from DSC thermograms and electron micrographs of PL HCl stored at 75% RH (Figures 3.17, 3.18 and Table 3.5).

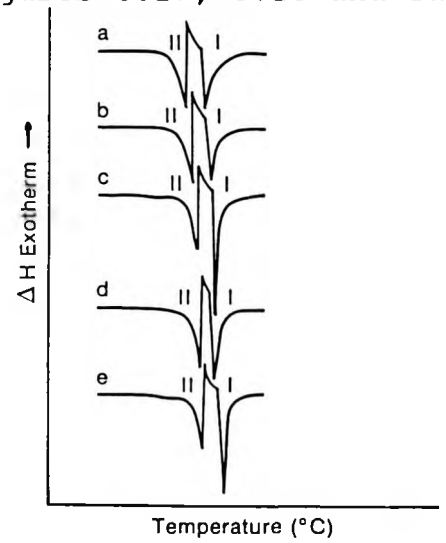


Figure 3.17 DSC traces of PL HCl a) as supplied; b) stored at 25°C, 11% RH; c)stored at 25°C, 75% RH; d) stored at 55°C, 11%RH and e) stored at 55°C, 75% RH.

A marked change in the ratio of the endothermic peak areas (I and II) which correspond to the enthalpies of melting of the metastable form II and the stable form I is observed (see Figure 3.17 and Table 3.5).

Table 3.5 Peak temperatures (T,°C) and enthalpies (H, J/G) of PL HCl obtained from the DSC thermograms under various storage conditions

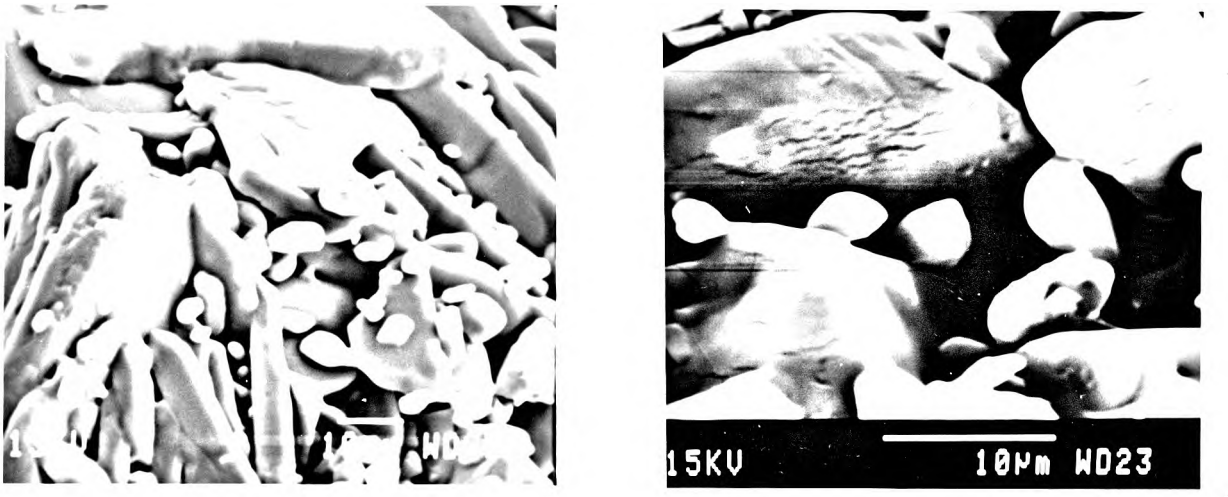
Sample	Storage Conditions	Peak II T	Peak II ΔH	Peak I T	Peak I ΔH	$\frac{\Delta H_{II}*}{\Delta H_I}$
a)	No storage	170.3	87.8	185.0	91.2	0.96
b)	25°C, 11% RH, 14 days	171.7	81.6	184.6	88.1	0.93
c)	25°C, 75% RH, 14 days	172.3	75.9	185.2	94.4	0.80
d)	40°C, 11% RH, 14 days	172.2	78.1	182.4	76.6	1.01
e)	40°C, 75% RH, 14 days	172.3	72.1	187.3	91.2	0.79

* The ratio of the enthalpies of melting corresponding to the two endothermic peak areas (I and II).

The decrease in the melting peak of the metastable form suggests a partial conversion to the stable polymorph. This correlates well with the observed moisture sorption isotherm (Figure 3.15) and the scanning electron micrographs , where partial conversion to thin, platy

crystals with large faces and well defined edges is evident at 75% RH (Figure 3.18).

a)



b)

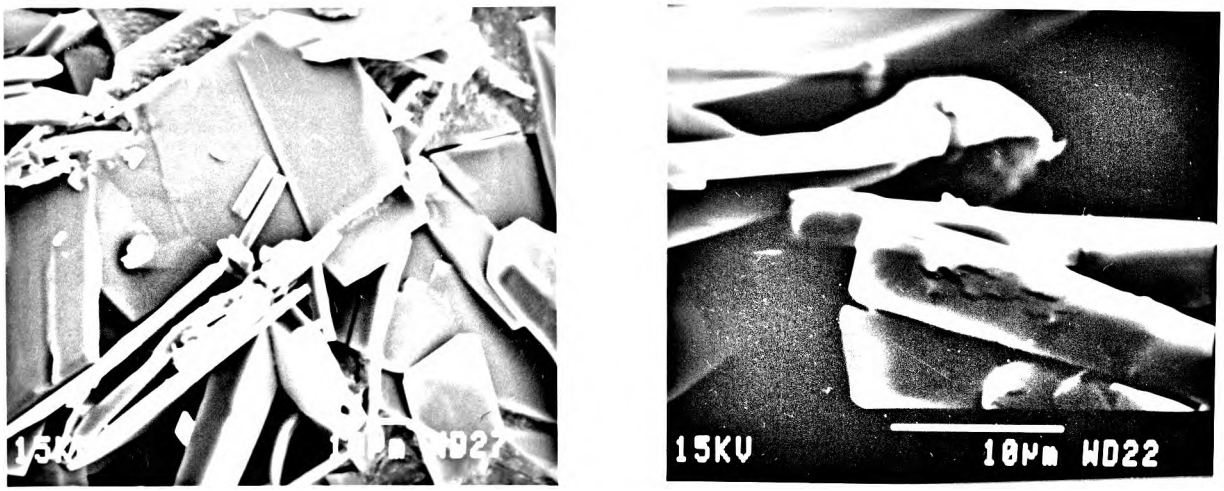


Figure 3.18 Scanning electron micrographs of a) PL HCl as supplied (x 900 and x 2700 magnifications respectively) and b) PL HCl stored at 75% RH (x 900 and x 2700 magnifications respectively).

3.3.4.4 CONCLUSION

PL HCl appears to be physically unstable at elevated levels of atmospheric moisture. The moisture adsorption isotherm and the thermal analysis indicate that in the region of 57-68% RH, PL undergoes a partial polymorphic transition resulting in a sudden decrease in its moisture adsorption capacity. This possibly occurs according to the mechanism of moisture sorption recently proposed by Ahlneck and Zografi (1990), where water is predominantely adsorbed into the metastable regions of the solid. Once sufficient water has been adsorbed, the system is plasticized. The resulting increased molecular mobility may then facilitate the conversion to a more stable molecular arrangement.

PL HCl is also a deliquescent solid and adsorbs large quantities of water above 70-75% RH. The increase in freely available water would be expected to significantly contribute to the decomposition of the solid (this is dicussed in detail in chapter 5).

In conclusion, there are strong indications that a polymorphic transformation to a stable, less hygroscopic form of PL HCl can be induced by exposure of the substance to elevated moisture levels. This stable polymorphic form of PL HCl is of interest as its lower hygroscopicity may enhance chemical stability, while the concurrent expected decrease in solubility is unlikely to be significant as PL HCL is a highly water soluble

substance. Furthermore it is apparent that precautions must be taken to protect PL HCl from the deleterious effects of moisture during manufacture and storage. Products containing PL HCl should be stored in air tight containers and a dessicant should be included.

CHAPTER FOUR

4 SOLUBILITY ANALYSIS

4.1 INTRODUCTION

The extent of absorption and bioavailability of a drug administered orally as a solid dosage form is strongly influenced by its solubility characteristics. This can be seen from the schematic representation of the processes involved in drug release and absorption in the gastrointestinal tract (GIT) (Figure 4.1).

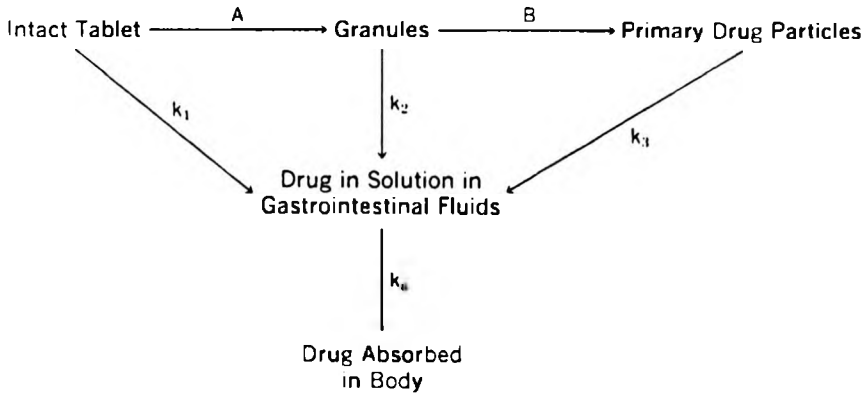


Figure 4.1 Disintegration (A, B) and dissolution processes (k_1, k_2, k_3) that precede drug absorption after administration of a tablet (Gibaldi, 1984).

The dissolution of the drug in the aqueous fluids of the GIT is a central and possibly rate limiting step in absorption. Important parameters characterizing the aqueous solubility are the equilibrium solubility, the intrinsic dissolution rate and the ionization constants of the drug.

Once in solution, the drug is available for absorption. At this point the relationship between the lipid solubility of the drug, its tendency to ionize and pH of the absorption site becomes critical. This relationship is the basis for the pH-partition theory of drug absorption (Gibaldi, 1980). The theory assumes that the cell membranes of the GIT present a simple lipoidal barrier to the transport of drugs. Thus, non-ionized acidic and basic drugs, if sufficiently lipophilic, would be absorbed. In contrast, the ionized forms would remain largely unabsorbed. Therefore, the larger the non-ionized fraction of a drug, the better its absorption. The extent of ionization of a drug in solution depends on the ionization constant (k_a) and the pH of the solution and can easily be calculated by the Henderson-Hasselbach equation.

However, the pH-partition theory fails to recognize the importance of the surface area of the absorption site, the retention time at the absorption site and the fact that organic ions can be absorbed slowly from the GIT. It is therefore not a universal theory of drug absorption (Gibaldi, 1984).

Nevertheless, in many instances the lipophilicity (measured as a partition coefficient) and the ionization constants considered together with the aqueous solubility of a drug provide a good base for predicting bioavailability problems (Nyqvist, 1986). Should such

problems arise, then various methods for enhancing the inherent solubility for the drug can be considered. These include salt formation, prodrug development, micronization, crystal modifications, solid dispersions and the formation of cyclodextrin inclusion compounds (Gibaldi, 1984; Motola and Agharkar, 1984; Halebian, 1975 and Duchene and Wouessidjewe, 1990). In this chapter the available solubility and ionization data for PL HCl are reviewed. In addition, solubility estimations are made and lastly the partition coefficient for PL HCl is determined.

4.2 THE SOLUBILITY OF PYRIDOXAL HCl

4.2.1 INTRODUCTION

Solubility is a complex phenomenon and depends on the physicochemical properties of the solvent and solute as well as on the temperature, pressure, agitation speed and pH of the solution. Quantitatively solubility can be defined as the concentration of solute found in a saturated solution at a certain temperature. This can be expressed in various ways eg. mass/volume, molality, molarity or as a percentage. For substances whose solubilities are not exactly known, the terms given in Table 4.1 can be used.

Table 4.1 Terms of approximate solubility

Term	Parts of solvent required per part of solute
very soluble	less than 1 part
freely soluble	1 to 10 parts
soluble	10 to 30 parts
sparingly soluble	30 to 100 parts
slightly soluble	100 to 1000 parts
very slightly soluble	1000 to 10000 parts
practicly insoluble	more than 10000 parts

(Adapted from Martin et al, 1983)

The solubility of solids in liquids is as yet incompletely understood and can therefore not be satisfactorily predicted except for ideal solutions (Martin et al, 1983).

4.2.2 METHODOLOGY

For most organic solvents there is no documented solubility data concerning PL HCl. A semi-quantitative procedure was therefore used to estimate the solubility of PL HCl in various organic solvents according to Table 4.1.

4.2.2.1 MATERIALS AND EQUIPMENT

The following analytical grade materials were used: 2-propanol (E Merck, Darmstadt), acetone (BDH, Poole), chloroform (BDH, Poole), ether (E Merck, Darmstadt), acetonitrile (Carlo Erba, Milan), tetrahydrofuran (E Merck, Darmstadt) and PL HCl (Fluka, Buchs). Solutions were stored in a temperature controlled oven (Memmert, Schwabach).

4.2.2.2 PROCEDURE

100µl of organic solvent was added to a test tube containing 10mg PL HCL. The tube was sealed, periodically shaken and stored for 24 hours at 25°C. If complete solubilization occurred, then PL HCl was judged "soluble" in that particular solvent (in accordance with Table 4.1). If undissolved solute was still visible, the volume of the solution was increased in multiples of ten and the procedure was repeated.

4.2.3 RESULTS

The experimentally determined solubility estimates and the literature solubility data are presented in table 4.2.

Table 4.2 The solubility of pyridoxal hydrochloride

solvent	solubility of PL HCL (25 C)	reference
water	50g /100ml	Budavari et al,1983
ethanol	1.7g/100ml	Budavari et al,1983
propanol	sparingly soluble	*
acetone	slightly soluble	*
chloroform	very slightly soluble/insoluble	*
ether	very slightly soluble/insoluble	*
acetonitrile	very slightly soluble/insoluble	*
tetrahydrofuran	very slightly soluble/insoluble	*

* Determined experimentally.

4.2.4 DISCUSSION AND CONCLUSION

PL HCl is freely soluble in aqueous solution and relatively soluble in aliphatic alcohols. However, solubility rapidly declines with decreasing polarity of the solvent. As a result PL HCl is relatively poorly soluble in most organic solvents. It has been suggested (Wells, 1988) that absorption problems may occur in compounds with aqueous solubilities which are lower than 10mg/ml (pH 1 - 7; 37°C). As PL HCl substantially exceeds this limit (500mg/ml, 25°C), it is unlikely that aqueous solubility will limit the rate of absorption.

4.3 THE IONIZATION CONSTANTS OF PYRIDOXAL HCl

4.3.1 INTRODUCTION

Most drugs, including PL HCl, are either weak acids or bases. When dissolved in water, these drugs will ionize to some extent and an equilibrium is reached between the ionized and non-ionized forms of the drug. For a weak acid (HA) this can be described as follows:

$$HA + H_2O \longrightarrow H_3O^+ + A^-$$

Assuming that water is in excess and that its concentration remains constant during the reaction, the following rate equation can be applied:

$$k_a = \frac{[H_3O^+]\ [A^-]}{[HA]}$$

The rate constant, k_a, is known as the ionization, dissociation or acidity constant.

Similarly, for a weak base:

$$k_b = \frac{[OH^-]\ [BH^+]}{[BOH]}$$

where k_b is the basicity constant. However, k_b is seldom used. Instead, the K^a values of the conjugate acids are usually employed in calculations involving ionization constants.

As previously discussed (section 4.1), the k_a values are important as they determine the extent of drug ionization at a specific pH. This in turn influences the partitioning of the drug between the lipid membranes and the aqueous fluids of the GIT. Furthermore, k_a values also provide information concerning the dependence of drug solubility on the pH of the surrounding environment (Motola and Agharkar, 1984). The relationship between the aqueous solubility of an acidic drug and pH is defined by a modified version of the Henderson-Hasselbach equation:

$$pH = pk_a + \log\frac{[Cs]}{[Ca]}$$

where Cs and Ca refer to the molar concentrations of the salt and free acid respectively.

4.3.2 THE MACROSCOPIC IONIZATION CONSTANTS OF PL HCL IN SOLUTION.

PL has three ionizable groups. In the case of the free aldehyde these are the pyridine group, the 3-hydroxy group and the 5-methyl hydroxy group. Furthermore in aqueous solution PL exists in three different molecular arrangements (Figure 4.2).

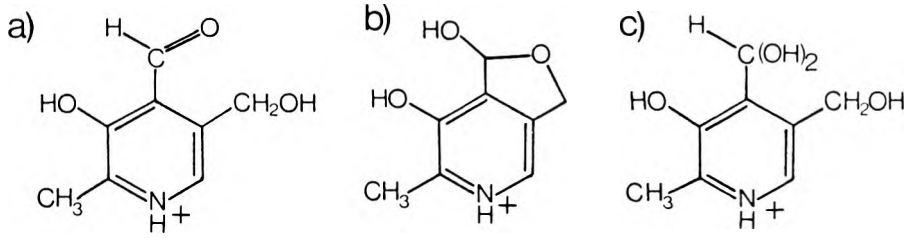


Figure 4.2 The various molecular structures of PL in aqueous solution. a) PL aldehyde; b) PL hemiacetal and c) PL hydrate.

This is due to the inherent instability of the 4-formyl group. In solution pyridine aldehydes show a marked tendency towards hydration, forming covalent hydrates of the general form $RCH(OH)_2$ (Kortnyk and Singh, 1963; Heinert and Martell, 1959). In addition, the free aldehyde group also readily interacts with the 5-hydroxy methyl group to form an internal hemiacetal (Nurnberg, 1961; Kortnyk and Singh, 1963; Morosov, 1986; and Metzler and Snell, 1955). Indeed, in acidic and neutral solutions pyridoxal exists almost exclusively as the hemiacetal (approximately 98.5% - 99.5%) with the free aldehyde and its hydrate only becoming significant at pH 10 - 11 (Harris et al, 1976; Morosov, 1986; and Kortnyk

and Singh, 1963). For our purposes the electronic state of pyridoxal need only be considered for the physiological pH range (2 - 7.4). The influence of the free aldehyde and its hydrate on the ionic equilibria is therefore negligible.

Given the three ionizable groups, PL can thus exist in five electronic states in solution (Figure 4.3).

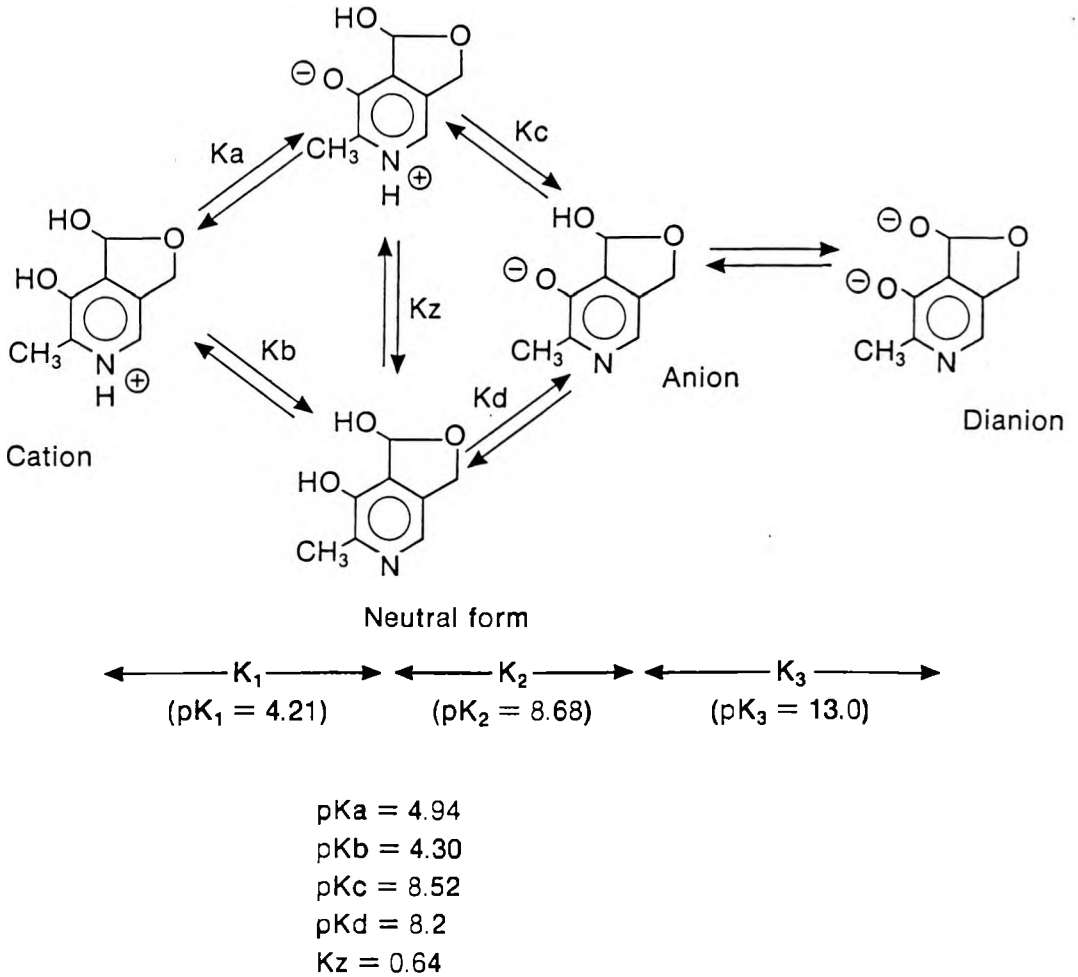


Figure 4.3 Simplified diagram of ionic equilibria of PL

The macroscopic ionization constants of PL (k_1, k_2 and k_3) have been the subject of numerous detailed studies and have been determined spectrophotometrically, spectrofluorometrically and titrimetrically (Metzler and Snell, 1955 ; Bridges et al, 1966 ; Morosov et al, 1967 and Harris et al, 1976). These ionization constants are summarized in Table 4.3 .

Table 4.3 Macroscopic ionization constants for PL

pk_1	pk_2	pk_3	*Reference*
4.20	8.66	13.0	(Metzler and Snell, 1955)
4.23	8.59		(Harris et al, 1976)
3.93	8.62		(Morosov et al, 1967)
3.9	8.9		(Bridges et al, 1966)

Due to the tautomeric relationship between the dipolar ion and the non-ionized form of PL (Figure 4.4), it is not possible to assign the pk_1 and pk_2 values to particular ionizable groups as they involve both pyridinium and 3-hydroxy groups. It is also clear that PL can never exist in the fully non-ionized form in solution, as a certain proportion of PL (dependent on the tautomeric constant, k_z) will always be present as the dipolar ion.

4.3.3 CALCULATION OF THE MICROSCOPIC IONIZATION CONSTANTS

It is apparent that the pk_1 and pk_2 can not be used to calculate the proportions of the various ionic species

present at a specific pH. For this purpose the ratio (k_z) of dipolar ion to the non-ionized form has to be known. The macroscopic ionization constants (pk_1, pk_2 and pk_3) are related to the ionization constants for the individual ionizable groups. (i.e. microscopic constants pk_a, pk_b, pk_c and pk_d) and the tautomeric ratio (k_z) as follows (Metzler and Snell, 1955) :

$$k_1 = k_a + k_b \quad \ldots\ldots\ldots A$$

$$\frac{1}{k_2} = \frac{1}{k_c} + \frac{1}{k_d} \quad \ldots\ldots\ldots B$$

$$k_z = \frac{k_a}{k_b} = \frac{k_c}{k_d} \quad \ldots\ldots\ldots C$$

Thus if pk_1, pk_2 and one other constant are known, all other constants can be determined. For pk_1 and pk_2 the values determined by Metzler and Snell (1955) were used. The value for pk_a corresponding to 4.94 was obtained from the literature (Likos and Kishore, 1986). Substituting these values into equations A, B and C the following results were obtained:

Table 4.4 Microscopic ionization constants and the tautomeric constant for PL

pk_a	=	4.94
pk_b	=	4.30
pk_c	=	8.52
pk_d	=	8.20
k_z	=	0.64

The proportions of the various ionic species present in solution at pH 5.5 and 7.4 were then calculated by applying the Henderson-Hasselbach equation (Appendix III)

Table 4.5 Proportions of ionic species present in solution at pH 5. and 7.4

Ionic Species	Cation	Neutral	Dipolar	Anionic
% at pH 5.5	4.88%	57.96%	37.1%	0.06%
% at pH 7.4	0.065%	57.89%	37.1%	4.98%

4.3.4 DISCUSSION AND CONCLUSION

In solution PL exists in various molecular arrangements, of which PL hemi-acetal is the dominant form. PL hemi-acetal itself exists as a series of ionic and tautomeric equilibria. As a result of the tautomeric relationship between the non-ionized molecule and the dipolar ion, PL is never 100% non- ionized. PL is mainly absorbed in the small intestine by passive diffusion (Buss et al, 1980). The proximal small intestine has a pH of approximately 5.5 . Under these conditions 58% of a dose of PL can be expected to be non-ionized, whereas 37% will occur as the dipolar ion. An increase in alkalinity to pH 7.4 does not significantly affect the extent of ionization. Ionization in the GIT fluids is therefore not expected to impede the absorption of PL to a great extent.

4.4 DETERMINATION OF THE PARTITION COEFFICIENT FOR PL HCl

4.4.1 BACKGROUND

If a solute is added to a system of two immiscible phases, then the solute distributes itself between the two phases so that, at equilibrium and constant temperature, the ratio of the solute concentrations in the two phases has a definite constant value which is independent of the actual amount dissolved (Glasstone and Lewis, 1976). This ratio is known as the distribution ratio or the partition coefficient (P).

For pharmaceuticals P is usually determined in water/non-polar organic systems. These can serve as model systems for drug transportation from aqueous fluids across lipid membranes during absorption and transportation to the site of action. Octanol is often chosen as the organic phase as it is weakly polar and can contain a significant amount of water in the form of hydrogen bonded complexes. To an extent this serves as a model for the complex lipid membranes which are mainly hydrophobic but also have hydrophilic regions containing bound water molecules (Smith et al, 1975). Other organic solvents that can be used include chloroform, ether and cyclohexane.

P may therefore provide an indication of a drugs

pharmacokinetic properties and biological activity. In particular, values of log P have been well correlated with specific pharmacokinetic properties and biological activity (eg. therapeutic effect, toxicity and pesticidal activity) in structure activity studies (Chou and Jurs, 1980).

Mathematically P can be represented as follows:

$$P = \frac{C_o}{C_w}$$

where P, C_o and C_w refer to the partition coefficient, the drug concentration in the organic phase and the drug concentration in the water phase respectively. It is assumed that the drug is fully non-ionized under the conditions of measurement. If the measurements are carried out at a pH such that the drug is partially ionized, then the ratio determined is the apparent partition coefficient (P_{app}). P is related to P_{app} by the following equation:

$$P_{app} = P \cdot f_u$$

where f_u is the fraction of non-ionized drug present (Pharmaceutical Codex, 1979).

It is apparent that as an indicator of drug transport across physiological barriers P is a valuable parameter for the formulation pharmacist. As no literature values of P or log P could be found for PL, it was decided to determine P_{app} for PL HCl at pH 7.4 in a water/octanol system. An estimate of the true P value was then

obtained by calculation.

4.4.2 METHODOLOGY

4.4.2.1 MATERIALS AND APPARATUS

PL HCl (Fluka AG, Buchs), 4-amino antipyrine (Fluka AG, Buchs), ammonium persulphate (BDH, Poole), sodium hydroxide (Merck, Darmstadt), hydrochloric acid (BDH, Poole) and 1-octanol (SAR Chem, Krugersdorp) were used in the study. All materials were of analytical grade quality. Double distilled water (Milli Q System, Millipore, Bedford M.A.) was used. Partitioning was carried out in a 200ml glass separating funnel with lid. A Phillips/Pye Unicam (Cambridge) PU8700 UV/VIS spectrophotometer with 1 cm quartz cells was used for absorbance measurements.

4.4.2.2 PROCEDURE

P was determined by using the "shake flask" method. Approximately 200ml 1-octanol and phosphate buffer (pH 7.4, BP 1990), were added together and pre-equilibrated overnight at 25°C. This was done to fully saturate the octanol with water and vice versa. Approximately 10mg PL HCl, accurately weighed, was dissolved in 50ml presaturated phosphate buffer. The buffer solution and 50ml presaturated 1-octanol were then mixed together in the glass separating funnel by inverting the funnel 100 times. The funnel was then allowed to stand at 25°C for 30 minutes. Aqueous phase (10 ml) was then drawn off

and adjusted to pH 2.1 with dilute HCl. The PL concentrations of 3 individual 1ml samples were then determined spetrophotometrically as previously described (section 2.2.2). The whole procedure was repeated 3 times. The P_{app} was calculated by difference, ie.

$$P_{app} = \frac{C_T - C_W}{C_W}$$

Where C_T is the total PL concentration in both the aqueous and oil phase and C_w is the PL concentration in the aqueous buffer phase. The true partition coefficient, P, was calculated using the previously described relationship between P_{app} and P (section 4.4.1):

$$P = \frac{P_{app}}{f_u}$$

4.4.3 RESULTS

A linear calibration curve (r=0.9998) was obtained over the concentration range of 15mg/l-25mg/l. Linear regression yielded the following equation:

Absorbance = 0.0125 PL concentration - 0.0083. P_{app} was determined as the average of 3 separate experiments. These are summarized in Table 4.6. A mean value of 0.44 (standard error (s_x) = 0.027) was determined for P_{app} with a confidence interval of 0.38 - 0.49 ($p > 0.1$). A value of 0.76 (log P = -0.119) was calculated for the true partition coefficient, P.

Table 4.6 Determination of P_{app}

Experiment	C_T (mg/l)	C_w (mg/l)	Mean C_w (mg/l)	$S_{\bar{x}}$ (%)	P_{app}
A	20.1	13.9 13.8 13.7	13.8	0.72	0.46
B	20.2	13.7 13.5 13.5	13.6	0.85	0.48
C	21.6	15.5 15.5 15.4	15.5	0.37	0.39

4.4.4 DISCUSSION AND CONCLUSION

Although P_{app} is a more realistic measure of partition in the body, P was determined to allow a comparison with the P values of other drugs. Compared with known log P values (Table 4.7), it can be seen that log P for PL is low (-0.119).

Table 4.7 Partition coefficients of various compounds (in octanol/water systems)

substance	logP
Ethanol	-1.00
propanol	-0.46
n-butanol	-0.18
thymol	2.98
sotalol	0.24
labetalol	3.09
propranolol	3.37
pindolol	1.75
timolol	1.98
atenolol	0.16
metoprolol	1.80
chlorpromazine	5.41
fluphenazine	5.67
thioridazine	6.51
alimemazine	5.01
perphenazine	5.28

(Martin *et al*.1983;Burgot *et al*,1990;Burgot and Burgot,1990)

However, the negative effect which the poor lipophilicity may have on absorption is counteracted by the excellent solubility of PL in the aqueous GIT fluids. Furthermore the small intestine, the principal site of absorption, provides a large surface area for absorption. Lastly, studies conducted on PN, which is also poorly soluble in non-polar solvents, show that single doses of up to 1000 mg are completely absorbed (Bauernfeind and Miller, 1978). Accordingly, bioavailability problems are unlikely to occur if PL HCl is administered in a solid dosage form.

CHAPTER 5

5 CHEMICAL STABILITY ANALYSIS OF PYRIDOXAL HCl

5.1 INTRODUCTION

The bulk of the research work on the stability of PL HCl has focused on the stability of PL HCl in aqueous solution. One of the first comparative stability studies of PN, PL and PM was carried out by Cunningham and Snell (1945). These workers found that compared to the other two vitamers PN had superior stability characteristics. In neutral and alkaline solution PL was found to be rapidly inactivated when exposed to light. When heated to 100°C in HCl and H_2SO_4, PL was found to be stable. However, decomposition occurred in alkaline conditions. A more recent study on the photosensitivity of vitamin B_6 found that when exposed to normal laboratory light, PL was stable for up to eight hours at pH 4.5. However, at pH 6 only 55% of the initial PL HCl was recovered (Ang, 1979). In a further study Morosov (1986) also observed the formation of 4-pyridoxic acid and its lactone when PL solutions were irradiated in the presence of oxygen. Saidi and Warthesen (1983) studied the influence of pH

on the kinetics of vitamin B6 degradation in solution. They also studied vitamin B_6 photolysis in dry food systems. It was found that at 40°C and 60°C PL solutions decomposed most rapidly at pH 5-6. The solutions were most stable at low pH. Decomposition was temperature dependent and appeared to follow first order kinetics. The major degradation product was 4-pyridoxic acid. PL HCl was found to be extremely light sensitive in the solid state. At 28°C, 32% RH and 400 ft-c light intensity PL HCl incorporated in a model food system had a half life of 6.4 days. This was increased to 10.3 days at 200 ft-c. Degradation followed apparent first order kinetics. Gregory and Kirk (1978) also assessed the stability of vitamin B6 in solid state model food systems. First order kinetics were observed for the degradation of PL at 37°C, 60% RH.

The stability characteristics of PL HCl in solution and the destabilizing effects of light and neutral and alkaline conditions have been well documented. The remainder of this chapter therefore focuses on the solid state stability of PL HCL. Initially the effect of moisture and temperature on the stability of solid PL HCl on its own is investigated. In the second part of this chapter the compatibility of PL HCl with several common tablet and capsule excipients is studied.

5.2 THE SOLID STATE STABILITY OF UNFORMULATED PYRIDOXAL HCl

5.2.1 CAUSES AND MECHANISMS OF DEGRADATION IN SOLID STATE PHARMACEUTICAL SYSTEMS

Pharmaceutical systems are generally characterized by a low level of entropy, this will often favour degradation. The underlying cause of the instability of most pharmaceutical systems is therefore of thermodynamic origin (Rhodes, 1984). Instability in drugs can be caused by physical, chemical or biological mechanisms. Instability in pharmaceutical systems can have the following consequences:

i) loss of active drug; ii) loss of vehicle; iii) loss of content uniformity; iv) reduction of bioavailability due to decreased solubility; v) impairment of pharmaceutical elegance, eg. discolouration and development of distasteful flavours; vi) production of potentially toxic materials (Rhodes, 1984). For tablet systems biological degradation is unlikely to be of major importance. The high temperatures and pressures reached during compression tend to inactivate harmful microbes (Fassihi and Parker, 1987). Selected aspects of physical stability have been discussed in chapter 3.

In contrast to chemical decomposition in solution, the chemical mechanisms of solid state decomposition are complex and often only partially understood (Connors et al, 1986a). Various reaction orders are possible,

depending on the mechanism involved. Degradation may occur via various competing or sequential reactions. The complexity of solid state decomposition reactions is also illustrated by the sigmoidal reaction rate curves observed by many workers (Figure 5.1). The curve can be divided into three stages: an induction period, an acceleration period and a decay period.

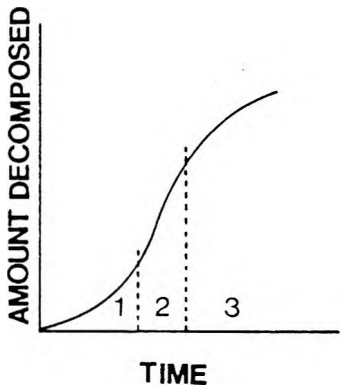


Figure 5.1 Typical sigmoidal decomposition curve a) induction period; b) acceleration period; c) decay period

This type of decomposition curve can be explained by the Prout-Tompkins model of solid state decomposition (Carstensen, 1974). A refined model was later put forward by Jacobs and Tompkins (Ng, 1975). The model assumes that decomposition is governed by the formation and growth of reaction nuclei or "hot spots" on the crystal surfaces. These centres of reactivity occur in disordered regions of the crystal, eg. crystal defect structures such as dislocations and stacking faults. Once decomposition products are formed these introduce additional strains on the crystal structure and promote the formation of new reaction nuclei. Such kinetics have

been reported for the solid state decomposition of p-aminosalicylic acid (Kornblum and Sciarrone, 1964), acetyl salicylic acid and derivatives (Hasegawa et al, 1975) and glutathione (Aruga et al, 1978).

Similar curves have also been observed for multi-phasic reactions. These reactions initially occur mainly in the solid state, however due to the formation of liquid decomposition product layers or the absorption of excessive amounts of water and deliquescence of the drug, later stages of the degradation process occur mainly in solution and follow first order solution kinetics. Such kinetics, also termed Bawn kinetics, have been observed for aminobenzoic acid which decomposes to liquid aniline and carbon dioxide (Carstensen, 1974). Sigmoidal decomposition curves have also been derived for propantheline bromide (a deliquescent drug) exposed to moist atmospheres (Yoshioka and Uchiyama, 1986; Carstensen et al, 1987).

An alternative mechanism for solid state drug degradation which has been suggested involves the concentrated suspension model. According to this model degradation primarily occurs in layers of drug saturated solutions surrounding the individual drug particles. The source of the solvent is critical. This may be a melt from a drug or excipient with low melting point, residual solvent from a wet granulation, adsorbed atmospheric moisture or a solvate which has released its

solvent (Connors et al, 1986b). The most common situation is probably that involving adsorbed atmospheric moisture. In contrast to the reaction nuclei model, moisture must be present in excess. Where only trace amounts of moisture are present a mechanism involving the penetration of water into energetic hot spots (regions of disorder), thus increasing molecular mobility and reactivity is more likely (Ahlneck and Zografi, 1989). The kinetics governing drug degradation in saturated aqueous layers have been elucidated by Leeson and Mattocks (1958). Leeson-Mattocks kinetics assume that decomposition can be totally accounted for by decomposition of the dissolved drug. The concentration of drug in solution is determined by the solubility of the drug and while solid drug remains available for dissolution, the model follows pseudo zero order kinetics analogous to those observed for drug degradation in suspension. The apparent zero order degradation rate constant being the product of the intrinsic first order degradation rate constant and the solubility of the drug in the solvent (Carstensen, 1988).

The complexity of solid state degradation often leads to limitations in solid state stability studies of new substances as not all the variables controlling solid state stability are known. In addition, degradation mechanisms and kinetics of the drug in its final dosage form usually differ from those of the drug in its pure

form. However, at the preformulation stage determination of exact mechanisms and kinetics (although useful if available) is not essential. The priority at this stage is the determination and knowledge of the effect of the major environmental factors affecting drug stability (Wadke et al, 1989; Monkhouse, 1984). Omnipresent environmental factors to which the drug will be exposed during its lifetime include heat, light, oxygen and water. In particular, moisture is thought to be the single most important destabilizing factor (Monkhouse, 1984). Moisture has been found to be the major destabilizing factor for sulpyrine (Yoshioka et al, 1979), doxylamine succinate (Van Tonder et al, 1991), moexipril (Gu et al, 1990), glutathion (Aruga et al, 1978), p-aminosalicylic acid (Kornblum and Sciarrone, 1964) and thiamine HCl (Carstensen et al, 1969). Associated factors such as the pH of the microenvironment may further modify the effects of any of the previously mentioned factors (Monkhouse, 1984; Rhodes, 1984).

The destabilizing effect of light on PL HCl has been well documented (section 5.1). The primary aim of this study was therefore to establish the effect of temperature and atmospheric moisture on the stability of solid PL HCl. The effects of acidic and basic additives and an antioxidant were also investigated. Degradation kinetics were not established.

5.2.2 METHODOLOGY

5.2.2.1 MATERIALS AND EQUIPMENT

Analytical grade PL HCl (Fluka Ag, Buchs), sodium bicarbonate (BDH, Poole) and ascorbic acid (Merck, Darmstadt) were used. Samples were stored in thermostatically controlled ovens (Memmert, Schwabach) during isothermal stress testing. Silica gel HF 60 chromatoplates (E Merck, Darmstadt) were used to detect decompositiion products by TLC. A Beckman System Gold liquid chromatograph (San Ramon, CA) equipped with a programmable solvent delivery module (model 126) and a diode array detector (model 168) was used to analyse the samples by HPLC.

5.2.2.2 PROCEDURE

Samples of PL HCl (approximately 10.0 mg) were accurately weighed into open glass vials. The samples were then placed into controlled humidity environments (0% RH, 11% RH, 54% RH) and stored at 25°C and 55°C respectively. The controlled humidity environments were created as previously described (section 3.3.4.2). A 0% RH was created by equilibrating phosphorous pentoxide in a glass dessicator. Samples containing sodium bicarbonate and ascorbic acid (approximately 10.0 mg) in addition to PL HCl were also prepared. After 14 days storage time the samples were analysed for the remaining

amount of PL HCl by HPLC and TLC as described previously (sections 2.3.2 and 2.4.2). To avoid sampling errors the whole sample was used in each case. Furthermore each sample was analysed three times.

5.2.3 RESULTS AND DISCUSSION

Table 5.1 shows that both high levels of temperature and relative humidity cause substantial degradation of PL HCl (for more detailed data refer to Appendix IVa). The combination of both high temperature and RH was found to be the most detrimental storage condition. The destabilizing effects of temperature and humidity are significant at the 90% level of confidence as shown by the factorial design experiment in section 5.2.2.2 .

Table 5.1 The solid state stability of PL HCl

Sample	Storage Condition	% Remaining
1	0% RH, 25°C	100.3
2	11% RH, 25°C	98.4
3	54% RH, 25°C	98.3
4	0% RH, 55°C	97.6
5	11% RH, 55°C	96.7
6	54% RH, 55°C	95.4
7*1	54% RH, 55°C	69.2
8*2	54% RH, 55°C	89.3

*1 PL HCl + sodium bicarbonate
*2 PL HCl + ascorbic acid

The results clearly show the destabilizing effect of sodium bicarbonate when compared to ascorbic acid. This correlates well with previously reported stability

studies which indicated that PL is relatively stable in acidic solutions while unstable in alkaline solutions (Cunningham and Snell, 1945; Saidi and Warthesen, 1983). It therefore seems that the alkaline sodium bicarbonate significantly alters the pH of the microenvironment, thereby accelerating chemical decomposition. It must however be noted that ascorbic acid also destabilizes PL HCl. The acidifying and antioxidant effects therefore seem to be overshadowed by a negative interaction between PL HCl and ascorbic acid.

The decomposition of PL HCl can be rationalized with the aid of the concentrated suspension model. PL HCl is highly water soluble and adsorbs large amounts of moisture at elevated humidity levels (section 3.3.4). Considerable surface dissolution can therefore be expected, resulting in the formation of a weakly acidic layer of solution. In strong acidic solution (pH<2) PL exists almost exclusively in the hemiacetal form. However, in weaker acidic solutions PL hemiacetal is partially hydrolysed (up to 1.5%) and a small amount of free aldehyde is formed (see section 4.3.2). Free aldehydes are much more reactive and oxidize readily when compared to their respective hemiacetals (Morrison and Boyd, 1983). This would also account for the increased decomposition observed under alkaline conditions, where a considerably greater percentage of free aldehyde is formed.

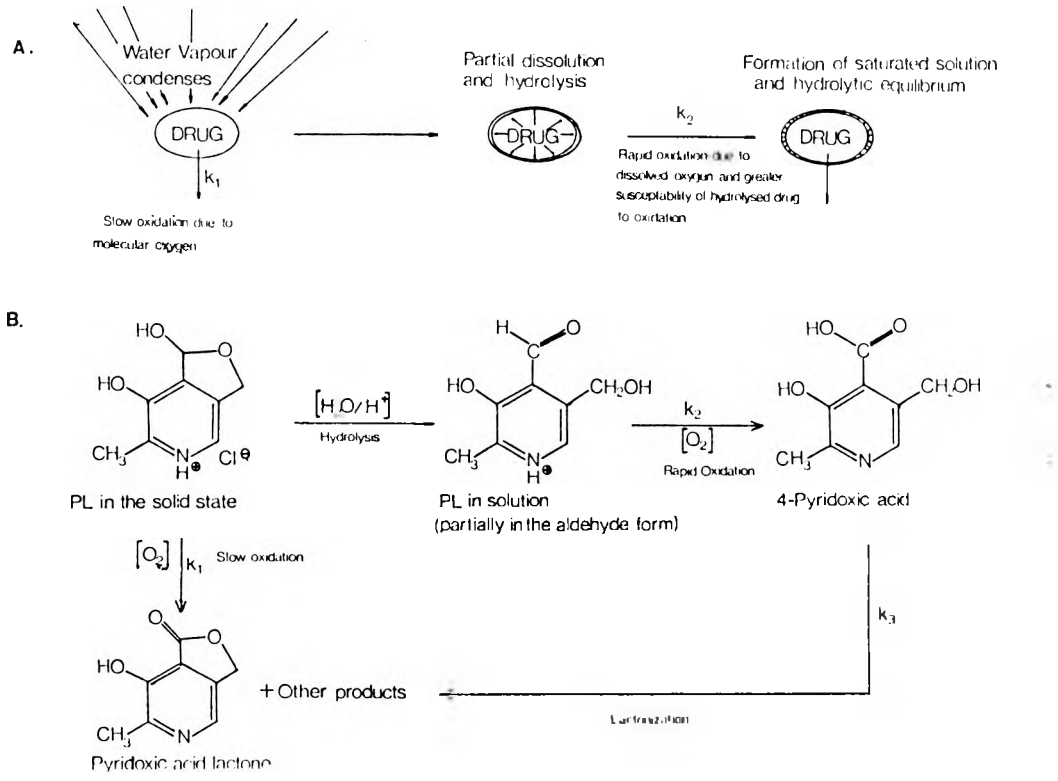


Figure 5.2 Proposed scheme for the solid state degradation of PL HCl

Solid state decomposition of PL HCl in the presence of atmospheric moisture is therefore likely to involve the partial hydrolysis of the PL hemiacetal and rapid oxidation of the resultant free aldehyde (Figure 5.2). As previously mentioned (section 2.4.3) decomposition products could not be identified by HPLC. However, TLC provides a slightly clearer picture of PL HCl decomposition. Samples exposed to high temperature and relative humidity show three distinct decomposition products (Figure 5.3) at R_f 0, 0.17 and 0.48 in addition to a trail of other decomposition products.

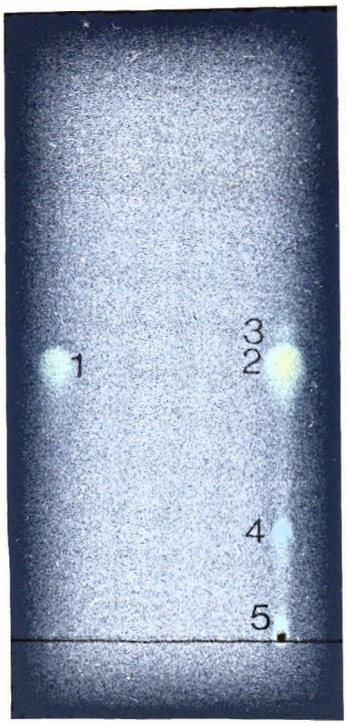


Figure 5.3 Photograph of a chromatoplate viewed at 256 nm showing PL HCl decomposition products. (1) PL HCl standard, (2) PL HCl sample exposed to 55°C, 75% RH for 2 weeks, (3) decomposition product R_f 0.48, (4) decomposition product R_f 0.17 and (5) decomposition products at R_f 0.

The pathway of decomposition is likely to involve 4-pyridoxic acid. This would then be partially converted to 4-pyridoxic acid lactone in the prevailing acidic

conditions. Both these substances have been identified as major decomposition products of PL (Saidi and Warthesen, 1983; Morosov, 1986). Simultaneously a limited amount of PL hemiacetal may also be broken down to 4-pyridoxic acid lactone by direct oxidation.

Degradation caused purely by heat in the absence of moisture (see sample 4, Table 5.1) can be attributed mainly to elemental oxygen and the increased reaction rates at higher temperatures. However even a relatively low increase in the humidity of the environment eg. 11% RH (sample 5, Table 5.1) results in an increase in decomposition. It appears that moisture causes decomposition of solid PL HCl by hydrolysis of the hemiacetal and by increasing the plasticity of the solid state system, thereby decreasing the energies of activation as suggested by Ahlneck and Zografi (1991).

5.3 EXCIPIENT COMPATIBILITY TESTING

5.3.1 INTRODUCTION

The formulation of a stable and effective dosage form requires careful selection of the excipients used to facilitate administration, promote consistent release and bioavailability of the drug, and protect it from degradation (Wells, 1988d). Although often regarded as "inert", pharmaceutical excipients can in fact readily interact with drugs (Monkhouse, 1984). This may occur in three different ways. The drug and excipient may react

with each other. Alternatively the excipient may alter the microscopic pH of the immediate drug environment. Lastly, hygroscopic excipients may increase the amount of water available for decomposition in the system (Ahlneck and Alderborn, 1988; Connors et al, 1986a). The detection of solid-solid interactions between drugs and excipients during preformulation is therefore of great importance.

The four techniques which can be employed for drug-excipient compatibility screening are isothermal stress testing utilizing chromatographic techniques (HPLC or TLC), thermal analysis using DTA (differential thermal analysis) or DSC, diffuse reflectance spectroscopy and assessment of discoloration after exposure to light stress (van Dooren, 1983; Carstens, 1974). However, from a review of the literature it appears that isothermal stress testing and thermal analysis are the methods of choice.

5.3.1.1 ISOTHERMAL STRESS TESTING: GENERAL BACKGROUND

In its simplest form isothermal stress testing involves the exposure of binary drug-excipient mixtures to elevated temperatures and moisture levels to accelerate drug ageing and drug-excipient interactions. Unstressed samples are used as controls. After a specific storage time (eg 2 weeks at 55°C) the samples can then be analysed by visual comparison as well as by chromatography (Carstensen et al, 1964; Carstensen,

1974). The main disadvantages of this method from a preformulation point of view are that it is relatively time consuming, requires the use and availability of a stability indicating method (is therefore labour intensive) and consumes relatively large amounts of sample. The extent to which these studies can be accelerated by increasing temperature stress is limited because the risk of inducing physicochemical changes that do not occur at ambient temperature increases (Monkhouse, 1984). Many workers therefore use temperatures not exceeding 50-60°C for isothermal excipient compatibility studies which require a minimum incubation period of 2-3 weeks (Gu et al, 1990; Nyqvist, 1986; van Dooren, 1983; Boatman and Johnson, 1981; Carstensen, 1974). However, the technique can be refined by employing fractional factorial designs which allow a reduction in the number of individual experiments and the amount of drug consumed (Motola and Agharkar, 1982; Ahlneck and Waltersson, 1986).

5.3.1.2 APPLICATION OF FACTORIAL DESIGNS TO EXCIPIENT COMPATIBILITY TESTING

Factorial designs are experimental designs that simultaneously evaluate the effects of various factors (eg temperature, moisture and presence of certain excipients) at several levels (eg high and low temperature). Depending on the choice of design, the effects caused by interactions of the various factors can also be evaluated. Statistical techniques are then

applied to test the significance of the calculated effects. Various factorial designs exist and choice of design depends largely on the particular application ie. the number of factors and levels, the type of factors and nature of the experiment, the maximum feasible number of individual experiments and whether interaction effects must be detected. A particularly useful factorial design for the evaluation of up to four components in a mixture experiment is the simplex lattice design (Huisman et al, 1984). Other well known factorial designs which are suitable for experiments involving up to 3 factors are the central composite, Box-Behnken, and full factorial designs (Mulholland, 1988). Ahlneck and Waltersson (1986) have reported the use of a 2^3 full factorial design to evaluate the compatibility of aspirin with 3 excipients. However, in preformulation excipient compatibility screening usually 10 or more excipients are evaluated. With such an increase in factors, the required number of experiments rapidly escalates, rendering these designs impractical and inconvenient. For such experimental situations Plackett-Burman designs are particularly suitable (Motola and Agharkar, 1984; Connors et al, 1986c)

The method of Plackett and Burman (1946) provides saturated fractional factorial designs that allow multifactorial experiments to be carried out with a minimum of trials without compromising accuracy (the individual combinations or mixtures tested within the

multifactorial experiment are referred to as trials). The advantages of this method in preformulation design are therefore the following: i) Efficiency is increased. This is vital when considering the limited amount of time and drug available at the preformulation stage. ii) Each variable is screened at two levels. iii) Each variable is screened in the presence of all other variables under investigation. iv) A study of the effect of environmental factors (eg temperature, humidity) can easily be incorporated in the experiment. v) Studies are carried out in a well designed experiment, the results of which can be supported with statistical significance (Motola and Agharkar, 1984).

The method also has some limitations: only main effects can be determined, ie. interactions between two or more variables can not be detected. However, these are generally not of major interest (Ahlneck and Waltersson, 1986; Box et al, 1978). Furthermore the smallest design that can be derived is an eight trial experiment.

5.3.1.3 EXCIPIENT COMPATIBILITY SCREENING USING DSC

An alternative preformulation compatibility screening method makes use of DSC. This technique does not require long term storage of mixtures and subsequent chromatographic analysis and uses only a few milligrams of drug per individual experiment. Thermograms of the individual excipients and of the drug are obtained and compared to the thermograms of drug-excipient mixtures.

Guidelines for the interpretation of the thermograms have been given by Smith (1982) and van Dooren (1983). Generally the thermogram of the mixture should broadly reflect the same thermal features as the superimposed thermograms of the drug and excipient. Any gross differences such as the appearance of new peaks, the disappearance of peaks or massive shifts in peak temperature or size are taken as a sign of interaction.

The value of employing thermal analysis as a predictive preformulation compatibility screen was demonstrated by Jacobson and Reier (1969). These workers found good correlation between data generated by DTA and accelerated ageing tests for mixtures of stearic acid with sodium oxacillin monohydrate, sodium dicloxacillin monohydrate, potassium penicillin G and ampicillin trihydrate. A similar approach was used to assess the compatibility of four excipients with cephradine (Jacobson and Gibbs, 1973). Again the results obtained by DTA and conventional isothermal stress studies correlated well. However, apart from these studies many of the published DSC studies have not provided direct verification of the predictions they make. Only one other detailed study comparing the accuracy of chemical compatibility predictions made by DSC screening to those made by isothermal stress testing could be found. In this study the DSC technique was judged to be unreliable (Chrzanowski et al, 1986). However, these authors applied a modified DSC technique which relies on

measuring differences in the heats of melting (EL-Shattaway et al, 1981). The study therefore does not really evaluate the more generally accepted, qualitative approach to DSC compatibility screening, which relies on detecting large aberrations in the thermograms of the drug-excipient mixtures. However, other authors have also stated that results obtained by DSC screening are often not conclusive (Gordon et al, 1984; van Dooren, 1983; Smith, 1982). Amongst the factors which have contributed to this observation are the high temperatures and the rapid heating rates which may give rise to degradation reactions that do not take place at room temperature. Furthermore the lack of moisture stress in DSC experiments may result in moisture mediated interactions being over looked. In addition, in various cases interpretation of thermograms becomes difficult. These include coincident thermal features (eg coincident melting), premature decomposition of one component (the interaction may then involve the degradation products), premature sublimation or vapourization or the formation of a mixed melting point entity (physical interactions do not necessarily affect chemical stability) (van Dooren, 1983; Smith, 1982). These difficulties have led van Dooren (1983) to propose an experimental design in which DSC screening is supplemented by isothermal stress tests. However, this largely negates the advantages of DSC screening.

Nonetheless DSC screening continues to be widely

regarded as a major method for preformulation compatibility testing (Wells, 1988d, Wadke et al, 1989) and compatibility studies utilizing DSC techniques continue to be published (Botha and Lotter, 1990a; Botha and Lotter, 1990b; Botha et al, 1987; Botha et al, 1986).

In this study the compatibility of PL HCl with various excipients is investigated by three different methods. A comparison is made between the DSC compatibility screening technique, conventional "one factor at a time" isothermal stress testing utilizing TLC and isothermal stress testing based on a Plackett-Burman factorial design. In contrast to Chrzanowski et al (1986) the more generally accepted qualitative approach to the interpretation of DSC thermograms is used. It should also be noted that although Plackett-Burman designs have been recommended for preformulation compatibility studies by several authors (Connors et al, 1986c; Motola and Agharkar, 1984), no recent applications of this approach to compatibility testing or comments on its validity could be found in the primary literature.

5.3.2 METHODOLOGY

5.3.2.1 MATERIALS AND EQUIPMENT

PL HCl (Fluka, Buchs) was used throughout. The following excipients were used: Eudragit RSPM (Rohm Pharma, Weiterstadt), ethyl cellulose 10 cps (Fluka, Buchs),

methyl cellulose 25 cps (Fluka, Buchs), Avicel pH 101 (FMC Corp, Philadelphia P.A.), anhydrous lactose (Sheffield Products, Norwhich N.Y.), Ludipress (BASF, Ludwigshafen), starch BP (Holpro Analytics, Johannesburg), mannitol (Riedel-de-Haen, Seelze), Aerosil 380 (Degussa, Frankfurt), magnesium stearate (Fluka, Buchs), stearic acid (Fluka, Buchs). DSC screening was performed in standard aluminium pans on a TA3000 system (Mettler, Greifensee) equipped with a DSC 20 cell. For isothermal stress testing samples were stored in a thermostatically controlled oven (Memmert, Schwabach). Silica gel HF 60 chromatoplates (E Merck, Darmstadt) were used to detect decomposition in the conventional isothermal stress study. The Beckman System Gold liquid chromatograph (San Ramon, C.A) as previously described (section 5.2.2) was used to obtain the quantitative results required for the Plackett-Burman experimental design.

5.3.2.2 DSC SCREENING

PL HCl-excipient mixtures containing lubricants or glidants (ie magnesium stearate, stearic acid and Aerosil) were prepared in a 5:1 ratio. All other PL HCl-excipient mixtures were prepared in a 1:5 ratio. Samples of the mixtures and the individual substances (3-8 mg) were weighed into standard aluminium pans. Before sealing the lids were perforated to allow even distribution of nitrogen (see section 2.6.3). The samples were then heated at a rate of 7°C/min over a

temperature range of 30-220°C in an atmosphere of flowing nitrogen. The thermograms were interpreted according to the guidelines of van Dooren (1983) and Smith (1982). A classification of no incompatibility, possible incompatibility and probable incompatibility as proposed by Smith (1982) was used.

5.3.2.3 CONVENTIONAL ISOTHERMAL STRESS STUDY

The ratios for the PL HCl-excipient mixtures used in DSC screening were also used in this study. Each excipient mixture (approximately 60 mg) was prepared by blending the components in glass vials with the aid of a vortexing device. The glass vials were then stored at 40°C, 75% RH (see section 3.3.4.2.2) for 21 days. Duplicate mixtures stored at 25°C, 11% RH were used as controls. A moderate temperature of 40°C was chosen to limit inherent PL HCl decomposition. The solid state stability studies of PL HCl on its own showed that PL HCl undergoes significant decomposition at 55°C. It is common to add water (up to 5%) directly to the powder mixtures and then to seal the vials before storage at elevated temperature (Carstensen, 1974; van Dooren 1983). In this study the powder mixtures were exposed to moisture stress by storage at constant humidity. This takes into account factors such as powder mixing, particle size, surface area and moisture penetration (Ahlneck and Lundgren, 1985) and therefore represents a more realistic experimental situation. After three weeks the TLC procedure described in section 2.3 was used to

test the various samples for decomposition products. The samples were also assessed visually.

5.3.2.4 ISOTHERMAL STRESS STUDY BASED ON A PLACKETT-BURMAN EXPERIMENTAL DESIGN

5.3.2.4.1 DERIVATION OF THE DESIGN

It was previously mentioned that the smallest Plackett-Burman design requires 8 trials. A general requirement for any two level Plackett-Burman design is that the sum of the trials (N) must be a multiple of 4, ie. designs can be derived for 8, 12, 16, 20, 24.....100 trials. For this study a 24 trial design was derived by following the procedure outlined by Plackett and Burman (1946). The procedure is described in detail in Appendix IVb. The design is shown in Table 5.2. In addition to the various excipients, the effect of temperature and relative humidity are also evaluated. The "+"and "-" symbols indicate whether a variable was present at a low or high level in a specific trial. The high and low levels for the variables are summarized in Table 5.3.

Variables N to W are dummy variables and are used to calculate the experimental error inherent in the design (Plackett and Burman, 1946). In turn this is used to calculate the minimum significant variable effect (E_{ms}). The calculation of E_{ms} and of the main effects is explained in Appendix IVb. Any main effect having an absolute value greater than E_{ms} is considered to be

statistically significant on the stability of the drug.

Table 5.2 Plackett-Burman design for study of 13 variables in 24 trials

	Variables													Dummy variables									
Trial	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**	**S**	**T**	**U**	**V**	**W**	%REC
1	+	+	+	+	+	−	+	−	+	+	−	−	+	+	−	−	+	−	+	−	−	−	−	Y1
2	−	+	+	+	+	+	−	+	−	+	+	−	−	+	+	−	−	+	−	+	−	−	−	Y2
3	−	−	+	+	+	+	+	−	+	−	+	+	−	−	+	+	−	−	+	−	+	−	−	Y3
4	−	−	−	+	+	+	+	+	−	+	−	+	+	−	−	+	+	−	−	+	−	+	−	Y4
5	−	−	−	−	+	+	+	+	+	−	+	−	+	+	−	−	+	+	−	−	+	−	+	Y5
6	+	−	−	−	−	+	+	+	+	+	−	+	−	+	+	−	−	+	+	−	−	+	−	Y6
7	−	+	−	−	−	−	+	+	+	+	+	−	+	−	+	+	−	−	+	+	−	−	+	Y7
8	+	−	+	−	−	−	−	+	+	+	+	+	−	+	−	+	+	−	−	+	+	−	−	Y8
9	−	+	−	+	−	−	−	−	+	+	+	+	+	−	+	−	+	+	−	−	+	+	−	Y9
10	−	−	+	−	+	−	−	−	−	+	+	+	+	+	−	+	−	+	+	−	−	+	+	Y10
11	+	−	−	+	−	+	−	−	−	−	+	+	+	+	+	−	+	−	+	+	−	−	+	Y11
12	+	+	−	−	+	−	+	−	−	−	−	+	+	+	+	+	−	+	−	+	+	−	−	Y12
13	−	+	+	−	−	+	−	+	−	−	−	−	+	+	+	+	+	−	+	−	+	+	−	Y13
14	−	−	+	+	−	−	+	−	+	−	−	−	−	+	+	+	+	+	−	+	−	+	+	Y14
15	+	−	−	+	+	−	−	+	−	+	−	−	−	−	+	+	+	+	+	−	+	−	+	Y15
16	+	+	−	−	+	+	−	−	+	−	+	−	−	−	−	+	+	+	+	+	−	+	−	Y16
17	−	+	+	−	−	+	+	−	−	+	−	+	−	−	−	−	+	+	+	+	+	−	+	Y17
18	+	−	+	+	−	−	+	+	−	−	+	−	+	−	−	−	−	+	+	+	+	+	−	Y18
19	−	+	−	+	+	−	−	+	+	−	−	+	−	+	−	−	−	−	+	+	+	+	+	Y19
20	+	−	+	−	+	+	−	−	+	+	−	−	+	−	+	−	−	−	−	+	+	+	+	Y20
21	+	+	−	+	−	+	+	−	−	+	+	−	−	+	−	+	−	−	−	−	+	+	+	Y21
22	+	+	+	−	+	−	+	+	−	−	+	+	−	−	+	−	+	−	−	−	−	+	+	Y22
23	+	+	+	+	−	+	−	+	+	−	−	+	+	−	−	+	−	+	−	−	−	−	+	Y23
24	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	Y24
Sum +																								
Sum −																								
Effect																								

Key: **A** stearic acid; **B** magnesium stearate; **C** Aerosil 380; **D** lactose; **E** Ludipress; **F** corn starch; **G** Avicel pH101; **H** methylcellulose; **I** ethylcellulose; **J** Eudragit RSPM; **K** mannitol; **L** relative humidity; **M** temperature; **N** to **W** dummy variables; **%REC** percentage of drug recovered;

Table 5.3 High (+) and low (-) levels for the variables used in the experiment

Variable	"+"	"-"
A: Stearic acid	2 mg	0 mg
B: Magnesium stearate	2 mg	0 mg
C: Aerosil 380	2 mg	0 mg
D: Lactose	50 mg	0 mg
E: Ludipress	50 mg	0 mg
F: Corn starch	50 mg	0 mg
G: Avicel pH101	50 mg	0 mg
H: Methylcellulose	50 mg	0 mg
I: Ethylcellulose	50 mg	0 mg
J: Eudragit RSPM	50 mg	0 mg
K: Mannitol	50 mg	0 mg
L: Relative humidity	75%	11%
M: Temperature	55°C	25°C

The use of 10 dummy variables in this study thus yields results with 10 degrees of freedom. Various authors have suggested that Plackett-Burman designs can be used to evaluate up to N-1 factors with N trials (eg up to 11 variables in 12 trials) (Connors et al, 1986; Nyqvist, 1986). This is possible, but such designs do not incorporate any dummy variables. The significance of the measured main effects can therefore not be determined correctly. This greatly reduces the value of the experiment. The scientist therefore has to weigh up the need to obtain statistically sound and meaningful information against the additional work required to analyse a greater number of samples and the additional amount of drug consumed. For 13 variables it is good practice to have at least 7 degrees of freedom (ie. 20 trials). In this case a 24 trial experiment (10 degrees of freedom) was selected.

5.3.2.4.2 PREPARATION AND STORAGE OF POWDER MIXTURES

Twenty four powder mixtures were prepared by weighing the required amounts of excipient into glass vials in accordance with the 24 trial factorial design (Table 5.2). Each mixture corresponds to one trial and only those excipients indicated as being present at a high level (+) for that particular trial were included. The amounts for each of the excipients have previously been given in Table 5.3. To each excipient combination approximately 10 mg PL HCl (accurately weighed) was added. The powders were then blended in the glass vials with the aid of a vortexing device. Each mixture was stored in a dessicator containing the relevant salt solution (see section 3.3.4.2.2). The dessicators were then placed into ovens providing the required temperatures (Table 5.3). A storage time of 14 days was allowed.

5.3.2.4.3 ANALYTICAL PROCEDURE

After 14 days storage the powder mixtures were analysed to determine the remaining amounts of PL HCl. For this purpose the previously described HPLC method was used (section 2.4.2). Each sample was analysed three times.

5.3.3 RESULTS AND DISCUSSION

5.3.3.1 DSC SCREENING

The DSC traces of PL HCl, the excipients and their corresponding mixtures are shown in Figures 5.4-5.14

The thermograms of the PL HCl mixtures containing Eudragit RSPM, Avicel pH101, methylcellulose, ethylcellulose, Aerosil 380 and stearic acid (figures 5.4-5.9) exhibit all the thermal features of the individual components. In general the broad melting endotherm of PL HCl (ca. 178°C) appears even broader and shallower and occurs at a slightly lower temperature. However, this may be attributed to the mixing process, which lowers the purity of each component in the mixture, thus resulting in slightly broader and lower melting points (Smith, 1982). Slight variations in peak shape and melting point may also be caused by varying sample geometries (Giron-Forest, 1984). The characteristic broad endotherms between 40°C and 140°C observed for the celluloses can be attributed to the loss of residual water from these polymers (Botha and Lotter, 1990a). No incompatibility could therefore be detected for Eudragit RSPM, Avicel, methylcellulose, ethylcellulose, Aerosil, and stearic acid.

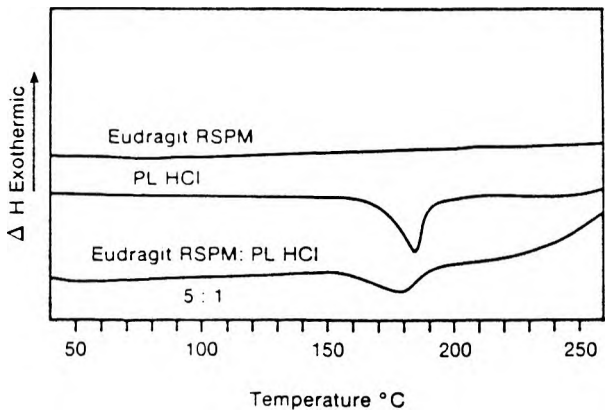


Figure 5.4 DSC thermograms of Eudragit RSPM, PL HCl and a PL HCl:Eudragit RSPM (1:5) mixture.

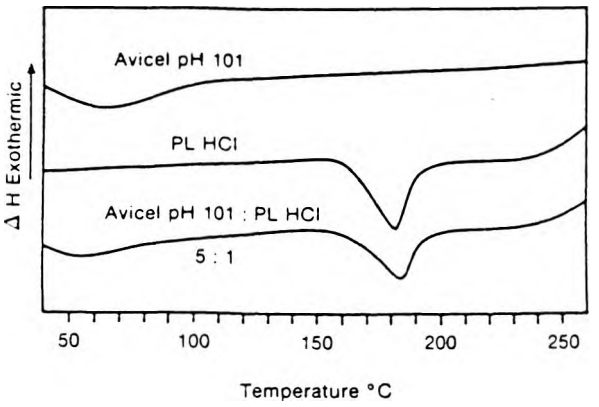


Figure 5.5 DSC thermograms of Avicel pH101, PL HCl and a PL HCl:Avicel pH101 (1:5) mixture.

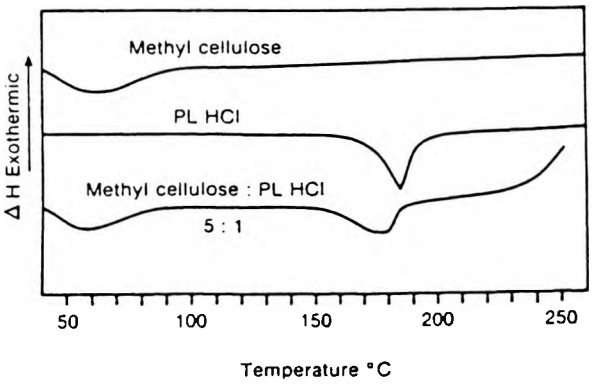


Figure 5.6 DSC thermograms of methylcellulose, PL HCl and a PL HCl:methylcellulose (1:5) mixture.

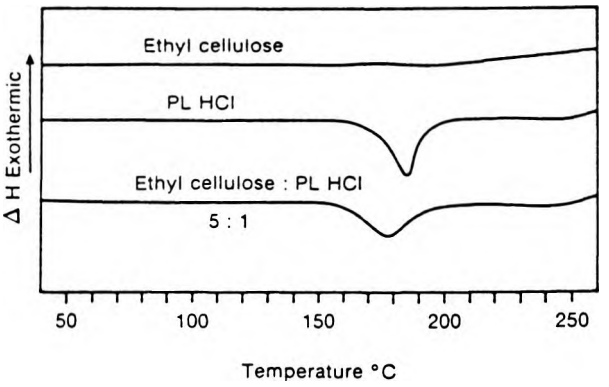


Figure 5.7 DSC thermograms of ethylcellulose, PL HCl and a PL HCl:ethylcellulose (1:5) mixture.

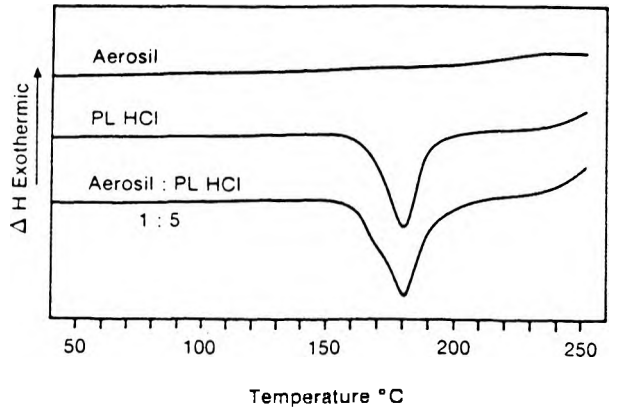


Figure 5.8 DSC thermograms of Aerosil 380, PL HCl and a PL HCl:Aerosil 380 (5:1) mixture.

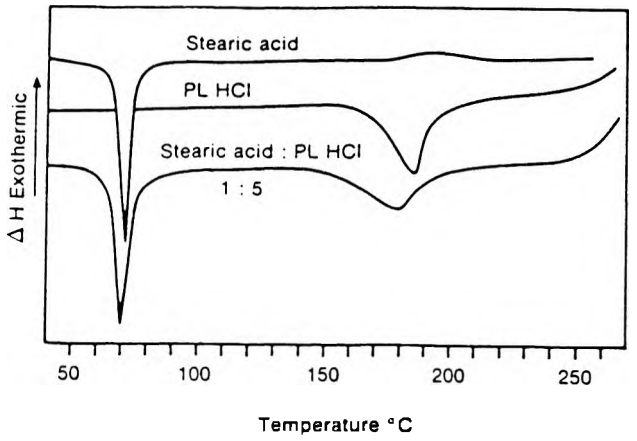


Figure 5.9 DSC thermograms of stearic acid, PL HCl and a PL HCl:stearic acid (5:1) mixture.

The DSC trace of magnesium stearate (figure 5.10) shows two shallow, broad endotherms in the region of 50°C to 110°C. The lower endothermic peak corresponds to the melting point of magnesium stearate. The DSC trace of the PL HCl : magnesium stearate (5:1) mixture also shows several small endothermic peaks in this region. The peaks could not be directly correlated with those of pure magnesium stearate. However, the PL HCl peak remains largely unchanged. Incompatibilities involving magnesium stearate are frequently observed by DSC. However, these are often of a physical nature and due to

the low concentrations of magnesium stearate in the final dosage form and are often irrelevant (Gordon et al, 1984; Wells, 1988d).

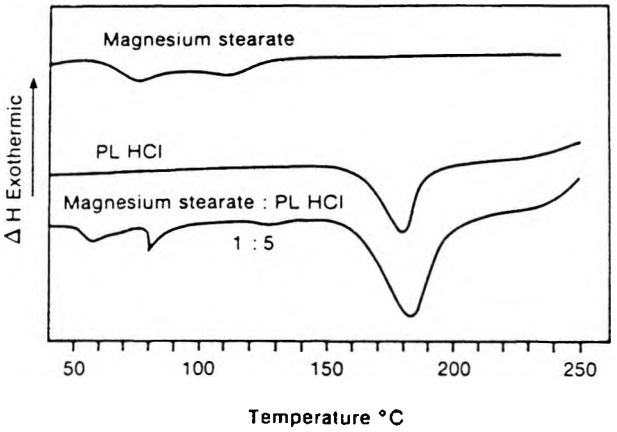


Figure 5.10 DSC thermograms for magnesium stearate, PL HCL and a PL HCl:magnesium stearate mixture.

Magnesium stearate is widely regarded as the lubricant of first choice and any implications for the stability of the formulation must be weighed up against the difficulties of finding a suitable substitute. Judging from the appearance of the DSC trace a significant solid-solid interaction is unlikely, but nonetheless possible.

Starch also exhibits a broad and shallow endotherm between 40°C and 120 °C (Figure 5.11). This may again be attributed to the loss of bound water. The DSC trace of the PL HCl : starch (1:5) mixture shows several interesting features. The PL HCl endotherm is broadened and peak temperature has been shifted upwards (185°C). An additional broad and shallow endotherm occurs at 220°C. This may indicate an incompatibility. However, the additional endotherm occurs after the melting point of

PL HCl and is very close to the pyrrolytic decomposition temperature for PL HCl.

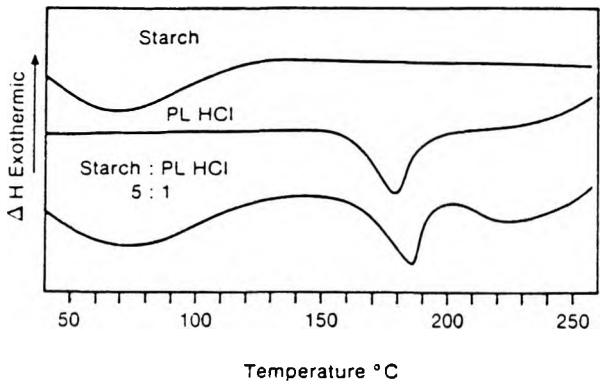


Figure 5.11 Thermograms for starch, PL HCl and a PL HCl:starch mixture

The observed interaction therefore involves a liquid phase and may also involve pyrrolytic degradation products. The evidence for a solid-solid incompatibility is therefore not conclusive. Nonetheless starch should be avoided until more information on its interaction with PL HCl can be obtained.

Mannitol exhibits a prominent sharp melting endotherm with a peak temperature of 168°C. This coincides with the onset of the PL HCl melting endotherm (Figure 5.12). The DSC trace of the PL HCl: mannitol mixture shows two large partially overlapping endothermic peaks. The peak temperatures occur at 145°C and 158°C respectively. Such large shifts in peak temperature are indicative of a strong interaction between the two components. Furthermore the second endotherm which probably corresponds to PL HCl is much larger and narrower when

compared to pure PL HCl. Mannitol and PL HCl are probably incompatible.

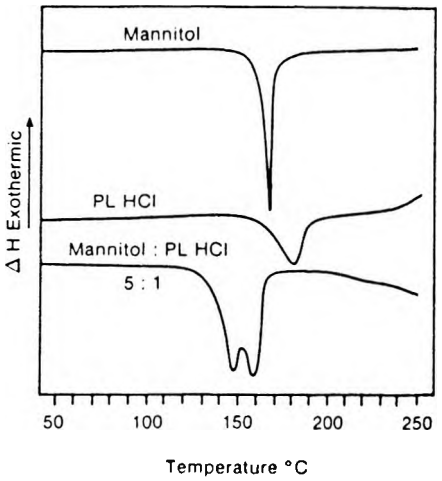


Figure 5.12 DSC thermograms of mannitol, PL HCl and a PL HCl:mannitol mixture (1:5).

An examination of Figures 5.13 and 5.14 reveals that lactose and Ludipress (which comprises lactose granulated with povidone and crospovidone) may also be incompatible with PL HCl.

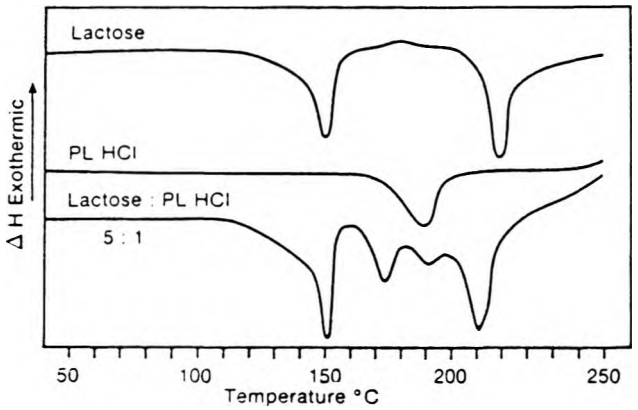


Figure 5.13 DSC thermograms for lactose, PL HCl and a PL HCl:lactose mixture.

The thermograms of the PL HCl:lactose and PL HCl:Ludipress (1:5) mixtures show an additional prominent endotherm in the region of 160°C to 170 °C. In both cases the small exothermic peak occurring between

the characteristic exotherms is obliterated.

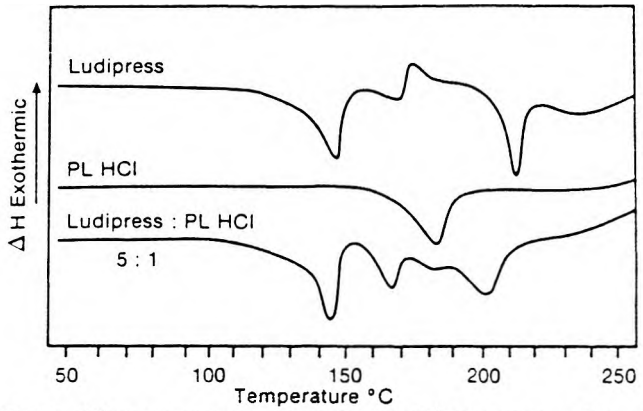


Figure 5.14 DSC thermograms of Ludipress, PL HCl and a mixture of PL HCl:Ludipress (1:5)

The results of the DSC compatibility screening can be summarized as follows:

No or unlikely incompatibility: Aerosil 380, Avicel pH101, ethylcellulose, Eudragit RSPM, magnesium stearate and stearic acid.

Possible incompatibility: corn starch.

Probable incompatibility: anhydrous lactose, Ludipress and mannitol.

It should be emphasized that the observed aberrations in the thermograms may be caused by physical rather than chemical interaction. Chemical incompatibility remains to be proven by stability testing.

5.3.3.2 CONVENTIIONAL ISOTHERMAL STRESS TESTS

The PL HCl-excipient mixtures stored at 40°C, 75% RH showed the following changes after 21 days:

Table 5.4 Visual assessment of PL HCl-excipient mixtures after 21 days storage at 40°C, 75% RH.

Excipient mixture	Observation
PL HCl:mannitol (1:5)	Extensive browning powder is lumpy and moist
PL HCL:Avicel pH101 (1:5)	No change,white and fine powder.
PL HCl:Eudragit RSPM (1:5)	White, powder caking has occured
PL HCl:starch (1:5)	White to slightly yellow
PL HCl:lactose (1:5)	Yellow
PL HCL:Ludipress (1:5)	Yellow, appears moist
PL HCl:methylcellulose (1:5)	Slightly yellow
PL HCl:ethylcellulose (1:5)	Slightly yellow
PL HCl:Aerosil 380 (5:1)	Yellow
PL HCl:magnesium stearate (5:1)	Slightly yellow
PL HCl:stearic acid (5:1)	Slightly yellow
PL HCl	Slightly yellow

In all cases the controls (stored at 25°C, 11% RH) showed no obvious change in appearance.

The results of the TLC analysis are shown in Figure 5.15. Significant levels of decomposition products could be observed near the origin for PL HCl:mannitol, PL

HCl:lactose and PL HCl:Ludipress samples. A smaller zone of decomposition products is also barely visible at the origin of the PL HCl:starch sample. It appears that the greatest decomposition was caused by mannitol. No differentiation could be made between the other excipients.

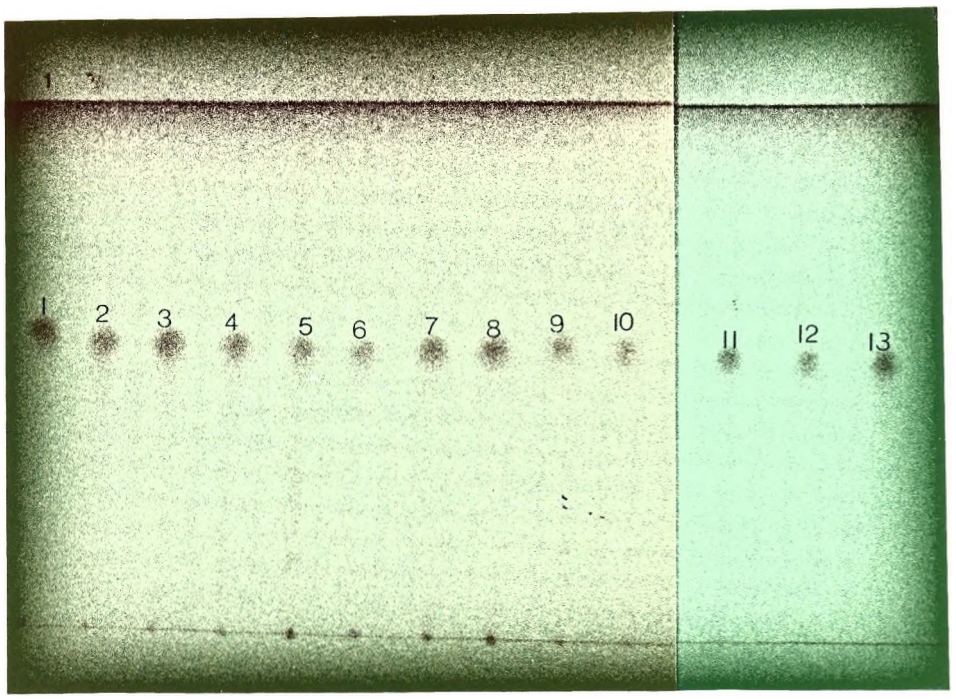


Figure 5.15 Chromatograms of the various PL HCL-excipient mixtures. Key: 1 PL HCl; 2 PL HCl:Avicel; 3 PL HCl:ethylcellulose; 4 methylcellulose; 5 PL HCl: mannitol; 6 PL HCl:starch; 7 PL HCl:Ludipress; 8 PL HCl:lactose; 9 PL HCl:Eudragit RSPM; 10 PL HCl: Aerosil 380; 11 PL HCl; 12 PL HCl:Magnesium stearate; 13 PL HCl:stearic acid;

TLC analysis of the controls showed that no significant breakdown occurred when stored at 25°C, 11% RH

for 3 weeks.

PL HCl has been identified as a moisture sensitive drug, undergoing both physical and chemical changes at elevated levels of atmospheric moisture (sections 3.4 and 5.1). Furthermore many drug-excipient interactions are moisture mediated. Mannitol was therefore selected as it is non hygroscopic and is often combined with moisture sensitive drugs (Peck et al, 1989; Handbook of Pharmaceutical Excipients, 1986a). In accelerated excipient compatibility tests on vitamins A, B1 and C it was found that mannitol was the excipient of choice due to its low moisture content (Wai et al, 1962). Furthermore mannitol can be used for direct compression. This would be advantageous for PL HCl as it is also known to react with aliphatic alcohols (Nurnberg, 1961)(section 2.3.1) often used as non aqueous granulating fluids. The reaction of the PL hemiacetal ring with alcohols may also be the cause of the strong interaction with mannitol which is a hexahydric alcohol molecule. At the higher moisture and temperature levels hydrolysis of the PL hemiacetal may also occur (section 5.1) resulting in the formation of the free aldehyde form of PL. The free carbonyl is highly reactive and may also react with the various mannitol hydroxy groups.

Lactose is a particularly useful diluent and filler as it is inexpensive, not excessively hygroscopic, has good compaction properties and is compatible with most drugs

(Peck et al, 1989; Handbook of Pharmaceutical Excipients, 1986b). However, lactose and Ludipress (which is lactose based) appear to be of limited value for any formulation containing PL HCl as they chemically interact with the latter. Lactose is known to undergo a non enzymatic browning reaction (generally known as the Maillard reaction) with amines (Duvall et al, 1964). The first step in the Maillard reaction involves a condensation reaction between an amino group and the carbonyl group of a reducing sugar. The end products of the complex reaction sequence are the characteristic brown nitrogenous polymers known as melanoidins (Labuza and Saltmarch, 1981). The reaction is promoted by alkaline and humid conditions (Duvall, 1964). In their compatibility study of various amphetamines with lactose Duvall et al (1965) found that the Maillard reaction occurred only in primary amine derivatives. It appears that this concept has since been generally accepted. As PL HCl has a tertiary amine group the Maillard reaction would therefore be ruled out as a possible mechanism for the interaction with lactose. However, in food systems vitamin B_6 has been reported to take part in Maillard type reactions (Klaui, 1979). Furthermore in their review of the Maillard reaction Labuza and Saltmarch (1981) do not rule out reactions involving secondary and tertiary amines, they merely state that primary amines react most rapidly. The possibility of an interaction between the protonated pyridine nitrogen of PL HCl and free lactose carbonyl groups should therefore be

considered. Alternatively, the reaction may again involve the unstable, reactive hemiacetal ring of PL and the free hydroxy groups of lactose. Such a reaction may also occur in the presence of starch.

It can be concluded that at elevated temperature and humidity mannitol, lactose, Ludipress and to a lesser extent corn starch adversely affect the stability of PL HCl . The results therefore correlate well with the probable incompatibilities detected by DSC screening. However, both methods are crude from a quantitative point of view and no conclusion could be reached on the extent of these negative interactions except that the reaction with mannitol appeared to be the most severe and starch seemed to react the least.

5.3.3.3 ISOTHERMAL STRESS TESTING USING A PLACKETT-BURMAN EXPERIMENTAL DESIGN

The full design showing % recovery of PL HCl per trial is shown in Table 5.5 . % Recovery of PL HCl was calculated from the mean of three HPLC determinations. To avoid sampling errors the whole sample was used. The relevant data can be found in Appendix IVc.

From the % recoveries the average effect was calculated for each variable as outlined in Appendix IVb. Using the dummy variables the standard deviation of the variable effect (s_{VE}) was calculated to be 0.968. From this the minimum significant variable effects (E_{ms}) were

calculated at the 90% and 60% levels of confidence (see to Appendix IVb). Table 5.6 compares the average effects (main effects) of the variables with the E_{ms} at various levels of confidence.

Table 5.5 Plackett-Burman design showing % PL HCl recovered per trial and the average effect of each variable

	Variable												Dummy variable										
Trial	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**	**S**	**T**	**U**	**V**	**W**	%REC
1	+	+	+	+	+	-	+	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-	-	95.15
2	-	+	+	+	+	+	-	+	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-	93.91
3	-	-	+	+	+	+	+	-	+	-	+	+	-	-	+	+	-	-	+	-	+	-	-	97.14
4	-	-	-	+	+	+	+	+	-	+	-	+	+	-	-	+	+	-	-	+	-	+	-	80.32
5	-	-	-	-	+	+	+	+	+	-	+	-	+	+	-	-	+	+	-	-	+	-	+	92.33
6	+	-	-	-	-	+	+	+	+	+	-	+	-	+	+	-	-	+	+	-	-	+	-	97.03
7	-	+	-	-	-	-	+	+	+	+	+	-	+	-	+	+	-	-	+	+	-	-	+	93.60
8	+	-	+	-	-	-	-	+	+	+	+	+	-	+	-	+	+	-	-	+	+	-	-	97.17
9	-	+	-	+	-	-	-	-	+	+	+	+	+	-	+	-	+	+	-	-	+	+	-	78.10
10	-	-	+	-	+	-	-	-	-	+	+	+	+	+	-	+	-	+	+	-	-	+	+	76.14
11	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+	-	+	-	+	+	-	-	+	68.28
12	+	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+	-	+	-	+	+	-	-	86.29
13	-	+	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+	-	+	-	+	+	-	100.6
14	-	-	+	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+	-	+	-	+	+	99.67
15	+	-	-	+	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+	-	+	-	+	98.06
16	+	+	-	-	+	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+	-	+	-	88.67
17	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+	-	+	96.33
18	+	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+	-	96.33
19	-	+	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+	100.3
20	+	-	+	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-	-	+	+	+	+	93.07
21	+	+	-	+	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-	-	+	+	+	96.47
22	+	+	+	-	+	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-	-	+	+	95.10
23	+	+	+	+	-	+	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-	-	+	87.18
24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	98.40

Key: **A** stearic acid; **B** magnesium stearate; **C** Aerosil 380; **D** lactose; **E** Ludipress; **F** corn starch; **G** Avicel pH101; **H** methylcellulose; **I** ethylcellulose; **J** Eudragit RSPM; **K** mannitol; **L** relative humidity; **M** temperature; **N** to **W** dummy variables; **%REC** percentage of PL HCl recovered;

Table 5.6 Summary of the compatibility test using a Plackett-Burman factorial design.

Variable	Average Effect	Significant 2P<0.1 (Ems=1.754)	Significant 2P<0.4 (Ems=0.851)
Stearic acid	-0.338	No	No
magnesium stearate	0.743	No	No
Aerosil 380	2.168	Yes	Yes
Lactose	-0.993	No	Yes
Ludipress	-0.529	No	No
Starch	-0.957	No	Yes
Avicel pH101	1.909	Yes	Yes
Methylcellulose	2.429	Yes	Yes
Ethylcellulose	1.382	No	Yes
Eudragit RSPM	-0.625	No	No
Mannitol	-2.468	Yes	Yes
Relative Humidity	-3.620	Yes	Yes
Temperature	-4.618	Yes	Yes

As would have been expected, relative humidity and temperature were found to be significant destabilizing factors. Their effects were larger than those of the excipients. Mannitol was the only excipient whose destabilizing effect was significant at the 90% level of confidence, thus confirming the findings of the previous two studies. Corn starch and lactose were also found to have relatively large destabilizing effects significant at the 60% level of confidence. Excipient compatibility studies are of a predictive and preventative nature. An excipient which is deemed to have a significant destabilizing effect at the 60% level of confidence represents a considerable risk to the stability of the final dosage form. Good stability characteristics are imperative. If possible such excipients should thus be avoided.

It is notable that according to this study starch has a relatively large destabilizing effect. This correlates with the possible interaction observed by DSC screening (section 5.3.3.1). However, upon accelerated ageing only a small amount of decomposition could be observed by TLC (section 5.3.3.2). An important difference between the design of conventional isothermal stress experiments and the Plackett-Burman design is that in the latter each excipient is evaluated 12 times, in each case in the presence of different levels and combinations of all the other factors. The conventional isothermal stress study is based on the analysis of a single sample. The potential for experimental error is therefore greater. Geometric factors due to inadequate sample mixing may play a role. Furthermore, the destabilizing effect of starch could be promoted by the presence of other excipients. Such secondary effects can not be determined by the Plackett-Burman design.

A slight discrepancy was also observed for Ludipress. DSC screening and TLC analysis after accelerated ageing of a PL HCl:Ludipress (1:5) mixture provided strong evidence of a chemical interaction. Using the Plackett-Burman design a relatively small destabilizing effect was calculated. Apart from the above mentioned possible reasons for the apparent differences between the studies, the following should be considered: Ludipress consists of lactose granulated with povidone and crospovidone. These two excipients could excercise a

protective effect on PL HCl. Crospovidone in particular is able to sorb large quantities of water, this may reduce the amount of water available for degradation (see discussion on celluloses below). Furthermore, the TLC analysis employed in the previous study is not quantitative and relies on visual estimation of the size and intensity of degradation zones and tails. This may account for the inability to correctly distinguish between the destabilizing effects of lactose, Ludipress and starch.

A relatively low destabilizing effect was also observed for Eudragit RSPM. The effect may largely be due to the alkalinity of the excipient (Handbook of Pharmaceutical Excipients, 1986c). Alkaline conditions have been identified as detrimental to the stability of PL HCl (section 5.1). Eudragit RSPM is unlikely to be used in the large proportions that were used in this study (Eudragit:PL HCl, 5:1) as it is used mainly for film coating and for inclusion in delayed release matrices. Therefore it is unlikely that Eudragit RSPM will cause stability problems.

The only other destabilizing effect observed was for stearic acid. However, the effect is small. Furthermore it is possible that the melting of stearic acid had an effect on the interaction with PL HCl. Various melting points have been reported for stearic acid: 59-64°C, 51-62.5°C and 63-69.2°C (Handbook of Pharmaceutical

Excipients, 1986d). A certain amount of melting can therefore be expected at 55°C. As the interaction does not exclusively occur in the solid state, no conclusions should be made. However,again it is unlikely that stearic acid would cause stability problems.

A common trend in this study are the relatively large positive effects observed for the cellulose derivatives ie. Avicel, methylcellulose and ethylcellulose. The effects of methylcellulose and Avicel are significant at the 90% level of confidence. These excipients (especially methylcellulose and Avicel) are relatively hygroscopic. Generally such excipients would be used with caution in conjunction with moisture sensitive drugs. However, it is known that ethylcellulose stabilizes ascorbic acid (Schmidt, 1982). Furthermore, in stability studies on acetyl salicylic acid in various cellulose mixtures it was demonstrated that a proportion of the water appears unavailable for degradation. It appears that this water becomes strongly bound to the free hydroxy groups in the amorphous regions of the cellulose (Ahlneck and Alderborn, 1988). It is possible that the celluloses tested in this experiment exercise a protective effect on PL HCl in a similar way. A considerable proportion of the moisture may therefore be strongly bound and unavailable for degradation reactions. The existence of various thermodynamic states of water in biopolymers such as cellulose has been extensively documented (Zografi, 1988; Zografi and

Kontny, 1986; Zografi et al, 1984;),although the rigidity of this concept has recently been challenged (Ahlneck and Zografi, 1990) (see also section 3.3.4.1). Nonetheless it appears that in this case a considerable proportion of the sorbed moisture is bound by the celluloses thus greatly reducing the molecular mobility in the system. Consequently the potential for interaction between moisture and PL HCl is reduced. The lower stabilizing effect of ethylcellulose may be due to the lower hygroscopicity.

The strong stabilizing effect (significant at the 90% level of confidence) observed for Aerosil 380 (colloidal silicon dioxide) may also be attributed to its moisture scavenging role. Colloidal silicone dioxide can sorb large quantities of water (up to 18% at 78% RH) and can be used as a drying agent for hygroscopic materials (Handbook of Pharmaceutical Excipients, 1986e). Furthermore, Aerosil is acidic and may also exert a stabilizing effect by maintaining an acidic microenvironment. PL has been shown to be very stable at low pH (see section 5.1).

The moderate stabilizing effect of magnesium stearate (significant at the 50% level of confidence) was unexpected. A possible explanation could be that magnesium stearate provides a hydrophobic coat for the PL HCl particles. Water soluble vitamins stabilized by coating with hydrophobic fatty acids and mono- and

diglycerides are commercially available (Schmidt, 1982).

Based on this study it can be recommended that mannitol, lactose and Ludipress and starch should not be combined with PL HCl.The destabilizing effect of Ludipress appears to be relatively low. However Ludipress is best avoided as it is used in relatively large quantities as a filler/diluent and its main component, lactose, has a substantial destabilizing effect on PL HCl.

Stearic acid and Eudragit RSPM have relatively small negative effects and are unlikely to be used in large quantities in solid dosage forms. They could therefore be used if no alternatives were available. However there is no apparent need for stearic acid as magnesium stearate, which has superior lubricity, appears to be compatible with PL HCl.

Methylcellulose, ethylcellulose, Avicel pH101 (microcrystalline cellulose), Aerosil 380 (colloidal silicone dioxide) and magnesium stearate are the excipients of first choice. They do not show any incompatibility with PL HCl and possibly have stabilizing effects.

The finding that the celluloses and colloidal silicone dioxide exert significant stabilizing effects on PL HCl deserves further investigation. This may provide new insights on simple ways to stabilize moisture sensitive

drugs.

5.3.4 CONCLUSION

The ability to assign a statistical significance to the observations made and to identify both destabilizing and stabilizing factors makes the isothermal stress study based on the Plackett-Burman experimental design the compatibility testing method of choice. DSC screening and conventional isothermal stress testing coupled with TLC analysis also proved to be reliable indicators of major chemical incompatibilities. Their shortcomings are their inability to give good estimates of the extent and significance of the destabilizing effects and their inability to detect stabilizing effects. An additional disadvantage of DSC screening is the uncertainty of whether the observed interaction is chemically significant or not. Additional isothermal stress studies are usually required to confirm the results. This largely negates the advantages of DSC screening.

It appears that in excipient compatibility testing the emphasis is often mainly on the detection of incompatibilities. However, the benefits of also investigating the stabilizing effects of various excipients are obvious, especially when formulating inherently unstable drugs such as PL HCl. This aspect may become increasingly important with the increasing use of drugs of biological origin such as peptide drugs developed by rDNA technology. From this point of view

isothermal stress testing based on a Plackett-Burman experimental design provides a superior method of excipient compatibility testing. DSC screening is suitable as a complementary method to isothermal stress studies and to gauge excipient compatibility during preliminary stages when no stability indicating method is available. In terms of excipient- drug interaction studies the most useful area of application for DSC would appear to be the study of pharmaceutically important physical interactions such as the formation of a solid dispersion of a poorly soluble drug in water soluble carrier materials (Ford and Francomb, 1985; Grant and Abougela, 1982).

An "ideal" preformulation excipient compatibility test which is rapid, consumes only small quantities of drug and provides accurate, reliable, quantitative data remains yet to be developed.

CHAPTER SIX

6. CONCLUSION

The aim of this study was to generate the necessary data required for the design of a stable, safe and effective solid dosage form containing PL HCl. The research work was therefore concentrated in four areas: analytical preformulation, elucidation of solid state properties of PL HCl, solubility analysis and stability analysis.

As PL HCl has not been previously used in pharmaceutical products, only a few suitable analytical methods were available for this study. Consequently new UV spectrophotometric and HPLC methods suitable for the routine analysis of PL HCl in pharmaceutical samples and dosage forms were developed. Furthermore, it is believed that this dissertation contains the first thermal analytical profile of PL HCl.

A notable feature of the thermal investigations is the observation of polymorphic transitions in PL HCl. In subsequent moisture sorption studies it was demonstrated that polymorphic transitions could also occur when PL

HCl was exposed to elevated levels of atmospheric moisture. This is significant for unstable and hygroscopic drugs such as PL HCl. Utilisation of the thermodynamically stable, less hygroscopic crystal form may provide a method whereby the destabilizing effect of moisture can be reduced.

The chemical stability studies further highlighted the critical importance of atmospheric moisture. By employing a Plackett-Burman experimental design it could be shown that moisture has a highly significant (90% level of confidence) destabilizing effect on PL HCl. The Plackett-Burman study also allowed the quantitative determination of the stabilizing and destabilizing effects of a series of common tableting excipients. Compared to excipient compatibility screens employing thermal analysis or conventional "one factor at a time" isothermal stress testing the factorial design was found to have two significant advantages: (1) Not only negative destabilizing interactions can be detected: positive stabilizing interactions can also be monitored. This aspect appears to have been previously disregarded by other workers. (2) The quantitative nature of the experiment allows excipients to be accurately graded in a hierarchy according to their stabilizing effects.

Due to the pragmatic and task orientated nature of preformulation studies, the results are usually presented in the form of a brief preformulation data

sheet rather than in the form of an extended research report. The most important findings are therefore summarized below.

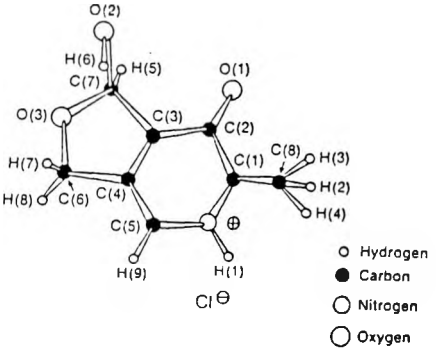
6.1 PREFORMULATION DATA SHEET FOR PYRIDOXAL HYDROCHLORIDE

1. Name: Pyridoxal hydrochloride

2. Source and batch No.: Fluka Ag, Buchs, Switzerland
   B/N 2912451089

3. Description: White, odourless, bitter/sour tasting powder

4. Structural formula:



5. Empirical formula: $C_8H_9NO_3$. HCl

6. Molecular weight: 203.63

7. Solid phase compatibility and stability:

   Compatible with: Most celluloses, Aerosil, magnesium stearate

   Can be used if necessary: stearic acid, Eudragit RSPM

   Incompatible with: Mannitol, starch, lactose, Ludipress

   Possible degradation routes: Hydrolysis

   Degradation products: Rf 0.00,0.17,0.48

   Method of detection: Thin layer chromatography

Adsorbent : Silica Gel 60HF
Solvents : Chloroform:Methanol (75:25)
Chamber : Unlined and unsaturated
Detection : Short wave UV light

Effect of environmental factors: Extremely photolabile, thermolabile and sensitive to moisture, stable in acidic environment.

8. Solid state properties:

Melting point: 165°C-167°C with decomposition if not hermetically enclosed.
DSC thermogram indicates polymorphic transition occurring with melting.

Particle characteristics:

Particle shape and topography:
A mixture of large platy and tabular forms and smaller anhedral prismatic forms. Smooth surfaces with occasional fissures.

Particle size:
Mean 15.8 µm
Range 3 - 60 µm

Hygroscopicity: (mg water/g after 14 days, 25° C)

at 11% RH = 1.93 , 54% RH = 3.73 ,

75% RH = 9.30 , 80% RH = 15.78

PL HCl is highly hygroscopic and deliquescent. Undergoes polymorphic transition to stable less hygroscopic form at RH 57% - 68%.

9. Solution data:

Solubility at 25°C (g/100ml)
Water : 50
Ethanol : 1.7
Propanol: sparingly soluble
Acetone : slightly soluble

pH of 10% aqueous solution: 2

Partition coefficient: log P = -0.119

pk_a : pk_1 4.2; pk_2 8.66; pk_3 13.0

10. Recommendation: PL HCl is highly water soluble and no bioavailability problems are anticipated.

Formulation of direct compression tablets or

capsules is feasible. Stable solid dosage forms can be used provided suitable excipients are used (see 7). Care should be taken during manufacture and storage to limit exposure to moisture and light.

It is apparent that PL HCl presents the pharmaceutical formulator with many challenging problems. This can be largely attributed to the chemical structure of PL HCl. The presence of the internal hemiacetal and its corresponding aldehyde functional group make PL HCl an inherently unstable and reactive drug molecule. A further contributing factor is the hygroscopicity of the compound. However, it is believed that the results of this study show that stable pharmaceutical products containing PL HCl can be formulated, thus providing the patient with benefits not available from current vitamin B_6 products.

It is trusted that this work will contribute to the development and design of new solid dosage forms containing PL HCl.

APPENDIX I

Ia) CALIBRATION DATA FOR UV SPECTROPHOTOMETRIC ASSAY

A calibration curve was constructed by measuring the absorbance of the 5 standard solutions given in the table below:

Standard Solution	Concentration (mg/l)	Absorbance
1	10.7	0.292
2	16.1	0.434
3	21.4	0.594
4	26.8	0.732
5	32.1	0.888

Least squares linear regression provides the following equation:

Absorbance = 0.0278 Concentration + 0.0085

The regression coefficient, r, is 0.9997.

Ib) DETECTION LIMIT FOR UV SPECTROPHOTOMETRIC ASSAY

The detection limit (DL) was determined from the measurement of 3 reagent blanks.

Reagent blank	Absorbance
1	0.031
2	0.029
3	0.025
X	0.0283
S	0.0031

DL was calculated at the 99% level of confidence as outlined by Cavenaghi *et al* (1978):

$$DL = \frac{3 \quad s}{\text{slope of calibration curve}}$$

ie $DL = \frac{3 \times 0.0031}{0.0278} = 0.33$ mg/l

Ic) SOLVENT STRENGTH PARAMETERS (E_o) FOR MOBILE PHASE SOLVENTS IN HPLC AND TLC

Elutropic series	Solvent strength (E_o)	Solvent
1	0	n-Pentane
2	0.01	Hexane
3	0.04	Cyclohexane
4	0.18	Carbon tetrachloride
5	0.29	2-Chloropropane
6	0.38	Diethylether
7	0.40	Chloroform
8	0.42	Dichloromethane
9	0.45	Tetrahydrofuran
10	0.49	1,2-Dichloroethane
11	0.56	Acetone
12	0.56	1,4-Dioxane
13	0.58	Ethyl acetate
14	0.62	Dimethyl sulfoxide
15	0.63	Diethylamine
16	0.65	Acetonitrile
17	0.82	2-Propanol
18	0.88	Ethanol
19	0.95	Methanol
20	high	Acetic acid
21	high	Water

Solvent strengths (E_o's) are additive. To calculate E_o for a combined solvent consisting of two or more components, eg Methanol: Acetonitrile (80:20), the proportional E_o's are calculated and added.

i.e. $E_o = 0.8 \times 0.95 + 0.2 \times 0.65$

$E_o = 0.89$

Id) CALIBRATION DATA FOR HPLC ASSAY

Seven standard solutions of PL were prepared. Each of these was injected three times to determine the average ratio of the PL and PN peaks.

PL Conc (mg/ml)	Ratio of Peak Areas 1	2	3	Mean Area X	Standard Error Sx
0.000100	0.000	0.000	0.000	0	0
0.000510	0.034995	0.033112	0.033882	0.03400	0.0005
0.001030	0.072815	0.075117	0.075598	0.07451	0.0008
0.005150	0.437893	0.440926	0.433386	0.4374	0.0022
0.010300	0.852225	0.877363	0.882008	0.8705	0.0093
0.051500	4.766038	4.892288	4.914985	4.8578	0.0803
0.103000	9.31375	9.475126	9.517522	9.435	0.0621

Regression analysis was performed using the System Gold (Beckman, San Ramon, CA) soft ware package. This yielded the following equation:

$$y = 0.010824\ x + 0.000241$$

where x is the ratio of the PL and PN peak areas and y refers to the PL concentration. A regression coefficient of r = 0.9995 was determined.

APPENDIX II

IIa) DERIVATION OF MOISTURE SORPTION ISOTHERM

At each humidity level three samples of PL HCl were analysed for their water content. Details are given below:

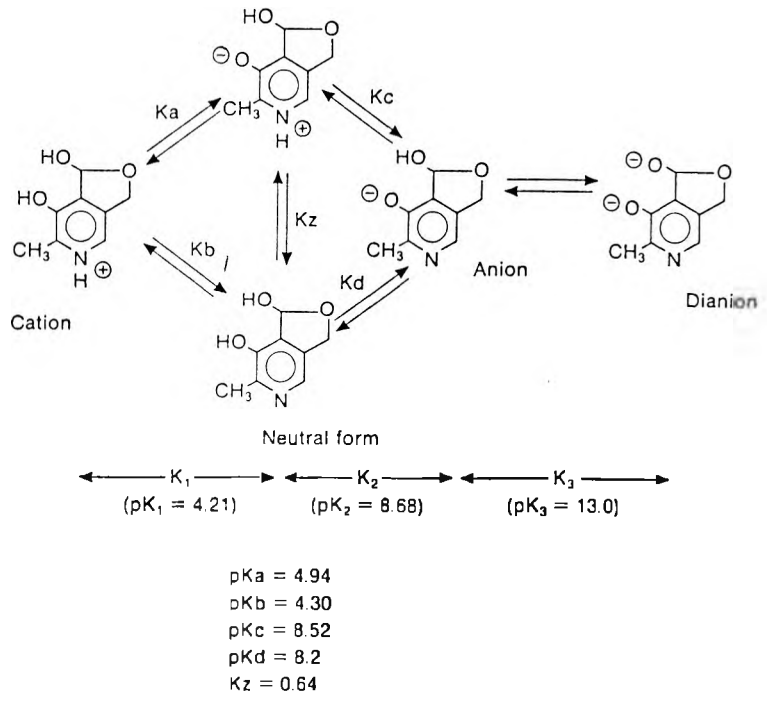
Sample	% MC	RH	Mean	Standard Error	95% CI
1	0.1777	11.3%	0.1927	0.0123	0.1395-
2	0.2172				0.2459
3	0.1831				
4	0.2773	21.6%	0.2716	0.0039	0.2547-
5	0.2641				0.2884
6	0.2734				
7	0.2983	32.8%	0.2889	0.0065	0.2611-
8	0.2921				0.3169
9	0.2765				
10	0.3769	54.4%	0.3736	0.0056	0.3495-
11	0.3812				0.3976
12	0.3627				
13	0.3842	57.5%	0.3872	0.0058	0.3622-
14	0.3829				0.4121
15	0.3983				
16	0.2341	68.6%	0.2447	0.0053	0.2218-
17	0.2508				0.2675
18	0.2491				
19	0.9715	75.3%	0.9296	0.0269	0.8137-
20	0.8793				1.0455
21	0.9388				
22	1.5473	80.9%	1.578	0.0249	1.4704-
23	1.5589				1.6852
24	1.627				
25	52.033	97.3%	51.721	0.3630	50.159-
26	50.997				53.282
27	52.132				

Key:
%MC = Percentage moisture content
RH = Relative humidity
95% CI = 95% confidence interval

APPENDIX III

IIIa) CALCULATION OF THE IONIC SPECIES OF PL IN SOLUTION

The following ionic equilibria exist for PL in solution (See section 4.3.2).



From the above diagram it can be seen that the ionization constant for the dianion is 13.0. At pH 5.5 and 7.4 its influence can therefore be assumed to be negligible.

CALCULATION OF THE PROPORTION OF PL IONS AT pH 5

The proportion of cation present can be calculated from the Henderson-Hasselbach equation:

$$pH = pk_1 + \log \frac{[Remainder]}{[Cation]}$$

where: $pk_1 = 4.21$

[Cation] = concentration of PL cation

[Remainder] = the concentration of remaining PL ions

thus: $5.5 = 4.21 + \log \frac{[\text{Remainder}]}{[\text{Cation}]}$

therefore [Remainder] = 19.49 [Cation].....equation 1

Furthermore: [Cation] + [Remainder] = 1.....equation 2

Substituting equation 1 into equation 2:

[Cation] + 19.49 [Cation] = 1

20.498 [Cation] = 1

Thus the proportion of cationic species at pH 5.5 is 4.87%.

THE PROPORTION OF ANIONS CAN NOW BE CALCULATED AS FOLLOWS:

[Anion] + [Remainder] = 0.9513.....equation 3

Substituting into the Henderson-Hasselbach equation, we have:

$5.5 = 8.68 + \log \frac{[\text{Anion}]}{[\text{Remainder}]}$

therefore [Anion] = 0.00066 [Remainder].....equation 4

Substituting equation 4 into equation 3, it can be seen that the proportion of the anionic species is 0.06%.

THE PROPORTION OF NEUTRAL AND DIPOLAR FORMS CAN BE CALCULATED WITH THE AID OF THE TAUTOMERIC CONSTANT Kz

ie $kz = 0.64 = \frac{[\text{Dipolar}]}{[\text{Neutral}]}$

thus 0.64 [Neutral] = [Dipolar].....equation 5

Furthermore,

[Dipolar] + [Neutral] = 0.9506.....equation 6

Substiting equation 5 into equation 6, we have:

[Neutral] + 0.64 [Neutral] = 0.9506

Therefore the proportion of the neutral PL species in solution at pH 5.5 is 57.96%. By subtraction the remaining dipolar species thus constitutes 37.1%. Similarly, it can be shown that at pH 7.4 the proportions of the ionic species are:
0.065% cations, 57.89% neutral, 37.1% dipolar and 4.98% anion.

APPENDIX IV

IVa) SOLID STATE STABILITY DATA FOR PL HCl
(see section 5.2.3)

Sample 1, Stored at 0% RH, 25°C

Original Mass	Amount found after two weeks	
11.0 mg	11.1 mg	
	11.1 mg	
	10.9 mg	
Mean	11.03	(100.3%)
Standard Error	0.07	

Sample 2, stored at 11% RH, 25°C

Original Mass	Amount found after two weeks	
10.4 mg	10.2 mg	
	10.3 mg	
	10.2 mg	
Mean	10.2	(98.4%)
Standard Error	0.03	

Sample 3, stored at 54% RH, 25°C

Original Mass	Amount found after two weeks	
10.5 mg	10.4 mg	
	10.3 mg	
	10.3 mg	
Mean	10.3	(98.4%)
Standard Error	0.03	

Sample 4, stored at 0% RH, 55°C

Original Mass	Amount found after two weeks	
10.0 mg	9.8 mg	
	9.7 mg	
	9.8 mg	
Mean	9.7	(97.6%)
Standard Error	0.03	

Sample 5, stored at 11% RH, 55°C

Original Mass	Amount found after two weeks	
11.3 mg	11.1 mg	
	10.8 mg	
	10.7 mg	
Mean	10.8	(96.17%)
Standard Error	0.120	

Sample 6, stored at 54% RH, 55°C

Original Mass	Amount found after two weeks	
10.3 mg	9.8 mg	
	9.5 mg	
	10.2 mg	
Mean	9.8	(95.47%)
Standard Error	0.203	

Sample 7, stored at 54% RH, 55°C

Original Mass	Amount found after two weeks	
10.7 mg	7.3 mg	
	7.4 mg	
	7.4 mg	
Mean	7.4	(69.2%)
Standard Error	0.033	

Sample 8, stored at 54% RH, 55°C

Original Mass	Amount found after two weeks	
10.0 mg	9.1 mg	
	8.8 mg	
	8.9 mg	
Mean	8.93	(89.33%)
Standard Error	0.088	

IVb) THE PLACKETT-BURMAN DESIGN

The method of Plackett and Burman (1946) provides saturated factorial designs that allow experiments to be carried out with a minimum of trials without compromising accuracy (the individual combinations tested within the multifactorial experiment are referred to as trials). Factors in the experiment are evaluated at 2 levels (ie a high (+) and a low (-) level). A constraint of the method is that the number of trials within a particular experiment has to be divisible by 4 for a 2 level factorial design. Furthermore the smallest design possible is an 8 trial experiment. The largest design that can be derived involves 100 trials. ie designs for experiments N = 8, 12, 16, 20, 24...100 can be derived.

Procedure for deriving a Plackett-Burman Design

In their work Plackett and Burman (1944) have provided the first line for each factorial design from N = 8 to N = 100. In section 5.3 a design with 24 trials is

employed. For a Plackett-Burman design with N = 24 the first line can be represented as follows:

+ + + + + - + - + + - - + + - - + - + - - - -

where "+" signifies the presence of a factor at a high level and "-" signifies the presence of a factor at a

low level.

Plackett-Burman designs are cyclical designs. The complete design is generated by cyclically shifting the first row by one place (N - 1) times and adding a final row of minus signs, thus

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
1	+	+	+	+	+	-	+	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-	-
2	-	+	+	+	+	+	-	+	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-
3	-	-	+	+	+	+	+	-	+	-	+	+	-	-	+	+	-	-	+	-	+	-	-
4	-	-	-	+	+	+	+	+	-	+	-	+	+	-	-	+	+	-	-	+	-	+	-
5	-	-	-	-	+	+	+	+	+	-	+	-	+	+	-	-	+	+	-	-	+	-	+
6	+	-	-	-	-	+	+	+	+	+	-	+	-	+	+	-	-	+	+	-	-	+	-
7	-	+	-	-	-	-	+	+	+	+	+	-	+	-	+	+	-	-	+	+	-	-	+
8	+	-	+	-	-	-	-	+	+	+	+	+	-	+	-	+	+	-	-	+	+	-	-
9	-	+	-	+	-	-	-	-	+	+	+	+	+	-	+	-	+	+	-	-	+	+	-
10	-	-	+	-	+	-	-	-	-	+	+	+	+	+	-	+	-	+	+	-	-	+	+
11	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+	-	+	-	+	+	-	-	+
12	+	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+	-	+	-	+	+	-	-
13	-	+	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+	-	+	-	+	+	-
14	-	-	+	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+	-	+	-	+	+
15	+	-	-	+	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+	-	+	-	+
16	+	+	-	-	+	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+	-	+	-
17	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+	-	+
18	+	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+	-
19	-	+	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+
20	+	-	+	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-	-	+	+	+	+
21	+	+	-	+	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-	-	+	+	+
22	+	+	+	-	+	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-	-	+	+
23	+	+	+	+	-	+	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-	-	+
24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

The rows of the design can then be taken as referring to trials (excipient mixtures) and the columns to variables affecting the formulation. In our case the experiment involves 11 excipients and 2 environmental factors (temperature and relative humidity). Only 13 columns therefore refer to assigned variables (real variables). Any 13 columns can be selected as the original variables. In this case the first 13 columns (A - M)

were used. The remaining columns (N - X) are "dummy" variables. The designation of variables A - M and their high and low levels is shown in table 5.3 (section 5.3.2.4.1).

The complete design can therefore be depicted as follows:

	Variable																						
Trial	**A**	B	C	D	E	F	G	H	I	J	K	L	**M**	N	O	P	Q	R	S	T	U	V	W	%REC
1	+	+	+	+	+	-	+	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-	-	Y1
2	-	+	+	+	+	+	-	+	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-	Y2
3	-	-	+	+	+	+	+	-	+	-	+	+	-	-	+	+	-	-	+	-	+	-	-	Y3
4	-	-	-	+	+	+	+	+	-	+	-	+	+	-	-	+	+	-	-	+	-	+	-	Y4
5	-	-	-	-	+	+	+	+	+	-	+	-	+	+	-	-	+	+	-	-	+	-	+	Y5
6	+	-	-	-	-	+	+	+	+	+	-	+	-	+	+	-	-	+	+	-	-	+	-	Y6
7	-	+	-	-	-	-	+	+	+	+	+	-	+	-	+	+	-	-	+	+	-	-	+	Y7
8	+	-	+	-	-	-	-	+	+	+	+	+	-	+	-	+	+	-	-	+	+	-	-	Y8
9	-	+	-	+	-	-	-	-	+	+	+	+	+	-	+	-	+	+	-	-	+	+	-	Y9
10	-	-	+	-	+	-	-	-	-	+	+	+	+	+	-	+	-	+	+	-	-	+	+	Y10
11	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+	-	+	-	+	+	-	-	+	Y11
12	+	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+	-	+	-	+	+	-	-	Y12
13	-	+	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+	-	+	-	+	+	-	Y13
14	-	-	+	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+	-	+	-	+	+	Y14
15	+	-	-	+	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+	-	+	-	+	Y15
16	+	+	-	-	+	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+	-	+	-	Y16
17	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+	-	+	Y17
18	+	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+	-	Y18
19	-	+	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+	Y19
20	+	-	+	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-	-	+	+	+	+	Y20
21	+	+	-	+	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-	-	+	+	+	Y21
22	+	+	+	-	+	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-	-	+	+	Y22
23	+	+	+	+	-	+	-	+	+	-	-	+	+	-	-	+	-	+	-	-	-	-	+	Y23
24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Y24
Sum +																								
Sum -																								
Effect																								

Key: A stearic acid; B magnesium stearate; C Aerosil 380; D lactose; E Ludipress; F corn starch; G Avicel pH101; H methylcellulose; I ethylcellulose; J Eudragit RSPM; K mannitol; L relative humidity; M temperature; N to W dummy variables; %REC percentage of drug recovered;

ANALYSIS OF THE RESULTS

The main effect of each variable is calculated as follows: The "Y" values (% drug recovered) for each variable where the variable is "+" are added and recorded as "sum +". Similarly the "Y" values for each variable where the variable is "-" are added and recorded as "sum -".

The average effect of the variable on the response is then calculated as follows:

$$\text{Effect} = \frac{(\text{sum } +) - (\text{sum } -)}{N/2}$$

for our particular study:

$$\text{Effect} = \frac{(\text{sum } +) - (\text{sum } -)}{12}$$

To calculate the significance of this effect, the standard deviation of the variable effect (s_{VE}) is calculated from the dummy variables as follows:

$$s_{VE} = \frac{(\text{Effect}_N)^2 + (\text{Effect}_O)^2 \ldots + (\text{Effect}_W)^2}{\text{number of dummy variables (ie 10)}}$$

by referring to a statistical table for t values, the minimum significant variable effect (E_{ms}) can be calculated as follows:

$$E_{ms} = t \cdot s_{VE}$$

Any main effect having an absolute value greater than E_{ms} is considered to be statistically significant on the stability of the drug.

IVc) ANALYTICAL RESULTS OBTAINED FOR THE PLACKETT-BURMAN EXCIPIENT COMPATIBILITY TEST

Trial	Original Amount of Drug(mg)	Amount found after two weeks (mg)			Mean % Recovered
		1	2	3	
1	10.3	9.8	9.8	9.8	95.15
2	10.4	9.7	9.8	9.8	93.91
3	10.5	10.2	10.2	10.2	97.14
4	10.5	8.5	8.4	8.4	80.32
5	10.0	9.2	9.2	9.3	92.33
6	10.1	9.8	9.8	9.8	97.03
7	10.9	10.2	10.2	10.2	93.60
8	10.6	10.3	10.3	10.3	97.17
9	10.2	8.0	7.9	8.0	78.10
10	10.2	7.8	7.7	7.8	76.14
11	10.3	7.0	7.0	7.1	68.28
12	10.7	9.1	9.6	9.0	86.29
13	10.4	10.4	10.4	10.6	100.64
14	10.1	10.1	10.0	10.1	99.67
15	10.3	10.1	9.9	10.3	98.06
16	10.0	8.9	8.8	8.9	88.67
17	10.0	9.7	9.6	9.6	96.33
18	10.0	9.9	9.5	9.5	96.33
19	10.2	10.3	10.2	10.2	100.33
20	10.1	9.4	9.4	9.4	93.07
21	10.4	10.0	10.1	10.0	96.47
22	10.2	9.5	9.9	9.7	95.10
23	10.4	9.3	9.0	9.1	87.82
24	10.4	10.2	10.3	10.2	98.40

CALCULATION OF EFFECTS

Variable effects were calculated using the following equation:

$$\text{Effect} = \frac{(\text{sum } Y_i+) - (\text{sum } Y_i-)}{24} \quad *$$

Variable	(sum Y_i+)	(sum Y_i-)	Effect
Stearic acid	1098.8	1106.91	-0.338
Mag. stearate	1111.77	1093.94	0.743
Aerosil	1129.92	1077.88	2.168
Lactose	1090.94	1114.77	-0.993
Ludipress	1096.51	1109.2	-0.529
Corn starch	1091.37	1114.34	-0.957
Avicel	1125.76	1079.95	1.909
Methylcellulose	1132.0	1073.71	2.429
Ethylcellulose	1119.44	1086.27	1.382
Eudragit RSPM	1095.35	1110.36	0.625
Mannitol	1073.24	1132.47	2.468
Relative Humidity	1059.41	1146.3	-3.620
Temperature	1047.43	1158.28	-4.618
N	1103.41	1102.3	0.046
O	1100.89	1104.82	-0.164
P	1101.35	1104.36	-0.125
Q	1089.82	1115.89	-1.086
R	1090.04	1115.67	-1.068
S	1107.7	1098.01	0.404
T	1093.97	1111.74	-0.740
U	1132.26	1073.45	2.450
V	1101.87	1103.84	-0.082
W	1096.56	1109.15	-0.525

* for individual Y_i values refer to table 5.5 (section 5.3.3.3).

Using the formula for the standard deviation of the variable effect (S_{VE}) a value of 0.967(6) was calculated for S_{VE}. Utilizing the appropriate student t values the minimum significant variable effects (E_{MS}) were then calculated:

For the 90% level of confidence E_{MS} = 1.754

For the 60% level of confidence E_{MS} = 0.851

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