DEVELOPMENT OF AN ANTIRETROVIRAL SOLID DOSAGE FORM USING MULTIVARIATE ANALYSIS

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DECLARATION

I hereby declare that the work on which this dissertation is based is original (except where acknowledgements have been made) and that neither the whole work nor any part thereof has been, is being or is to be submitted for another degree at this or any other university.

_____

L. Nqabeni

On this_____ day of _____at the Nelson Mandela Metropolitan University

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LIST OF KEY ABBREVIATIONS

ANOVA : Analysis of variances

API : Active pharmaceutical ingredient

AUC : Under curve response

COST : Changing one separate factor

° C : Degrees Celsius

D. time : Disintegration

DoE : Design of experiments

DSC : Differential scanning calirometry

FDA : Food and Drug Administration

HBV : Hepatitis B virus

HCl : Hydrochloric acid

HIV-1 : Human Immunodeficiency Virus Type 1

HPLC : High performance liquid chromatography

HSDSC : High sensitive differential scanning calirometry

ICH : International Conference on Harmonization

KF : Karl Fischer

M : Molarity

m/m : mass/mass

m/v : mass/volume

MCC : Microcrystalline cellulose

µm : micrograms

mg : milligrams

min : Minute

MMR : Multivariate multiple regression

MS : Microsoft

N : Newton

NaOH : Sodium hydroxide

NRTI : Nucleoside reverse transcriptase inhibitor

OFAT : One factor at a time

OD : Outside diameter

PEG : Polyethylene glycol

PSD : Particle size distribution

PVP : Polyvinylpyrrolidone

RSD : Relative Standard Deviation

rpm : Revolutions per minute

sec : Second

SEM : Scanning electron microscopy

U. mass : Uniformity of mass

USA : United States of America

UV : Ultra-violet

vs **:** Versus

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SUMMARY

Introduction: The aim of pharmaceutical development is to design a quality product and the manufacturing process to deliver the product in a reproducible manner. The development of a new and generic formulation is based on a large number of experiments. Statistics provides many tools for studying the conditions of formulations and processes and enables us to optimize the same while being able to minimize our experimentation. The purpose of this study was to apply experimental design methodology (DOE) and multivariate analysis to the development and optimization of tablet formulations containing 150 mg lamivudine manufactured by direct compression. **Methodology:** The formulation was developed by using a three step approach namely; preliminary study, design of experiments and the optimization study. Following the trial formulation, both formulation and process factors to be used were identified. Sixteen formulations involving four factors evaluated at two levels (2^4) were generated by full factorial design with different proportion of API particle size, lubrication blending times, magnesium stearate levels and starch glycollate. To minimize bias, the experiments were executed in a randomized order. Standard pharmacopoeial methods were used to test for physical properties of the tablets. Analysis of variances (ANOVA) was used to determine the main effect and the interaction effect of the considered factors on the responses. The interaction plots were generated for each response so as to determine the extent of the interactions between the factors. Multiple multivariate regression (MMR) analysis was used for formulation optimization. **Results and discussion:** The ANOVA revealed that only the API particle size and magnesium stearate concentration considerably affected tablet uniformity of mass. The disintegration and dissolution were considerably affected by the disintegrant content. None of the four factors considerably affected the tablets friability. The ANOVA revealed interactions amongst these factors therefore, to affect (change) any of the responses, the level of one factor could not be changed independently of the level of the other factors. The MMR revealed that the coarse grade lamivudine material, 3.0 % of sodium starch glycollate, 1.5 % of magnesium stearate and 1 minute lubrication blending time were selected as the optimum factor settings that would represent the optimum responses for this formulation. An optimum formulation generated

by the use of statistical tools was then produced and analyzed. **Conclusion:** Although most formulations met the required specifications, this study has demonstrated and described the efficiency and effectiveness of using a statistically designed methodology to develop a lamivudine 150 mg tablet formulation.

Keywords: Analysis of variances (ANOVA), Design of experiments (DoE), multivariate analysis, pharmaceutical development, lamivudine, antiretroviral agents.

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Peck et al. (1989) describes tablet formulation and design as the process whereby the formulator ensures that the correct amount of drug in the right form is delivered at the proper time at the proper rate and in the desired location, while its chemical integrity is protected from that point.

There is an increased competition amongst manufacturers that necessitates products and processes to be cost efficient. Cost of raw material or manufacturing process should be considered before a final tablet formulation is selected. Tablets can be manufactured by mainly two methods, namely granulation and direct compression. Most powders cannot be compressed directly into tablets due to the lack of proper characteristics of binding into compact entity and lack of disintegrating and lubricating properties. For these reasons, the drug is first pretreated with the other excipient to form granules that can be tabletted (Bandelin, 1989:148-181). Due to the space, time and equipment involved in granulation, the method has proven to be cost and labour intensive. The direct compression method involves tablet compression from powder blends of the active ingredient and suitable excipients. The most obvious advantage of direct compression is economy since fewer steps are involved during manufacture. However, due mainly to poor flow and poor compression properties of some active pharmaceutical ingredients, it is not always possible to use the direct compression approach. The availability of new directly compressible vehicles that posses both fluidity and compressibility have made it possible for most drugs to be directly compressed. This method requires a new and critical approach to the selection of raw material, flow properties of powder blends and effect of process and formulation variables on the formulation (Shangraw, 1989:195-213).

One method used to select and optimize formulation is the statistical design of experiments (DoE) together with analysis of variances (ANOVA). Unlike conventional methods, namely trial-and-error and changing one factor at a time (OFAT), design of experiments allows us to evaluate many factors concurrently while minimizing experimentation. It uncovers the main and interaction effects of independent variables on dependent variables (Tye, 2004:485-491).

1.2 PROBLEM DEFINITION

A vast majority of tablets produced are manufactured by a process requiring granulation of the powdered constituents prior to tabletting. The primary purpose of granulation is to produce a free-flowing and compressible mixture of the excipients and the active pharmaceutical ingredient (API). The availability of new excipients or new forms of old excipients, particularly fillers and binders have allowed production of tablets by a much simpler method of direct compression. Shangraw (1989) once quoted that 'direct compression should not be conceived as a simplified modification of the granulation process'. It requires a new and critical approach to the selection of raw material, flow properties of powder blends and effects of formulation variables on compressibility.

An alternative approach on tablet formulation is design of experiments (DoE) and multivariate statistical analysis. Unlike the conventional approach, DoE often leads to the real optimum and requires fewer experiments to evaluate many factors concurrently. Although this method looks ideal for drug formulation, most researchers are still using older methods. One of the main reasons for not using DoE is based on the fear of statistics. With the sophisticated computer packages that are specially designed for DoE, this is no longer a valid excuse (Tye, 2004:485-491). Lamivudine raw material powder has been reported to have poor flowability properties. The wet granulation method could prove to be the solution to formulate this drug into a tablet form but with the application of statistical experimental design and analysis methods, an optimized direct compression method may also be possible.

1.3 AIM AND OBJECTIVES

1.3.1 Primary aim

The main aim of this study was to formulate an oral solid dosage form containing 150 mg of lamivudine using a direct compression method of manufacture and applying design of experiments (DoE) and statistical multivariate analysis.

1.3.2 Objectives

The objectives derived from the aim were thus identified as follows:

1. To develop a primary formulation using a direct compression method of manufacture.
2. To identify physicochemical properties that could affect the choice of manufacturing.
3. To identify the process and formulation parameters that could be altered to influence overall tablet quality.
4. To apply statistical multivariate analysis methodology to asses the influence that the process and formulation parameters have on tablet quality.
5. To propose a final formulation and efficient method of manufacture, ensuring that it meets all required specifications

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

After an initially slow start, the development of new antiviral agents and their generics has now entered an accelerated growth phase. There are now more than 15 antiviral agents that have been formally licensed for treatment of human immunodeficiency virus infections. Lamivudine and other several other compounds (such as adefovir dipivoxil) have been approved for treatment of the hepatitis B virus infections. (DeClercq, 2001:73-89). The FDA generic approval of lamivudine was granted in 1995 for adult and paediatric use (Coffey & Peiperl, 2001).

High-precision dosing, manufacturing efficiency and patient compliance make tablets the most popular dosage form. As a class, tablets are one of the most challenging of all pharmaceutical products to design. These challenges include difficulty in achieving reliable drug bioavailability for hydrophic drugs to poor mixing as a result of poor flow (Banker & Anderson, 1986: 295-329). Tablets can be manufactured by either wet granulation, direct compression or dry granulation. Direct compression is the preferred method of manufacture due to fewer steps involved in manufacturing (less cost) and causing fewer problems with bioavailability (Peck et al., 1989:75-93). When it comes to formulation, trial and error methods do not allow the formulator to know how close any particular formulation is to the optimum formulation (Banker & Anderson, 1986:295-329). Design of experiments (DoE) and statistical analysis are now used for process and formulation optimization. Design of experiment allows one to evaluate the effect of changing both the formulation and process variables (independent factors) on tablet responses (dependent factors). However, in order for effective formulation to take place, a thorough knowledge of the active pharmaceutical ingredient (API) must be obtained. Therefore, the physicochemical properties of the API, lamivudine will now be discussed.

2.2 LAMIVUDINE

2.2.1 Physicochemical properties of lamivudine

Lamivudine is a white to off white crystalline substance and has a melting point of 176 ºC and a solubility of approximately 70 mg/ml in water at 20 °C. The pH of a 1% w/v solution of lamivudine in water is approximately 6.9 and the pKa determined by UV is 4.30 (USP, 2004:1064). The structure of lamivudine is shown in Figure 2.1 below.

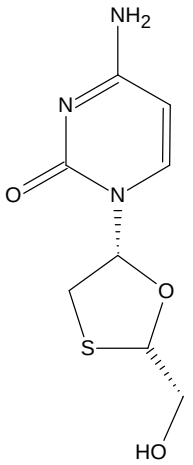


Figure 2.1: Lamivudine (2(1H)-Pyrimidinone, 4-amino-1-[2-(hydroxymethyl)-1, 3-oxathiolan-5-yl]-, (2R-cis)); Molecular mass = 229.3 g/mol; Empirical formula = $C_8H_{11}N_3O_3S$ (USP Convention, 2004:1064)

2.2.2 Action and uses

Lamivudine is a nucleoside reverse transcriptase inhibitor (NRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1) and hepatitis B virus (HBV). It is phosphorylated to active metabolites that compete for incorporation into viral DNA. They competitively inhibit the HIV reverse transcriptase and act as chain terminators of DNA synthesis (as shown on Figure 2.2 below). The lack of a 3'-OH group in the incorporated nucleoside analogue prevents the formation of the 5' to 3' phosphodiester linkage

essential for DNA chain elongation, and therefore, the viral DNA growth is terminated. Lamivudine is a synthetic nucleoside analogue and is also incorporated into viral DNA by HBV polymerase, resulting in DNA chain termination. (Safrin, 2001:p831-842)

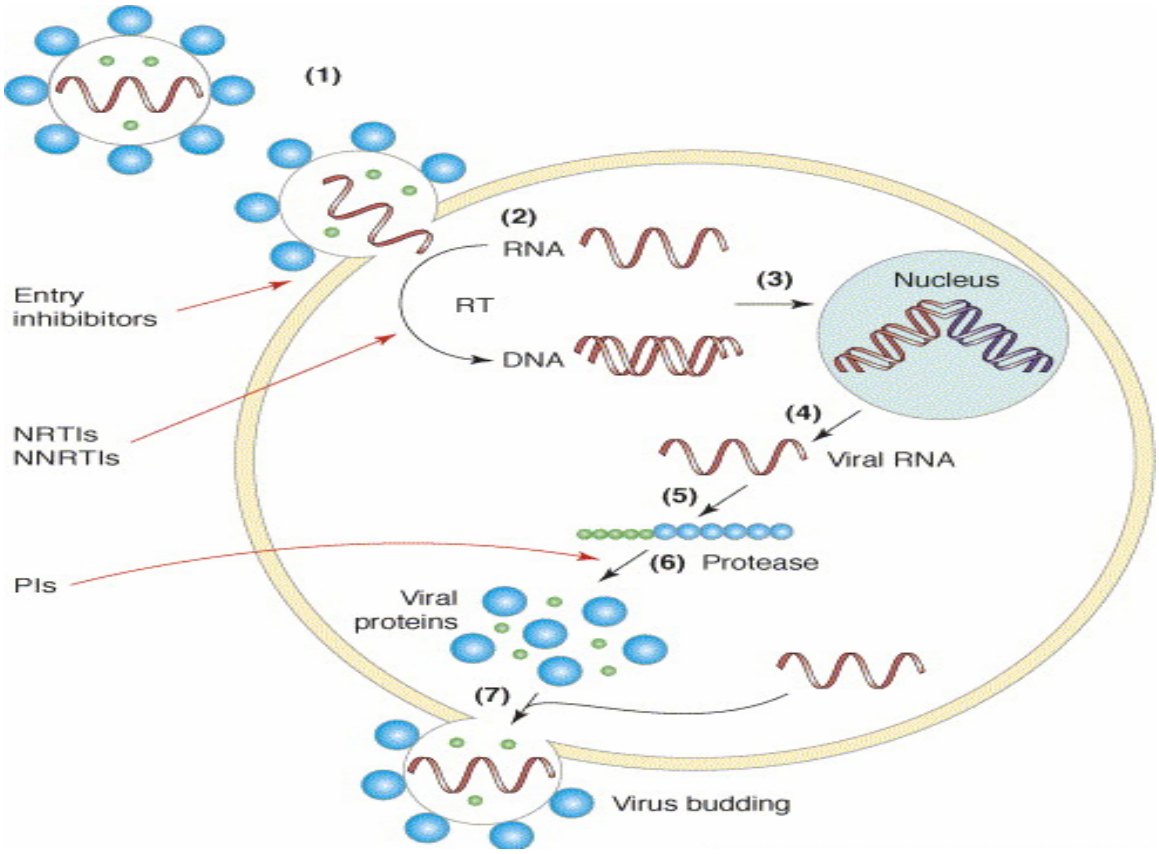


Figure 2.2: Schematic diagram of human immunodeficiency virus indicating antiretrovirals actions (Source: Chen et al., 2006:149)

2.2.3 Marketed products

There is an increased number of antiretroviral products currently on the market. The list of some of the antiretroviral solid dosage forms containing lamivudine, the excipients used and the manufacturers that are currently on the market is shown in Table 2.1.

Table 2.1: Marketed products containing lamivudine

Trade name	Active Pharmaceutical Ingredient (API)	Dosage Form	Excipients used	Manufacturers
Epivir®	Lamivudine 150 mg	Oral Film Coated Tablets	Hypromellose, magnesium stearate, microcrystalline cellulose, polyethylene glycol, polysorbate 80, sodium starch glycolate, and titanium dioxide	GlaxoSmithKline
	Lamivudine 300 mg		300mg contains black iron oxide as a colourant	
Aspen Lamivudine	Lamivudine 150 mg	Oral Film Coated Tablets	Colloidal silicon dioxide, Magnesium stearate, microcrystalline cellulose, povidone, sodium starch glycollate	Aspen Pharmacare
Lamivir®	Lamivudine 150 mg	Oral Film Coated Tablet	Hypromellose, magnesium stearate, microcrystalline cellulose, propylene glycol, sodium starch glycollate, talc, titanium dioxide	Cipla Ltd
Lamivir®	Lamivudine 100 mg	Oral Film Coated Tablet		

(**Source**: GSK, 2006; WHO, 2006)

The formulations listed in Table 2.1 contain almost the same excipients. The manufacturers are not protective about the constituents used, thus the main challenge for the generic formulator would be to use these excipients with the correct quantities that would produce quality products. The most significant barrier to access the ARVs is the high cost of these drugs and therefore there is an increased drive for the development and production of low cost generic antiretrovirals in developing countries. This is the biggest challenge for the manufacturers, to produce quality antiretrovirals at low cost.

2.3 DEVELOPMENT OF AN ORAL SOLID DOSAGE FORM

The aim of pharmaceutical development is to design a quality product and its manufacturing process to consistently deliver the intended performance of the product. The information and knowledge gained from pharmaceutical development studies and manufacturing experience provide scientific understanding to support the establishment of the design space, specifications, and manufacturing controls. It is important to recognize that quality cannot be tested into products; i.e., quality should be built in by design. Changes in formulation and manufacturing processes during development and lifecycle management should be looked upon as opportunities to gain additional knowledge and further support establishment of the design space. (ICH, 2005)

According to the International Conference on Harmonization (ICH) and Food and Drug Administration (FDA) guidelines on product development, there are no regulations that require a product development report, although companies are required to produce scientific data which justifies the formulation, the manufacturing and control processes. Most companies have used product development reports, technology transfer reports and others to summarize the scientific data that justifies the product and process (FDA, 1994). The product development report should therefore satisfy the needs of the company and has no legislative requirements. The development data found in these reports are indicated in the Subsections below (2.3.1-2.3.4)

2.3.1 Drug Substance Characterization

Characterization of the chemical and physical properties of the drug substance is one of the most important steps in the development of a solid dosage form. It is important to identify the physicochemical and biological properties of the drug substance that can influence the performance of the drug product and its manufacturability. Examples of physicochemical and biological properties that might need to be examined include solubility, water content, particle size, crystal properties, biological activity (toxicity), and permeability. (Fiese & Hagen, 1986:171-184) In addition, the physical properties such as solubility, polymorphism, hygroscopicity, particle size and density must also be addressed.

Review of the literature demonstrates that the physical quality, for example, particle size of raw materials, can sometimes produce a significant impact on the availability and clinical effect of a dosage form. Therefore, it is appropriate that the physical characteristics of a drug substance be characterized, that the impact of the physical characteristics be determined and that a specification for the bulk drug product be established if necessary. (Wadke et al., 1989:53-57)

Development data will vary between new drugs and generics. Characterization and establishment of specifications for the drug substance is one example. Therefore, the finished dosage form manufacturer must perform the appropriate test to characterize the drug substance chemically and physically and establish appropriate specifications. This may require developing analytical methods to identify impurities. In some cases this information can be obtained from literature searches or from the raw material supplier. (Wadke et al., 1989:53-57)

2.3.2 Use of excipients

Excipients are critical to tablet formulation since their functions and characteristics may affect drug product performance. It is important for the formulator to establish any

incompatibilities with the excipients used. The ability of excipients (e.g., antioxidants, penetration enhancers, disintegrants, release controlling agents) to provide their intended functionality, should also be demonstrated. The information on excipient performance can be used, as appropriate, to justify the choice and quality attributes of the excipient, and to support the justification of the drug product specification (Banker & Anderson, 1986:295-329). Control of the physical characteristics of the excipient is also important because variations in such characteristics may also affect the performance of the dosage form. Changes in particle size of some excipients, for example, may affect content uniformity. In other cases, a change in the supplier of an excipient or lubricant may affect dissolution or bioavailability. (Banker & Anderson, 1986:295-329)

2.3.3 Formulation Development

A summary should be provided describing the development of the formulation, including identification of those attributes that are critical to the quality of the drug product, taking into consideration the intended usage and route of administration. Information from formal experimental designs can be useful in identifying critical or interacting variables that might be important to ensure the quality of the drug products. The summary should clearly show the formulation design from initial concept up to the final design. The choice of drug product components (e.g., the properties of the drug substance, excipients used, container closure system, any relevant dosing device), the manufacturing process, and, if appropriate, knowledge gained from the development of similar drug product(s) should be taken into consideration. (ICH, 2005)

The design of a tablet usually involves a series of compromises on the part of the formulator, since producing the desired properties (e.g. rapid disintegration and dissolution) frequently involves competing objectives. The correct selection and balance of excipients for each active ingredient or ingredients combination in a tablet formulation to achieve the desired response is not a simple goal to achieve. Increased competition among manufacturers has necessitated that products and processes be cost efficient and

thus cost of a raw material or a particular processing step must be considered before a final tablet formulation or manufacturing is selected. (Peck et al., 1989:75-93)

2.3.4 Manufacturing Process Development

After identifying all the physicochemical properties that may affect the drug product, the manufacturing process should be selected, described and explained. The selection of the equipment used and its appropriateness for the intended products should be discussed. The knowledge gained from process development studies can be used, as appropriate, to justify the drug product specification. The statistical approach for experimental design helps the manufacturing process development programme to identify any critical process or formulation parameters that should be monitored or controlled (e.g., lubrication blending time) and optimizes to ensure that the product is of the desired quality. (Peck et al., 1989:75-93)

Two reasons for monitoring processing quality during development are to optimize the process as well as the product, and to establish in-process quality control tests for routine production (Peck et al., 1989:75-93). Changes in the tablet manufacturing process may change the purity profile of the active pharmaceutical ingredient or physical characteristics of the tablets and therefore causes problems with the finished dosage form.

2.4 PREFORMULATION STUDIES

Preformulation testing is the first step in the rational development of dosage forms of a drug substance. It can be defined as an investigation of physical and chemical properties of a drug substance alone or when combined with the excipients. The overall objective of preformulation studies is to generate information useful to the formulator in developing stable and bioavailable dosage forms that can be mass produced. (Wadke et al., 1989:1-7) In the simplest case, these preformulation investigations may merely confirm that there are no significant barriers to the compound's development (Fiese & Hagen, 1989:171-195). Not all the preformulation parameters are determined for every new product. Data,

as they are generated must be reviewed to decide what additional studies must be undertaken. (Wadke et al., 1989:1-7)

This literature review will only describe some of the physicochemical properties needed to support the development of a lamivudine 150 mg tablets. These include drug-excipient compatibilities, properties of flow (bulk and tapped density and angle of repose) and particle characterization.

2.4.1 Bulk Density

Bulk density of a compound varies substantially with the method of crystallization, milling or formulation. Usually bulk density is of great importance when one considers the homogeneity of a low-dose formulation in which there are large differences in drug and excipient densities. Once a density problem is identified, it is often easily corrected by milling, slugging or formulation (Fiese & Hagen, 1989:171-195). The density of solids also affects their flow properties. In the case of a physical mixture of powders, significant difference in the absolute densities of the components could lead to segregation (Wadke et al., 1989:53-57). Apparent bulk density (g/ml) is determined by pouring presieved bulk drug into a graduated measuring cylinder via large funnel and measuring the volume and weight. In addition to bulk density it is frequently desirable to know the true density of a powder for calculation of void volume or porosity of packed powder beds (Fiese & Hagen, 1986:171-195). A simple test has been developed to evaluate the flowability of a powder by comparing the bulk density (ρ_{Bmin}) and tapped density (ρ_{Bmax}) of a powder. A useful guide is given by the Carr's compressibility index as shown in Equation 2.1 below.

$$\text{Carr's index} = \frac{\text{Tapped} - \text{Bulk density}}{\text{Tapped density}} \times 100 \qquad \text{(Equation: 2.1)}$$

Carr's index can be determined on small quantities of powders and may be interpreted as shown in Table 2.2. Values less than 20 % Carr's index indicate good flow, whereas

greater than 33 % Carr' index indicate poor flow. Between 20 % and 40 % added glidant improves the flow. (Wells, 2002:133-136)

Table 2.2: Carr's index as an indication of powder flow (Source: Wells, 2002:134)

Carr's index (%)	**Type of flow**
5-15	Excellent
12-16	Good
18-21	Fair
23-35	Poor
33-38	Very poor
> 40	Extremely poor

2.4.2 Angle of repose

When a heap of powder is allowed to stand with only the gravitational force acting on it, the angle between the free surface of the static heap and the horizontal plane can achieve a certain maximum value for a given powder. This angle is defined as the angle of repose and is the common way of expressing flow characteristics of powders and granules. (Wadke et al., 1989:53-57) The angle of repose is best suited for particles greater than or equal to 150 µm as in this size range, cohesive effects will be minimal and the coefficient of friction will be largely dependent upon the normal component of the weight of the test specimen. Values for angles of repose less than or equal to 30^{o} generally indicate a free-flowing material while angles greater than or equal to 40^{o} suggests a poorly flowing material. (Rosanske, et al., 1989:245-348)

There are number of ways to determine the angle of repose. Examples are fixed height cone method, fixed base cone method, tilting table and rotating cylinder methods (Wells, 2002:133-136). The angle of repose measurement has some drawbacks as a predictor of powder flow in that it lacks sensitivity. For example, in a study reported by Wadke et al.,

(1989) sodium chloride, spray-dried lactose and Fast-Flo® lactose showed similar angles of repose, but their rates of flow through the orifice were quite different.

2.4.3 Fine particle characterization

Bulk flow, formulation homogeneity, and surface area controlled processes such as dissolution and chemical reactivity are directly affected by size, shape and surface morphology of the drug particles (Fiese & Hagen, 1989:171-195). The effect is not only on the physical properties of solid drugs but also, in some instances, on their biopharmaceutical behaviour. Particle size plays a role in the homogeneity of the final tablet. When large differences in particle size exists between the active components and excipients, mutual sieving (demixing) effects can occur, making thorough mixing difficult or, if attained, difficult to maintain during the subsequent processing steps. Other parameters being equal, reasonably fine materials interdisperse more readily and randomly. However, if a material becomes too fine, then undesirable properties such as electrostatic effects and other surface active properties may cause undue stickiness and lack of flowability. Not only size but shape too influences the flow and mixing efficiency of powders and granules. (Wadke et al., 1989:1-7)

Size can also be a factor in stability; fine materials are relatively more open to attack from atmospheric oxygen, heat, light, humidity, and interacting excipients than coarse materials. Because of these significant roles, it is important to decide on a desired size range, and subsequently to maintain and control it. Reducing particle size to a very small dimension often leads to aggregation and apparent increase in hydrophobicity, possibly lowering the dissolution rate and making handling more troublesome. (Wadke et al., 1989:1-7)

Several tools are commonly employed to monitor particle size and shape. The most rapid technique allowing for a quick appraisal is microscopy. For a quantitative particle size distribution analysis of materials that range upward from about 50 µm, sieving or screening is appropriate, although shape has a strong influence on the results. Other

techniques used to measure particle size are sedimentation, centrifugal sedimentation, permeability and light scattering techniques. (Wadke et al., 1989:1-7) The above physical parameters are very important in the formulation of a direct compression oral solid dosage form as they affect the overall flow characteristics of the powder mix, and controlling powder flow is one of the greatest challenges in any direct compression process.

2.4.4 Active-excipient compatibility

Drug-excipient compatibility is one of the most important preformulation study. A new drug in which a formulator lacks experience, the most challenging part would be the selection of excipient of that would be both chemically and physically compatible with the drug (Peck et al., 1989:75-93). Excipients are often regarded as 'inert' substances, although it is not known that they can interact with drugs giving rise to changes in stability, dissolution rates and bioavailability of the drug (Mura et al., 2005:65-71).

Differential scanning calorimetry (DSC) has shown to be an important tool to quickly obtain information about possible interactions between the active and the excipients, according to the appearance, shift or disappearance of endothermic or exothermic peaks and/or variations in the corresponding enthalpy values in thermal curves of drug-excipient mixtures (Mura et al.,2005:65-71). More often a 1:1 ratio is used even though this is not the ratio used for the final formulation, in order to maximize the probability of detecting a physical or chemical reaction. The thermograms obtained with the drug-excipient mixture are compared to thermograms of the drug alone and excipient alone. A typical thermogram showing an endothermic reaction is shown in Figure 2.3. , indicating an interaction between an active and the excipient. (Peck et al., 1989:75-93)

Conventional DSC is sometimes not considered fully reliable due to the fact that differences in the DSC scans of binary mixtures compared to the individual constituent may be caused by other factors other than chemical incompatibility. Stepwise isothermal mode high sensitive DSC (HSDSC) may prove to be a potential alternative approach to

the conventional DSC to study active-excipients incompatibilities. HSDSC operates on a similar principle as conventional DSC but with greater sensitivity and accuracy. The most important advantage of HSDSC is that the temperature may be changed with ease, which makes it possible to perform isothermal runs with greater sensitivity. (Wissing et al., 1999:141-150)

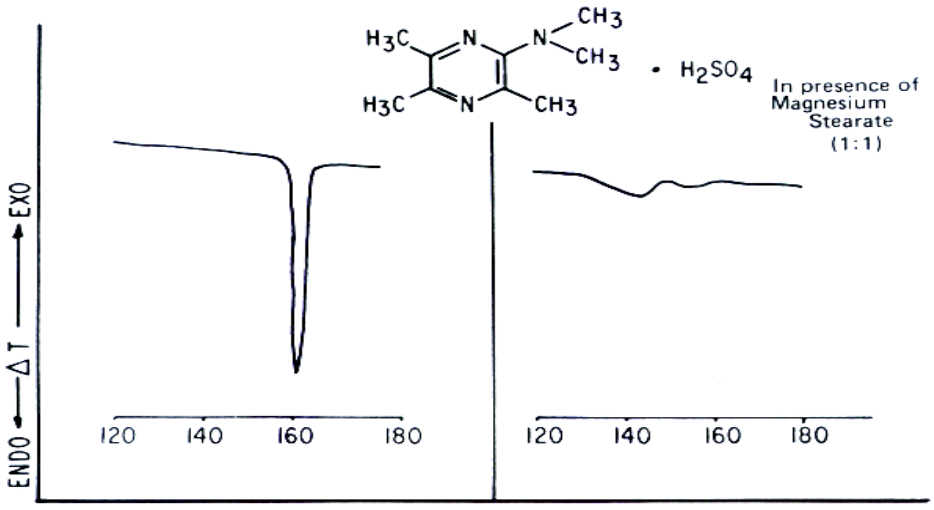


Figure 2.3: DSC curves of pure component and the mixture with magnesium stearate (Source: Peck et al., 1989:79)

2.5 TABLET MANUFACTURING

The objective of the design and manufacture of the tablets is to deliver orally the correct amount of drug in the proper form, at or over the proper time and in the desired location, and to have its chemical integrity protected to that point (Banker & Anderson, 1986:295). The sub-sections below (Sections 2.5.1-2.5.6) will describe the different manufacturing method, examples of commonly used excipients, equipment, evaluation of tablets, manufacturing problems and factors involved in formulation developments.

2.5.1 Different methods of tablet manufacturing

Compacted or compressed tablets are produced from powder mixtures or granulations made by one of the following general techniques:

- Direct compression
- Wet granulation
- Dry granulation (by Roller compaction or Slugging)

The choice of excipients in a tablet formulation depends on the active ingredient, the type of tablet, the desired tablet characteristics, and the process used to manufacture the tablet.

2.5.1.1 Direct compression method

The availability of the new excipients or new forms of old excipients, particularly fillers and binders, and the invention of new (or the modification of old) tablet machinery have allowed the production of tablets by the much simpler procedure of direct compression. The term direct compression was long used to identify the compression of a single crystalline compound (usually inorganic salts with cubic crystal structures such as sodium chloride) into a compact without the addition of other substances. (Shangraw, 1989:195-213) The term direct compression is now used to define the process by which tablets are compressed directly from powder blends of the active ingredient and suitable excipients (including diluents, disintegrants, and lubricants), which will flow uniformly into a die cavity and form into a firm compact (Shangraw, 1989:195-213).

The simplicity of the direct compression process is obvious. However, it is apparent simplicity that has caused so many initial failures in changing formulations from wet granulation to direct compression. Direct compression should not be conceived as a simplified modification of the granulation process for making tablets. It requires a new and critical approach to the selection of raw materials, flow properties of powder blends, and effects of formulation variables on compressibility. (Shangraw, 1989:195-213)

The most obvious advantage of direct compression is economy. Savings can occur in a number of areas, including reduced processing time and thus reduced labour costs, fewer manufacturing steps and pieces of equipment. The most significant advantage in terms of tablet quality is that of processing without the need for moisture and heat which is inherent in most wet granulation processes, and avoidance of high compaction pressures involved in producing tablets by slugging or roller compaction. One of the least recognized advantages of direct compression is the optimization of the tablet disintegration. Disintegrating agents added prior to wet granulation are known to be less effective than those added just prior to direct compression. In direct compression all of the disintegrant is able to perform optimally, and when properly formulated, tablets made by direct compression should disintegrate rapidly to the primary particle state. However, it is important that sufficient disintegrant be used to separate each drug particle if ideal dissolution is to occur. Fewer chemical stability problems would be encountered in tablets prepared by direct compression as compared to those made by wet granulation process. The primary cause of instability is moisture. Other aspect of stability is the effect of tablet aging on dissolution rates. Changes in dissolution profiles are less likely to occur in tablets made by direct compression than those made from wet granulation. (Shangraw, 1989:195-213)

Considering all the advantages mentioned above, it is difficult to understand why more tablets are not made by a direct-compression process. The technological limitations revolve mainly about the flow and bonding of particles to form strong compacts, and the speed at which this must be accomplished in an era of ever-increasing production rates. The choice of excipient is extremely critical in formulating direct-compression tablets. This is most true of the diluent, which often serves as the matrix around which revolves the success or failure of the formulation. Direct compression fillers must possess good compressibility and fluidity properties. (Shangraw, 1989:195-213)

Outside of compressibility failures, the area of concern most often mentioned by the formulators of direct compression is content uniformity. Direct compression blends are subject to unblending in post-blending steps. The lack of moisture in the blends may give

rise to static charges that can lead to unblending. Differences in particle size or density between actives and excipient particles may also lead to unblending in the hopper or feed frame of the tablet press. The problems of blending can be approached in two ways; namely, the traditional approach involves trying to keep particle sizes or densities uniform and the problem can also be solved by ordered blending. In order to reduce the likelihood of raw material failure, it is advisable to set quality specifications on particle size, bulk density, fluidity, and compressibility. (Shangraw, 1989:195-213)

2.5.1.2 Dry granulation methods

Another process for making the 'running' powder blend for tabletting is the dry granulation process. The same excipients that are used in direct compression can be used in dry granulation. This method is mostly used in situations where the effective dose of a drug is too high for direct compression and the drug is sensitive to heat, moisture or both which precludes wet granulation. It involves the compaction of the components in a tablet press, followed by milling and screening, prior to final compression into a tablet. (Shangraw, 1989:195-213)

2.5.1.3 Wet granulation method

Wet granulation is the oldest and most conventional method of making tablets. Although it is the most labour-intensive and most expensive of the available methods, it persists because of its versatility. In wet granulations the bonding properties of the liquid binders available are usually sufficient to produce bonding with a minimum of additives. (Bandelin, 1989:148-181)

The advantages of wet granulation process are well established and the advent of high-shear mixers, fluidized bed granulation and drying equipment has made wet granulation a more efficient process today than it was a quarter of a century ago (Shangraw, 1989:195-213). The advantages include the fact that the cohesiveness and compressibility of powders is improved due to the added binder that coats the individual powder particles,

causing them to adhere to each other so they can be formed into agglomerates called granules. Drugs having a high dosage and poor flow and/or compressibility must be granulated by the wet method to obtain suitable flow and cohesion for compression. Good distribution and uniform content for soluble, low-dosage drugs and colour additives are obtained if these are dissolved in the binder solution. Bulky and dusty powders can be handled without producing a great deal of dust and airborne contamination. Wet granulation prevents segregation of components of a homogeneous powder mixture during processing, transferring and handling. The dissolution rate of an insoluble drug may be improved by wet granulation with the proper choice of solvent and binder. (Bandelin, 1989:148-181)

On the other hand granulation is subject to many problems. The greatest disadvantage of wet granulation is its cost because of the space, time and equipment involved. In addition to cost, other problems include the fact that, due to the large number of processing steps, it requires a large area with temperature and humidity control and a number of pieces of expensive equipment. This method is also time consuming, especially the wetting and drying steps. There is a possibility of material loss during processing due to transfer of material from one unit operation to another. There is greater possibility of cross-contamination than with direct compression method. It can slow the dissolution of drugs from inside granules after tablet disintegration if not properly formulated and processed. This method is not suitable for hydrolysable drugs and/ or thermolabile drugs and also incompatibilities between ingredients will be aggravated as the binder solvent will bring them into closer contact. (Bandelin, 1989:148-181)

The complex nature of wet granulation is still not well understood, which accounts for the continuing interest in research on the process. One significant problem is the degree of wetting or massing of the powders. Wetting plays important role in the compression characteristics of the granules, and also in the rate of drug release from the final tablet. Although the wet granulation method is labour-intensive and time-consuming, requiring number of steps, it continues to find extensive application for a number of reasons. One reason is because of its universal use in the past, the method persists with established

products, where for one reason or another, it cannot be replaced by direct compression. Although a number of these products might lend themselves to the direct-compression method, to do so would require a change in ingredients to other excipients. (Bandelin, 1989:148-181)

2.5.2 Examples of commonly used excipients

In addition to the active ingredient(s), a series of excipients are normally included in a tablet and their role is to ensure that the tabletting operation can run satisfactorily and ensure that tablets of specified quality are prepared. Depending on the intended main function, excipients to be used in tablets are subcategorized into different groups. However, one excipient can affect the properties of a powder or the tablet in a series of ways, and many substances used in formulations can thus be described as multifunctional. (Banker & Anderson, 1986: 295-329)

To assure that no excipient interferes with the utilization of the drug, the formulator must carefully and critically evaluate combinations of the drug with each of the contemplated excipients and must ascertain compliance of each ingredient with existing standards and regulations. The screening of drug-excipient and excipient-excipient interactions should be carried out routinely in preformulation studies. (Bandelin, 1989:148-181)

The excipients discussed below represents 80 to 90% of currently used tablet excipients. Some of the tablets excipients can be used in all three manufacturing processes for example magnesium stearate is a lubricant in wet granulation and direct compression methods (Banker & Anderson, 1986: 295-329).

2.5.2.1 Diluents/Fillers

Diluents are fillers designed to make up the required bulk of the tablets when the drug dosage itself is inadequate to produce this bulk (Banker & Anderson, 1986: 295-329). Since they often comprise the bulk of the tablet, selection of a candidate from this group

as a carrier for a drug is of prime importance. Since combinations are also possible, consideration should be given to possible mixtures. (Bandelin, 1989:148-181)

Microcrystalline cellulose (MCC) is used as a diluent in direct compression (Banker & Anderson, 1986: 295-329). It improves flow and has good lubrication and disintegration properties. Tablets prepared with MCC generally exhibit excellent hardness and low friability. MCC can also be used as a binder in wet granulation. (Wheatley, 2000:1-12)

Lactose is the most commonly used filler in tablet formulations. Hydrous lactose does not flow well and its use is limited to tablet formulations prepared by wet granulation. Spray-dried lactose monohydrate and anhydrous lactose have good flowability and compressibility. Spray-dried lactose monohydrate is specifically engineered for direct compression and is ideally suited for drugs that do not compress well. (Wheatley, 2000:1-12)

Starch and starch derivatives are among the most commonly used excipients in tablet formulations. They can function as disintegrants, binders or fillers. Because of poor flow, loss of binding and compressibility in the presence of a lubricant, they are less suitable for direct compression tablet formulations.(Wheatley, 2000:1-12) Starch normally possesses a high typical moisture content of between 11 to 14 % and can therefore can lead to stability problems of ingredients sensitive to hydrolysis. (Banker & Anderson, 1986: 295-329)

Dibasic Calcium Phosphate in its unmilled form has good flow properties and compressibility. Because it has no inherent lubricating or disintegrating properties, other excipients must be added to prepare a satisfactory tablet formulation. Mannitol is a popular excipient for chewable tablets, owing to its pleasant taste. Mannitol has poor flow and compression properties. Dextrose has poor compression properties and tablets compacts are soft. (Wheatley, 2000:1-12)

2.5.2.2 Binders

Binders are ‘glue’ that hold powders together to form granules. They are the adhesives that are added to tablet formulations to provide the cohesiveness required for bonding together of granules under compaction to form a tablet. These materials are added either in dry or liquid form during wet granulation to form granules or to promote cohesive compacts for directly compressed drugs. (Bandelin, 1989:148-181)

Binders are either sugars or polymeric materials, with the latter falling into two classes; namely, natural polymers such as starches or gums including acacia, tragacanth, and gelatin and synthetic polymers such as polyvinylpyrrolidone (PVP), methyl- and ethylcellulose and hydroxypropylcellulose. Binders of both types may be added to the powder mix and the mixture wetted with water, alcohol-water mixtures, solvent or the binder may be put into solution in the water or solvent and added to the powder. (Bandelin, 1989:148-181)

2.5.2.3 Disintegrants

Disintegrant is the term applied to various agents added to tablet granulation for the purpose of causing the compressed tablet to break apart (disintegrate) when placed in an aqueous environment. Ideally, it should cause the tablet to disrupt, not only into the granules from which it was compressed, but also into the powder particles from which the granulation was prepared. (Bandelin, 1989:148-181)

Starch is the oldest and was the first most commonly used disintegrant in compressed tablets. Because of requirements for faster dissolution and problems with compression and tablet softening, starch is being replaced with newly developed ‘super disintegrants’. The name super disintegrant comes from the low use levels at which they are effective. Croscarmellose sodium, sodium starch glycollate, and crospovidone are examples of cross-linked cellulose, cross-lined starch and a cross-linked polymer respectively. Cross-linking serves to greatly reduce water solubility, while allowing the excipient to swell and

absorb many times its weight of water, causing the tablet to break apart or disintegrate. (Wheatley, 2000:1-12)

2.5.2.4 Lubricants

Lubricants are used to prevent adhesion of the tablet material to the surface of the dies and punches and to facilitate the ejection of the tablet from the dye. Commonly used lubricants include magnesium stearate, calcium stearate, stearic acid, talc and polyethylene glycol (PEG). Magnesium stearate is the most commonly used and effective lubricant for tablets. Lubricants are mixed in low concentration, into the final tablet blend in dry form just before compression. (Wheatley, 2000:1-12)

2.5.2.5 Glidants

Glidants are materials that improve the flow characteristics of granulation by reducing interparticulate friction. The effects produced by different glidants depend on their chemical nature in relation to that of the powder or granule and the physical factors including particle size, shape, and distribution of the glidant. In general, hydrophilic glidants tend to be more effective on hydrophilic powders, and the opposite is true for hydrophic glidants. For any particular system there is usually an optimum concentration above which the glidant may act as an anti-glidant. (Bandelin, 1989:148-181) Colloidal silicon dioxide is the most commonly used glidant. Talc is also used and may serve the dual purpose of lubricant and glidant. In certain formulations, the alkali stearates and starch are employed. (Wheatley, 2000:1-12) Colloidal silicon dioxide eliminates the negative effect of magnesium stearate on interparticle bonding while maintaining the lubrication action.

2.5.3 Manufacturing process

This sub-section will describe the processes, stages and equipment involved in all three manufacturing methods. These stages and processes are illustrated in Figure 2.4 below.

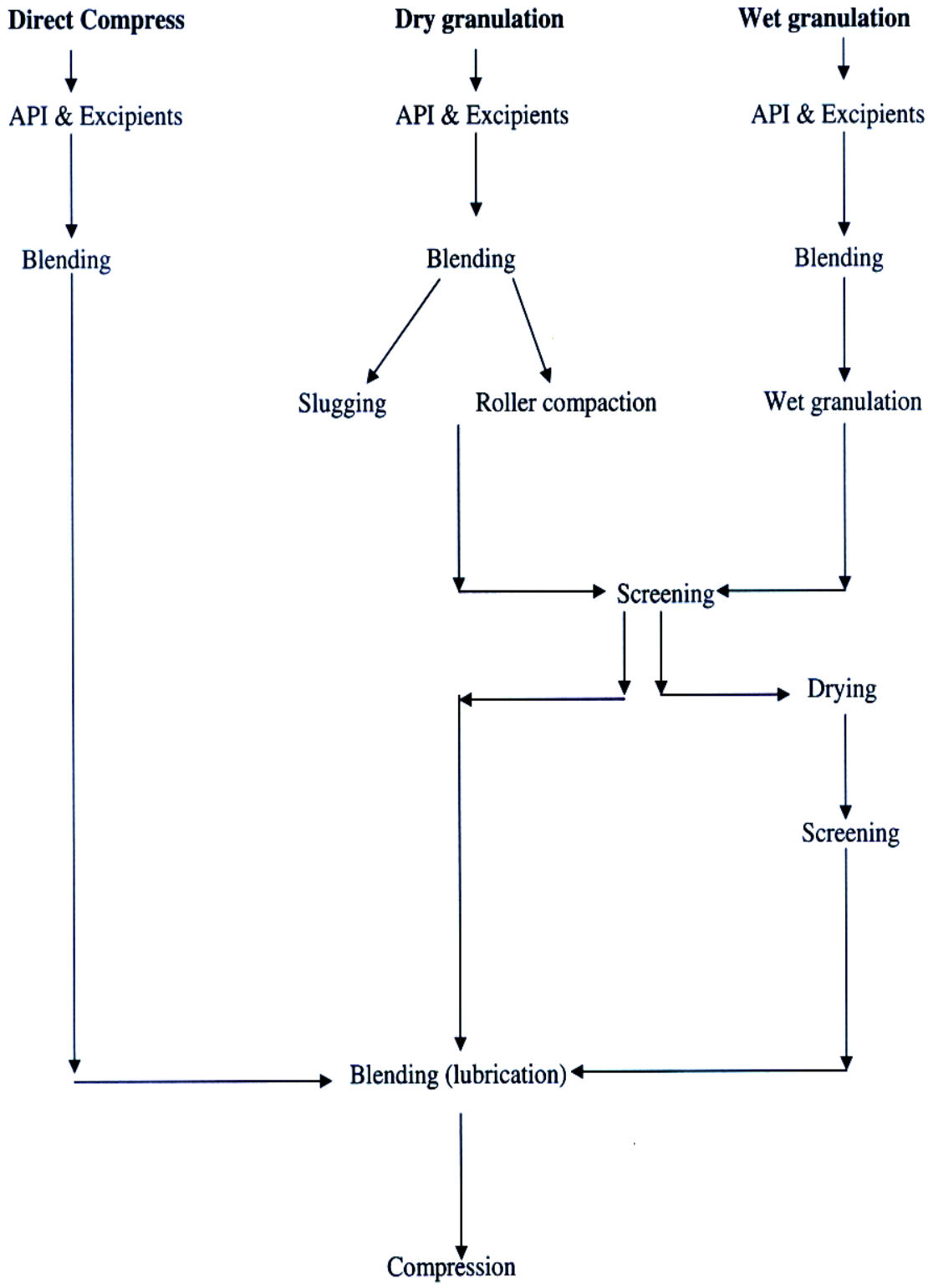


Figure 2.4: Processes involved in tablet manufacturing methods

2.5.3.1 Blending

In all three methods, the raw material of the active ingredient is blended with the appropriate excipient(s). Direct compression consists of compressing tablets directly from powdered material without modifying the physical nature of the material itself. Direct compression excipients must exhibit low lubricant sensitivity to compression, have good stability, promote tablet disintegration and drug dissolution and exhibit non-interference with the bioavailability of the active ingredient. (Wheatley, 2000:1-12) Usually a lubricant is added just prior to compression.

Some of the examples of the mixers used for dry powders include the v-shaped and double cone blenders. They mix the dry powders with a minimum of energy imparted to the powder bed as a result of tumbling the powder. High shear mixers provide more shearing but dusting may be prevalent causing segregation of fine particle to settle on top of the powder blend after blending has ceased (Twitchell, 2002: p191-196)

2.5.3.2 Granulation

Most powders cannot be compressed directly into tablets because they lack the proper characteristics of binding or bonding together into a compact entity and they do not ordinarily posses the lubricating and disintegrating properties required for tableting. Also the flow into the die may be impeded by poor flow behaviour. For these reasons, drugs must first be treated, either alone or in combination, with a filler, to form granules that lend themselves to tableting. This process is known as granulation. (Bandelin, 1989:148-181)

Granulation is any process of size enlargement whereby small particles are gathered together into larger, permanent aggregates to render them into a free-flowing state similar to that of dry sand (Bandelin, 1989:148-181). The primary purpose of the granulation step is to produce a free-flowing and compressible mixture of the active ingredients and

excipients. Tablets can be prepared by granulation via two methods, namely dry granulation and wet granulation methods.

Dry granulation can be further processed via two methods: Slugging or roller compaction. In slugging, a blend of powders is forced into the dies of a large-capacity tablet press and is compacted by means of flat-faced punches to form slugs. The slugs are then screened or milled to produce granules which now flow more uniformly than the original powder mixture. On a large scale, dry granulation can be performed on a specially designed machine called a roller compactor. Roller compactors, utilize two rollers that revolve toward each other. Powdered material is fed between the rollers by a screw conveyor system. After passing through the rollers, the compressed mass resembles a thin wide ribbon that has fallen apart into large segments. These are equivalent to the slugs produced by slugging process. The segments are then screened or milled for the production of granules. (Wheatley, TA, 2000:1-12)

Wet granulation forms the granules by binding the powders together with an adhesive. The wet granulation technique employs a solution, suspension, or slurry containing a binder, which is usually added to the powder mixture, however the binder may be incorporated dry into the powder mix. Liquid bridges are developed between particles, and the tensile strength of these bonds increases as the amount of liquid added is increased. Once the granulating liquid has been added, mixing continues until a uniform dispersion is attained and all the binder has been activated. Granulation in large blenders requires 15 minutes to an hour. The length of time depends on the wetting properties of the powder mixture and the granulating fluid. The wet mass is forced through a screen or wet granulator. Wet screening involves converting the moist mass into coarse, granular aggregates by passage through a hammer mill or oscillating granulator, equipped with screens having large perforations. The wet granules are dried in an oven or a fluidized bed dryer. A drying process is required to remove the solvent that was used in forming the aggregates and to reduce the moisture content to an optimum level of concentration within the granules. The dried granules are screened to a suitable size for compression. The size of the screen depends on the grinding equipment used. A lubricant agent is

mixed with the dried granules. The granules are then compressed into the finished tablet. (Banker & Anderson, 1986: 295-329)

The mixer used for wet granulation should produce the mixing mechanisms appropriate for the formulation. For example, diffusive mixing is generally preferable for potent drugs, and high shear is needed to break up aggregates of cohesive materials and ensure mixing at a particulate level. (Twitchell, 2002:191-196) The following are the examples of the type of mixers used during wet granulation process.

High-speed mixer-granulator, as the name suggests, it can both mix and granulate a product, thereby removing the need to transfer the product between pieces of equipment and so reducing the opportunity for segregation to occur. This type of a mixer is not used for lubrication as it may cause over-mixing of lubricants due to its high speed. (Twitchell, 2002: p191-196)

Fluidized-bed mixers are mainly used in drying of granules and coating of multiparticulates, however they can be used to mix powders prior to granulation in the same bowl (Twitchell, 2002: p191-196).

Planetary mixers are commonly found in domestic kitchen (e.g. Kenwood-type mixers) and larger machines which operate on the same principles are used in the industry. Planetary mixers are sometimes used to mix powders, particularly if a wet mass for granulation is required. (Twitchell, 2002: p191-196)

The wet mass is formed into granules by forcing through a screen to form granules. Some of the above mentioned mixers can also function as granulators, but the most commonly used granulator includes: Oscillating granulator that has rotor bars that oscillate and force the moist mass through the sieve screen, the size of the sieve determines the granule size. It is also used in dry granulation size reduction.

2.5.3.3 Drying

The granules can be collected on trays and transferred to a drying oven or fluid bed drier. The examples of most common drying equipment for pharmaceutical granulations include tray drier, fluidized bed drier and vacuum drying. In tray drying, wet granules are placed on trays which are then placed in the drying oven. Although tray drying method is slow and relatively inefficient, it is still a commonly used method of drying. Fluid bed drying involves a drying gas forced through a solid bed at a velocity sufficient to partially suspend the granules. It is efficient for the drying of solids due to its ability in promoting heat and mass transfer. Vacuum drying has ability to dry substances at low temperature and is more rapid than tray drying but not as rapid as fluid bed drying. Other advantage of vacuum drier is its ability to reduce oxidation.

2.5.3.4 Compression

In pharmaceutical tabletting an appropriate volume of granules/powders in a die cavity is compressed between an upper and lower punch to consolidate the material into single solid matrix, which is subsequently ejected from the die cavity as an intact tablet (Parrot, 1989: 201-207).

The process of tabletting is divided into three stages, namely: Die filling, which is accomplished by gravitational flow of the powder from a hopper via the die table into the die. The die is closed at its lower end by the lower punch. The second stage is the tablet formation, whereby the upper punch descends and enters the die and the powder is compressed until a tablet is formed (Alderborn, 2002: 397-410). The subsequent events that occur during this stage are transitional repacking, deformation at points of contact, fragmentation and/or deformation, bonding, deformation of the solid body, decompression (Parrot, 1989:201-207). The third stage is tablet ejection which occurs when the lower punch rises up until its tip reaches the level of the top of the die. The tablet is subsequently removed from the die and the die table by a pushing device. (Alderborn, 2002: 397-410)

There are two types of tablet presses that are commonly used during tablet production, namely, the single-punch press (eccentric press) and the rotary press. Single-punch press has one die and one pair of punches as shown in Figure 2.5. Single-punch tablet press has its primary use in the production of small batches of tablets, such as during formulation development. The output from the single-punch press is about 200 tablets per minute. (Alderborn, 2002: 397-410)

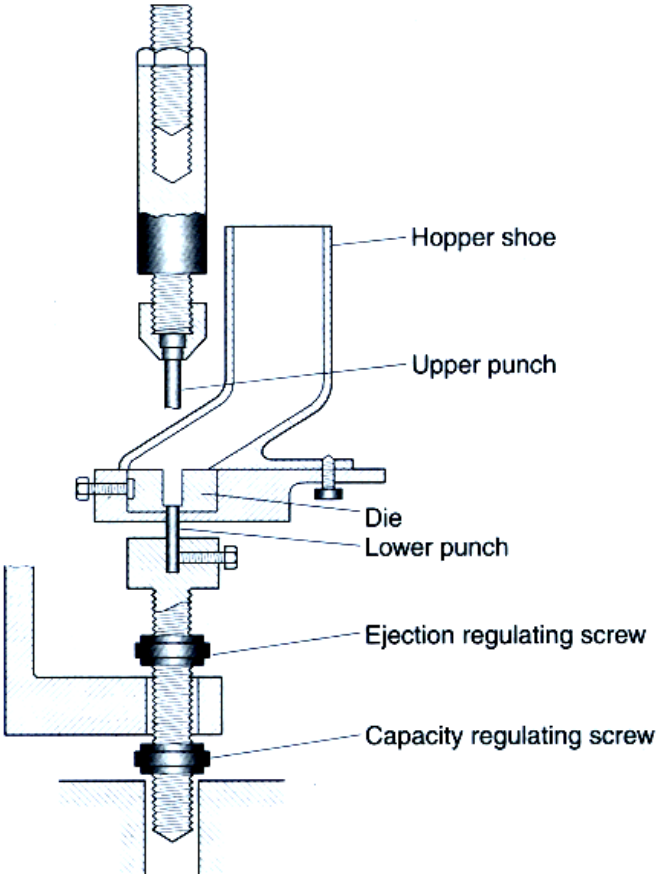


Figure 2.5: A single-punch tablet press (Source: Alderborn, 2002:400)

A rotary press operates with a number of dies and sets of punches (Figure 2.6), which can vary from three for small rotary presses up to 60 or more for large presses. Rotary press has its primary use during scale-up in the latter part of the formulation development, and during large-scale manufacturing. Outputs of over 10 000 tablets per minute can be achieved by rotary press. (Alderborn, 2002: 397-410)

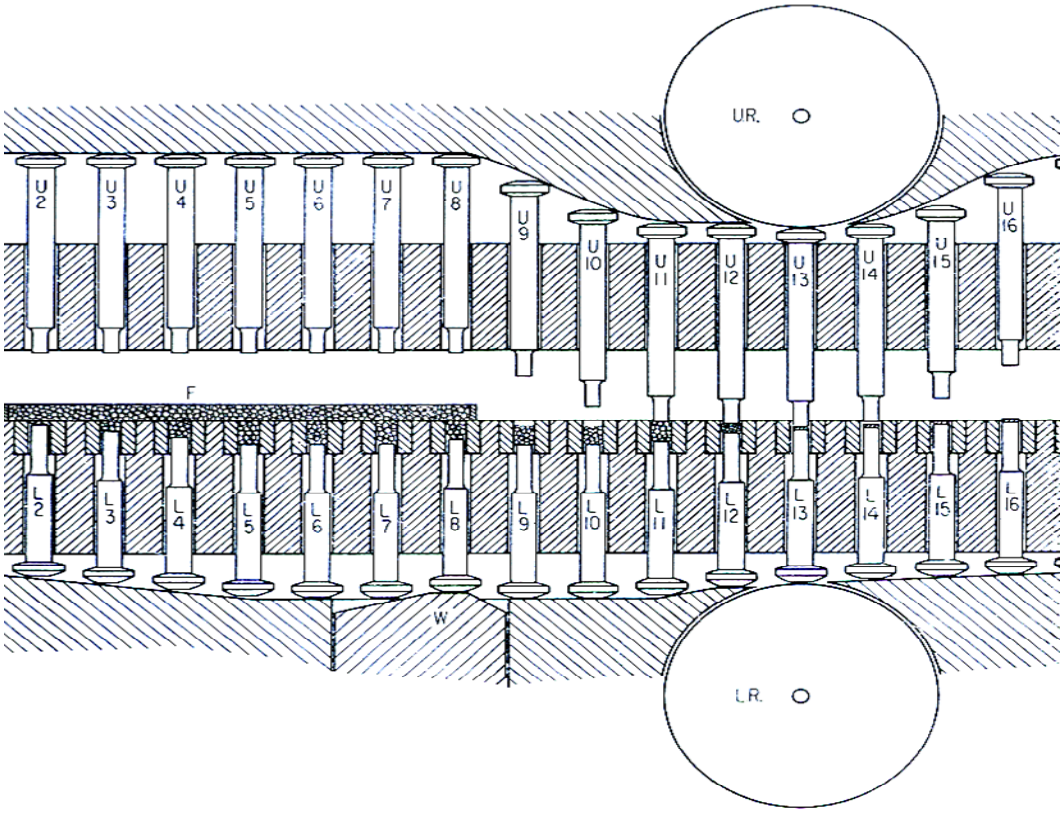


Figure 2.6: Punch tracks of a rotary tablet press (Source: Alderborn, 2002:401)

The size and shape of a tablet as well as certain identification markings are determined by the compression machine tooling (Banker & Anderson, 1986: 295-329). Compression machine toolings are used in research and development and in production of tablets. In research and development, tablet toolings are used to provide basic information on the mechanical and compaction properties of powders that should be used in tablet formulation. This work is normally carried out by single-punch presses. In production tablet tooling are used to control the tabletting operation to ensure that tablet of consistent quality are produced. (Alderborn, 2002: 397-410)

The most common tools employed are referred to as BB and are 133. 4 millimeters (mm) in length, and have nominal barrel diameter of 19.0 mm and 25.4 mm head diameter. B tooling is identical to the BB type except that lower punch is only 90.5 mm long. D tooling is popular for large tablets, utilizing a 25.4 mm barrel diameter, 31.8 mm diameter, and 133.4 mm length. The dies that are used with the above punches are either

a 24.0 mm outside diameter (OD) die capable of making an 11.1 mm round tablet or 14.3 mm capsule-shaped tablet; or a 30.2 mm OD die capable of handling a 14.3 mm round or 19.1 mm capsule shaped tablet. (Banker & Anderson, 1986: 295-329)

2.5.4 Evaluation of tablets

Pharmaceutical tablets are evaluated for their chemical, physical and biological properties. In case of physical or chemical properties, a series of characteristics are generally required to fully identify the particular property. All three property classes may be interrelated. For example, chemical breakdown or interactions between tablets components may alter physical tablet properties in such way that the biological properties are significantly changed. Even without chemical breakdown, various physical properties of tablets can undergo change under environmental or artificial stress conditions, and these changes may be of more significance and concern in some tablets systems than chemical stability.

During the initial formulation screening for a new tablet product, only a minimum number of measurable tablet properties are involved. These may include such properties as tablet size and shape, thickness, colour and sometimes any unique identification markings to be placed on the tablets. (Rosanske, et al., 1989:317-337)

Once the feasibility studies have indicated likelihood for success, a more formal development program is initiated. During the development phase, significant attention is paid to critical quality characteristics of the tablet. This phase is often characterized by the use of statistical design or other means to evaluate and, where possible, quantify those formulation and frequently process variables that have significant effect on the established tablet quality characteristics. Tablet properties often in this phase of development include general appearance, potency/content uniformity, weight variation, hardness, friability, disintegration, dissolution, and stability. (Rosanske, et al., 1989:317-337)

2.5.4.1 General Appearance

General appearance of the tablets is a highly important parameter as viewed by the consumer. As such, all tablets should have an aesthetic appearance that is free of any kind of visual defects. (Rosanske, et al., 1989:317-337)

2.5.4.2 Potency and Content Uniformity

The potency of tablet is generally expressed in terms of grams (g), milligrams (mg) or micrograms (μg) (for very potent drugs) of drug per tablet. Compendial or other standards provide an acceptable potency range around the label potency. For most drugs in the tablet form, the stated compendial range for acceptability is not less than 95 % and not more than 105 % of the labeled amount. The usual method of determining potency of tablet products (average assay content) involves taking 20 tablets, powdering them, taking an accurately weighed sample of the powder, analyzing that powder by an appropriate analytical technique, and calculating the average assay content of the product. To assure uniform potency for tablets of low-dose drugs, a content uniformity test is applied, in this test, not less than 30 tablets are randomly selected for the sample, and at least 10 of them are assayed individually. Nine of the ten tablets must contain not less than 85 % or not more than 115 % of the label claim, Relative Standard Deviation must be less than or equal 6.0 %. The tenth tablet may not contain less than 75 % or more than 125 % of the labeled amount. If either of these conditions is not met, an additional 20 tablets must be individually assayed. None of the additional 20 tablets may fall outside of the 85 % to 115 % range and the Relative Standard Deviation of the 30 tablets may not exceed 7.8 %. (Rosanske, et al., 1989:317-337; USP, 2004:2396)

What appears to be a wide acceptance range (85% to 115%) for content uniformity can often be difficult to achieve. Three factors that can contribute directly content uniformity problems in tablets include nonuniform distribution of the drug substance throughout the powder mixture or granulation, segregation of the powder mixture or granulation during

the various manufacturing processes, and tablet weight variation. (Rosanske, et al., 1989:317-337; Banker & Anderson, 1986:295-329)

2.5.4.3 Uniformity of mass and average mass

A tablet is designed to contain a specific amount of drug in specific amount of tablet formula. As a check that tablet contains the proper amount of drug, tablet weight is routinely measured. Composite samples (usually 20) are taken and weighed throughout the compression process. Weight uniformity can be considered indicative of dosage uniformity provided that major component of the tablet is active drug and the uniformity of drug distribution of the granulation or powder from which the tablets are made is perfect. The USP uniformity of mass test is performed by weighing 20 tablets individually, calculating the average mass, and comparing the individually tablet weight to the average. The tablets meet the USP test if no more than 2 tablets are outside percentage limit (5 %) and if no tablet differs by more than 2 times the percentage limit. (Banker & Anderson, 1986:295-329)

Granulation as well as mechanical problems can contribute to tablet weight variation. If everything is working well mechanically, the weight can be caused to vary by a poorly flowing granulation, which causes spasmodic filling of the dies. Granulation having a wide particle size distribution may have a localized nonuniformity of density within granulation. (Rosanske, et al., 1989:317-337)

2.5.4.4 Hardness

Tablet hardness must be an important consideration to a formulator from the early stages of formulation development, as it can have significant influence on such quality tablet parameters such as disintegration and dissolution properties. It is important to know the relationship between tablet hardness and critical tablet quality characteristics and also the formulation or processing variables that significantly affect the tablet hardness. (Rosanske, et al., 1989:317-337)

Hardness can be defined as being the force required to break a tablet in a diametrical compression test. A hardness test consists of placing the tablet between two anvils and applying pressure to the anvils until the tablet breaks. The crushing strength that causes the tablet to break is recorded. Hardness is sometimes referred as ”tablet crushing strength”. Units for hardness vary depending on the instrument used (e.g. kilo-Ponds, Newtons and kilo-Newtons)

Hardness is a function of applied compressional force and is therefore a function of those factors that cause the force to vary. Tablet size, shape and orientation in the tester will also affect the measured hardness values for a given formulation. Large tablets require a greater force to cause fracture and are therefore often considered “harder” than small tablets. Factors that may alter tablet hardness in the course of a production run are substantial alterations in machine speed and changes in the particle size distribution of the granulation mix during the course of a compression run. These latter changes tend to affect the dies fills. Dies having a light fill (large particles, low density) will produce a softer tablet than dies receiving a heavy fill (small particles, high density). Tablet hardness is often monitored as an in-process test during a compression run, although tablets are often harder several hours after compression than they are immediately after compression. Lubricants can have significant effect on tablet hardness when used in too high concentration or when mixed too long. The lubricant will coat the granulation particles and interfere with tablet bonding. (Rosanske, et al., 1989:317-337; Banker & Anderson, 1986:295-329)

2.5.4.5 Friability

Another measure of a tablet’s strength is its friability. Friability is a measure of the tablet’s ability to withstand both shock and abrasion without crumbling during handling of manufacturing, packing, shipping and consumer use. Normally, a pre-weighed tablet sample is placed in the friabilator chamber which is then rotated for 100 revolutions. Conventional tablets that lose less than 0.5 to1.0 % in weight are generally considered acceptable and when capping is observed during the friability testing, tablets should not

be considered acceptable, regardless of the percentage weight loss. Very dry granulations that contain only fractional percentages of moisture will often produce more friable tablets than will granulations containing 2 to 4 %. (Rosanske, et al., 1989:317-337)

2.5.4.6 Disintegration

It is generally accepted that in order for a drug to be available to the body, it must first be in solution. For most conventional tablets, the first important step in the sequence is the breakdown of the tablet into smaller particles or granules. This process is known as disintegration. Figure 2.7 illustrates the way in which a tablet disintegrates and becomes available in the systemic circulation. The time it takes for a tablet to disintegrate in aqueous medium is measured using a device described in the USP.

The medium used, the temperature of the medium, and the operator performing the test can all have a significant effect on the recorded disintegration times. In addition, many factors involved with a tablet's formulation and method of manufacture can affect the disintegration. The disintegration times can be affected by the nature of the drug, the diluents used, the binders or amount of binder, as well as the manner in which these are incorporated into the tablet. The type and amount of disintegrating agent can profoundly affect disintegration times. The presence of excess amounts of lubricants or excessive lubrication times can increase disintegration times. The compaction pressure used to compress the tablets also influences disintegration, with an increase in pressure generally resulting in an increase in disintegration times. (Rosanske, et al., 1989:317-337)

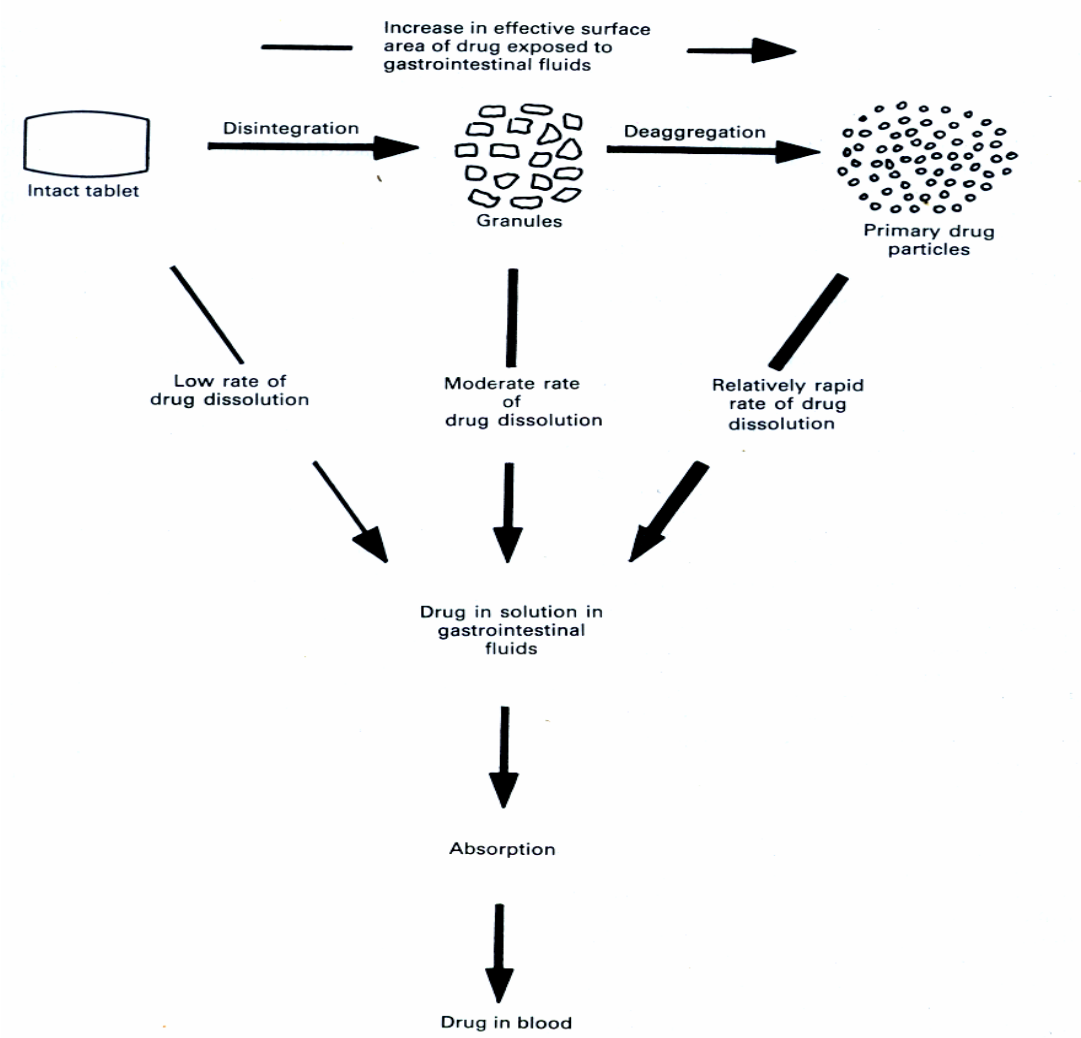


Figure 2.7: Illustration of processes involved for the drug release from a tablet by disintegration and dissolution (Source: Alderborn, 2002:407)

2.5.4.7 Dissolution

Disintegration tests offer no assurance that the formulation will release the drug, even in the form of small particles. Since a drug must normally be in solution before absorption can take place, drugs given via orally administered tablets must dissolve in the contents of the gastrointestinal tract before systemic absorption can occur. Often, the rate of drug absorption is determined by the rate of dissolution from the tablet. Therefore, if it is important to achieve peak blood levels quickly, it will usually be important to obtain rapid drug dissolution from the tablet. For drugs that are absorbed in the upper part of the

gastrointestinal tract, i.e. acidic drugs, rapid dissolution may be especially important. In such cases, the design of the tablet and dissolution profiles may determine the total amount of drug absorbed as well as the rate of absorption. (Rosanske, et al., 1989:317-337)

USP apparatus II method is commonly used to test dissolution. The most commonly used volume for the dissolution flasks with a USP apparatus II is 900 ml. The temperature of the medium is generally 37 ± 0.5 ^{o}C maintained throughout the test with paddles rotating at about 50 revolutions per minute. Water has been used as the preferred medium in many cases, but it is hardly a universal medium because of solubility restrictions and pH changes as the drug dissolves. Addition of buffers can solve this problem. The pH of the medium is an important factor regarding the solubility and stability of most drugs and formulations. (Rosanske, et al., 1989:317-337; USP, 2004:2303)

Various parameters of the dissolution testing can affect results. The dissolution medium used is extremely important. Both the dissolution vessel and the water bath used to warm the dissolution medium should be covered to minimize medium losses due to evaporation. The agitation of the dissolution medium should be widely variable but highly controlled. Both high and low agitation speeds can cause problems in dissolution testing. Dissolution results may be expressed in terms of the concentration of drug in the drug dissolution medium versus time, the amount of drug released from the dosage form versus time, or the time required for a stated amount of the drug to dissolve. (Rosanske, et al., 1989:317-337)

2.5.5 Manufacturing problems

In the normal process of developing formulations, and in the routine manufacture of tablets, various problems occur. Sometimes, the source of the problem is the formulation, the compression equipment, or a combination of the two (Banker & Anderson, 1986: 295-329). The following sections will deal with common problems associated with the manufactured tablets.

2.5.5.1 Binding

Binding in the die or difficult ejection is usually due to insufficient lubrication. It is the resistance of the tablet to ejection from the die. This results in tablets with rough edges. This may be overcome by, increasing lubrication, using tapered dies, and compressing at lower temperature and/or humidity. (Bandelin, 1989:188-190)

2.5.5.2 Sticking and picking

Sticking refers to tablet material adhering to the die wall. Sticking does not allow the lower punches free movement and therefore can place unusual stresses on the cam tracks and punch heads, resulting in their damage. Sticking can also apply to the buildup of material on the punch faces. Picking is a term used to describe the surface material from a tablet that is sticking to and being removed from the tablet's surface by a punch. (Banker & Anderson, 1986: 295-329) These may be overcome by, changing or decreasing the lubricant, adding an adsorbent, and polishing the punch faces (Bandelin, 1989:188-190).

2.5.5.3 Capping and laminating

Capping occurs when the upper segment of the tablet separates from the main portion of the tablet and comes off as a cap. It is usually due to air entrapped in the granulation or powder material that is compressed in the die during compression stroke and then expands when the pressure is released. (Bandelin, 1989:188-190) Lamination is due to the same causes as capping except that the tablet splits and comes apart at the sides and is ejected in two parts. These may be overcome by decreasing the upper punch diameter, changing the lubrication, and increasing the binder. (Bandelin, 1989:188-190)

2.5.5.4 Chipping and cracking

Chipping refers to tablets having pieces broken out or chipped, usually around the edges. This may be due to damaged tooling. Cracked tablets are usually cracked in the centre of

the top due to expansion of the tablet, which is different from capping. It may occur along with chipping and laminating and/or it may be due to binding and sticking. It often occurs where deep concave punches are used. These problems may be reduced by polishing the punches, replacing nicked or chipped punches and adding dry binder. (Bandelin, 1989:188-190)

2.5.5.5 Uniformity of mass

Weight variation is one of the important in-process measurements. The mass of a tablet being compressed is determined by the amount of granulation or powder in the die prior to compression. Therefore anything that can alter the die-filling process can alter tablet mass. (Banker & Anderson, 1986: 295-329)

2.5.5.6 Poor flow

When granulation does not flow readily, it tends to move spasmodically through the feed frame so that some dies are incompletely filled. With poor flow, the addition of a glidant may be helpful. (Banker & Anderson, 1986: 295-329)

2.5.5.7 Poor mixing

Sometimes, the lubricants and glidants are not thoroughly distributed. The flow of particles is then impaired, and the granules do not move efficiently into the dies. (Banker & Anderson, 1986: 295-329)

2.5.6 Factors in formulation development

More than in any other type of tablets, successful formulation of direct compression tablets depend on careful consideration of excipient properties and optimization of the compressibility, fluidity, and lubricability of powder blends. Preformulation studies are

essential in direct compression tabletting even for what would appear to be a simple formulation. (Shangraw, 1989:214-220)

2.5.6.1 Compressibility

The formulation should be directed at optimizing tablet hardness without applying excessive compression force while at the same time assuring rapid tablet disintegration and drug dissolution. In those cases where the drug makes up the greater part of the final tablet weight, the functional properties of the active ingredient, the type and concentration of the excipient dominate the problem. In regard to the active ingredient it is important to determine the effect of particle size on compressibility as well as the effect of crystalline form on compressibility. It may be necessary to granulate the active ingredient by slugging to improve compressibility and increase density. (Shangraw, 1989:214-220)

2.5.6.2 Fluidity

The fluidity of tablet blends is important not only from direct effect on uniformity of die fill and thus uniformity of tablet weight, but also from the role it plays in blending and powder homogeneity. Because of the overall smaller particles size encountered in direct-compression blends, fluidity is a much more serious problem than in the case of granulations. It is important that fluidity specifications be placed on all active ingredients and fillers that make up more than 5 % of a final tablet formulation. If the amount of drug is small, this problem can be overcome by proper choice of excipient fillers. However, when the drug makes up higher proportions of the tablet weight, the use of glidants in addition to careful selection of tablet fillers is necessary. Most direct compression fillers are purposely designed to give good flow properties. (Shangraw, 1989:214-220)

2.5.6.3 Uniformity of content

Highly fluid powder blends facilitate unblending. The narrower the particle size range of all components and the more alike the particle densities, the less chance for unblending or segregation. It is important to note that it is the particle density and not the bulk density that is important in segregation. Major problems with segregation can occur in spherically shaped fillers, particularly if the particle is large and spherical, such as in the case with compressible dextrose. In such case it is necessary to select other excipients to fill the empty spaces or to purposely pre-blend a micronized active ingredient with the large-particle filler. (Shangraw, 1989:214-220)

2.5.6.4 Lubrication

The lubrication of direct-compression powder blend is, if anything more complicated than that of classical granulations. In general, the problems associated with lubricating direct-compression blends can be divided into two categories, namely, the type and amount needed to produce adequate lubrication and the softening effect of lubrication. Because there are already many more surfaces covered with lubricant in direct-compression blends, the softening effect upon compression is magnified. This is particularly true in direct-compression fillers that exhibit almost no fracture or plastic flow on compression. In most instances standard blending times will result in complete coverage of these surfaces. The same blending times in direct compression blends may or may not cover all primary surfaces. Thus the length of blending becomes much more critical in direct compression than in lubrication of tablet granulation. If blended long enough, alkaline stearate lubricants will shear off and completely cover all exposed particle surfaces. (Shangraw, 1989:214-220) Because many lubricants are hydrophobic, tablet disintegration and dissolution are often retarded by the addition of a lubricant (Alderborn, 2002:404-410).

The most common approach to overcome the softening as well hydrophobic effects of alkaline stearate lubricants is to substantially limit the length of time of lubricant

blending often to as little as 2 to 5 min. It is probably advisable in all direct compression powder blending not to include the lubricant during the majority of the blending period.

2.6 MULTIVARIATE METHODS AND STASTICAL ANALYSIS IN PHARMACEUTICAL PRODUCT DEVELOPMENT

2.6.1 Background

Statistics is a branch of mathematics that provides many tools for studying the conditions of formulations and processes and enables us to optimize the same while being able to minimize our experimentation. It is important to keep in mind that as scientists, we bear the responsibility to ensure that our analysis of data remains true and accurate. (Gomez, 2005: 42-45)

Formulation of medical products (e.g., tablets) was previously performed mainly on the basis of the experience of the formulator often in combination with the approach of changing one separate factor at a time, but use of statistical experiment design in connection with commercial statistical software has found widespread use. The drawback with the former alternative is that to keep the number of experiments on an acceptable level, only a few variables can be used for each experiment class. The problem with the latter alternative is that the number of necessary experiments drastically increases with many levels (i.e., excipients) in the qualitative design factor. In a previous study that recognized the need for a more general strategy in tablet formulation, the implementation of multivariate methods for both screening and optimization proved successful. The screening of excipients and the resultant models will enable the identification of suitable excipients for the formulation. (Gabrielsson, et al., 2003:1053-1075)

The development of a pharmaceutical product is a resource-draining endeavor which includes scientists performing preformulation studies, stability studies and formulation development, as well as process development studies. The development of a pharmaceutical product, generic or new chemical entity can take anywhere from six

months to a few years. The end result in most cases is a pharmaceutical dosage form that is robust, uniform, and stable and can be manufactured reproducibly. (Gomez, 2005: 42-45)

2.6.2 Design of experiments (DoE) vs. changing one separate factor at a time (COST) or one factor at a time (OFAT)

The 'COST' approach often does not lead to the real optimum and gives different implications, depending on the starting point. The COST approach also requires many experiments for little gain in information about the system under investigation. (Tye, 2004:485-491) One of the advantages of a multivariate approach lies in the knowledge and material gathered during the screening phase. For instance, if any of the chosen excipients do not work in the ensuing optimization, there are alternatives with documented qualities to choose from. (Gabrielsson, et al., 2003:1053-1075)

DoE is a tool that allows us to evaluate a multitude of factors concurrently while minimizing experimentation. It is important that DoE is not confused with trial and error. Trial and error involves the evaluation of one variable at a time. The advantage of experimenting with multiple variables concurrently is that we learn about interaction effects that would be hidden if we only observed one variable at a time. Another tool available is Analysis of Variance (ANOVA), which allows us to test for significant differences between means and uncovers the main and interaction effects of independent variables on dependent variables. (Gomez, 2005: 42-45).

Independent factors are the tools for our control of formulas/processes. These include for example: blend times, target tablet weights and formulation ingredients and many others. Dependent factors on the other hand allow us to measure the control of our system. Examples are hardness, blend uniformity, dissolution, and many others. (Shiromani, 2004:30-34; Gomez, 2005: 42-45)

A well designed DoE will give us very valuable information and can result in identification of cause and effect relationship between variables. (Shiromani, 2004:30-34; Gomez, 2005: 42-45). The statistical design method often provides a more economical use of resources, especially when many factors exist and provides a greater chance of finding optimum conditions. Also predictions can be made about future experiments and formulations.

One of the main reasons for not using DoE is based on a fear of statistics, which many researchers consider to be a complication. With the advent of sophisticated computer packages that are specially designed for DoE, this is no longer a valid excuse. A further reason for not using DoE is the number of experiments that have to be performed in parallel; these can be reasonably large, depending on the complexity of the design. (Tye, 2004:485-491)

2.6.3 Experimental design

There are several types of statistical design for pharmaceutical formulations, including:
Factorial Design: (both full and fractional factorials)
Response surface Methodology

- Sequential Simplex Technologies
- D-Optimal Technique
- I-Optimal Technique (Shiromani, 2004:30-34)

The objective of experimental design is to plan and conduct experiments in such that maximum information regarding the experimental domain is extracted in the fewest possible experiments. The most commonly used method in experimental design is factorial design method. The experimental variables are given maximum and minimum values based on preexisting knowledge on the topic. In a full factorial experimental design all factors are changed simultaneously. Not only does this cover the entire area of interest with few experiments as possible, it also makes it possible to examine interactions. (Gabrielsson, et al., 2003:1053-1075)

The geometrical representation of the experimental design is a square with two factors and with three factors it is a cube, four factors make up a hypercube, and so on. If *k* variables are investigated at two levels, the number of experiments in the full factorial design is given by 2^k. The number of experiments in an experimental design method grows rapidly with an increasing number of variables. When dealing with many variables ($k>5$), a full factorial design is not the best option, at least not for the purpose of screening. It more appropriate to use only a fractional factorial design. Depending on the number of factors and experiments, the drawback is lost information caused by confounding of interaction effects with main and/or interaction effects. However, confounded effects can be resolved by performing additional experiments. (Gabrielsson, et al., 2003:1053-1075)

The first step is the identification of the variables involved. Depending on the trial formulation these can be formulation or process variables. Once all the critical factors involved have been identified, the levels at which the experimentation is to occur are determined. Having identified all critical factors and the levels at which the experimentation would occur, the scientist can now design the study protocol using any one of the many commercially available statistics programs. The experiments are performed in random order to eliminate the influence of systematic errors. The next step is actual experimentation, whereby all experiments are carried out and samples tested. At this point the data generated can be evaluated by ANOVA. (Gomez, 2005: 42-45).

As an example, paracetamol is known to have poor flowability and compressibility characteristics as a result, the production of paracetamol tablets is almost exclusively by wet granulation, a disadvantageous method when compared to direct compression. The experimental design methodology (DOE) was applied to the development and optimization of tablet formulations containing paracetamol 500 mg and manufactured by direct compression. The physical and chemical data found from the optimized formulation met all pharmaceutical specification, demonstrating that the design of experiments is a great tool for the research and development of new formulations.
(Martinello et al., 2006:87–95)

CHAPTER 3

HIGH PERFOMANCE LIQUID CHROMATOGRAPHY ANALYTICAL METHOD FOR QUANTIFICATION OF LAMIVUDINE

3.1 INTRODUCTION

Analytical method transfer is defined as the process that qualifies a laboratory to use an analytical test procedure (Scypinsk et al., 2002:84-88). The most common variations of method transfer are comparative testing between two laboratories or sites and complete or partial method validation or revalidation. Comparative testing involves two or more laboratories or sites executing a pre-approved protocol that details the criteria by which the receiving laboratory is deemed to be qualified to use the method(s) being transferred. The resulting data are compared with a set of predetermined acceptance criteria. Method validation or revalidation involves the receiving laboratory repeating some or all of the validation experiments depending on the type of method being transferred. During the method validation, one must identify which validation parameters are to be generated or challenged by the sending and receiving laboratories and to accomplish method transfer, it is for the receiving laboratory to repeat some or all of the validation experiments. After validation is completed, the second laboratory is deemed qualified to perform the method. (Scypinsk et al., 2002:84-88).

After the completion of the experiments, the success of an analytical method transfer, in most cases, is tested by comparing statistical results such as means or standard deviations that the participating laboratories obtained after analyzing samples of the same substance. If the test results suggest the rejection of a method transfer, there are two possibilities i.e. the correct rejection of an inappropriate transfer as well as rejecting a good method transfer. The decision has to be made without the knowledge of the true situation. On the other hand, if the results suggest to accept method transfer, therefore the decision to accept the transfer will be correct based on statistical tests (analysis). (Schepers & Wätzig, 2005:310-314)

International Conference on Harmonization (ICH)-Q2 (R1) guideline (ICH, 2005) discusses the characteristics that should be considered during the validation of an analytical method. It is the responsibility of the applicant to choose the validation procedures and protocol suitable for a product. However, it must be remembered that the main objective of validation of analytical method is to demonstrate that it is suitable for its intended use.

For this study an analytical method was obtained from a generic pharmaceutical manufacturer and was validated by means of a reverse-phase high performance liquid chromatography (RP-HPLC) to quantify lamivudine in tablets. The validation study was carried out fulfilling the ICH guidelines in order to prove that the new analytical method meets the reliability characteristics, and that these characteristics show the capacity of an analytical method to keep, throughout the time, the fundamental criteria for validation namely specificity, linearity, precision and accuracy. The method will be applied during the assay of lamivudine tablets, dissolution and content uniformity testing in order to quantify the drug.

3.2 EXPERIMENTAL

3.2.1 Equipment

The chromatographic system consisted of a Waters® 1500 Series HPLC system (Waters® Milford , USA) equipped with a Waters® 1500 series column heater, a Waters® 1525 binary HPLC pump, Rheodyne manual sample injector **(**Rheodyne LLC, Rohnert Park, USA) and a Waters® 2487 Dual Wavelength Absorbance Detector. Data acquisition was performed using Waters® Breeze Chromatography software (Version 3.30).

3.2.2 Materials and reagents

Lamivudine (100.0 % m/m) (Hetero Labs Limited, Gaddapotharam, India) was kindly donated by Aspen Pharmacare, Port Elizabeth, South Africa. Ammonium acetate (97.0 %

m/m) and glacial acetic acid (99.8 % v/v) were obtained from UNIVAR® (Redmond, USA). Methanol Chromasolv® for HPLC (99.9% v/v) was obtained from Sigma-Aldrich (Steinheim, Germany). Hydrochloric acid (32.25 % v/v) (BDH, West Chester, USA) supplied by Merck Chemicals (Pty) Ltd (Midrand, South Africa). Sodium hydroxide pellets AR was obtained from MINEMA (Johannesburg, South Africa).

Tablets used corresponded to the 'Lamivudine 150mg' tablet preparation and its declared excipients, which included Microcrystalline cellulose (Vivapur® PH102), Sodium starch glycollate (Vivastar®) (JRS Pharma, Weissenborg, Germany) and Magnesium stearate (Liga Magnesium Stearate MF-2-V) (Peter Green, Edisonstraat, Netherlands). A placebo product for the validation study was prepared with these excipients. These chemicals were kindly donated by Aspen Pharmacare

3.2.3 Chromatographic conditions

Analysis was performed using a Luna C8 (2) 5 µm 100 Å chromatographic column with dimensions 150 mm × 4.6 mm internal diameter (Phenomenex®, Torrance, USA). Separation was achieved at a flow rate of 1.0 ml min^{-1} at 40 °C. Column back pressure ranged from 1082 - 1100 psi. A 10 µl aliquot of sample was injected using the manual injector and an observation time of 15 minutes was allowed. UV detection was at 265 nm.

3.2.4 Mobile phase preparation

Ammonium acetate buffer (pH = 3.8) was prepared by dissolving 1.9 g ammonium acetate in 1000 ml of water (reverse osmosis) and then 5 ml of acetic acid was added and the pH adjusted to 3.8 using acetic acid. The mobile phase was prepared off line by mixing ammonium acetate buffer with methanol at a ratio of 90 % acetate buffer: 10 % methanol. The mobile phase was degassed for 5 minutes by ultrasonication (Ultrasonic LC 130, Labotec, Göltingen, Germany) and filtered through a 47 mm G.H Polypro

hydrophilic polypropylene 0.45 µm membrane filter (Pall®, Ann Arbol, USA) prior to its use. The mobile phase was also used as a solvent during validation studies

3.2.5 Sample preparation

Full details about the specific sample preparation are described under the Validation Study (Section 3.3). The mobile phase was used as a solvent for sample preparations and all the samples were degassed for 5 minutes by sonication and then filtered through 33 mm Millex®-HV Millipore Hydrophilic PVDF 0.45 µm filter units (Millipore, Billerica, USA).

3.3 VALIDATION STUDY

3.3.1 Specificity

Specificity of an analytical procedure is its ability to assess or identify unequivocally the analyte in the presence of formulation components, which may be expected to be present (Ermer, 2005: 3-19).

For the specificity study, identification of the active, the samples corresponding to placebo and final product were studied under different degrees of stress conditions such as exposure to sodium hydroxide 1M, hydrochloric acid 1M and high temperatures.

The following list states the stress conditions, dilutions and the quantities used for each sample analysed for the specificity study. All the samples were prepared as explained in Section 3.2.5.

Details for the specificity study are given below:

1. Mobile phase as solvent.
2. Placebo at working concentration: 350 mg of the product placebo dissolved in 500 ml solvent, diluted 15 ml to 100 ml with solvent and filtered.

3. Active at working concentration: 45 mg of lamivudine dissolved in 100 ml solvent, diluted 10 ml to 100 ml with solvent and filtered.
4. Product at working concentration: 350 mg of the product dissolved in 500 ml solvent, diluted 15 ml to 100 ml with solvent and filtered.
5. Product stressed for 24 hours at 60 °C: 350 mg of the product dissolved in 500 ml solvent, diluted 15 ml to 100 ml with solvent and filtered.
6. Product stressed for 24 hours at 105 °C: 350 mg of the product dissolved in 500 ml solvent, diluted 15 ml to 100 ml with solvent and filtered.
7. Acid hydrolysis (1.0 M hydrochloric acid (HCl) hydrolysis, standing-6 hours): 875 mg of the product dissolved in 50 ml solvent. Pipetted 5.0 ml into a 10.0 ml volumetric flask. Added 0.2 ml of 1.0 M hydrochloric acid. Allowed to stand for 6 hours. Added 0.2 ml of 1.0 M sodium hydroxide. Made up to10 ml with solvent and filtered.
8. Base hydrolysis (1.0 M sodium hydroxide (NaOH) hydrolysis, standing-6 hours): 875 mg of the product dissolved in 50 ml solvent. Pipetted 5.0 ml into a 10.0 ml volumetric flask. Added 0.2 ml of 1.0 M sodium hydroxide. Allowed to stand for 6 hours. Added 0.2 ml of 1.0 M hydrochloric acid. Made up to10 ml with solvent and filtered.
9. Peroxide oxidation (5 % hydrogen peroxide, standing-6 hours): 875 mg of the product dissolved in 50 ml solvent. Pipetted 5.0 ml into a 10.0 ml volumetric flask. Added 0.2 ml of 5 % hydrogen peroxide. Allowed to stand for 6 hours. Made up to10 ml with solvent and filtered.

After the stress assays, each solution was analyzed under the conditions specified in Section 3.2.3 above.

The requirements for specificity for this method are: The solvent, placebo and degradation products must contain no components that co-elute with the lamivudine peak**.**

3.3.2 Linearity

Linearity of an analytical procedure is its ability, within a given range, to obtain test results that are directly proportional to the concentration of analyte in the sample. (Ermer, 2005: 3-19)

To carry out this study, five analyte concentrations within the range 50–150 % of the working concentration (75.00 µg/ml) were prepared by dissolving 75 mg and 112.5 mg of lamivudine raw material in a 100 ml of the solvent to make up 100 % and 150 % of the solution respectively. An 80 ml aliquot of the solvent was added and the samples were sonicated for 5 minutes and the solutions were allowed to cool and made up to volume with solvent. A 10 ml aliquot of each solution was then transferred into a 100 ml volumetric flask and made up to volume with the solvent. A 5 in 10 and 7.5 in 10 dilution was used to prepare the 50 % and 75 % concentrations respectively from the working concentration (75.00 µg/ml). An 8.33 in 10 dilution was used to prepare 125 % concentration from the 112.50 µg/ml (150 %) solution. Each solution was analyzed in triplicate. A graph of area under curve response (AUC) was plotted as a function of theoretical lamivudine concentration and linear regression analysis was performed using the software packaging Microsoft (MS) Excel® (version 2003).

The requirements for linearity for this method are that the correlation co-efficient of the regression line (R^2) must be greater than or equal to 0.999 and the y-intercept must not be significantly different from zero (i.e. z-value should be not be greater than 2 % and not smaller than -2 % of the 100 % response). Z-value is equal to the y-intercept as the percentage of the analytical response at the 100 % analyte level (Carr & Wahlich, 1990: 615-616).

3.3.3 Precision

Precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions (Ermer, 2005: 3-19).

Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Repeatability precision expresses the analytical variability under the same operating condition over a short period of time. Intermediate precision expresses within-laboratory variations (i.e. different days, different analysts and different equipment) and reproducibility precision is done between laboratories (inter-laboratories studies). (Ermer, 2005: 3-19). For the purpose of this study only reproducibility and repeatability precision studies would be considered, since the method is transferred from one laboratory to another and only one HPLC system would be used.

Five different solutions of lamivudine analyte within the range of 50 %-150 % were prepared, using the same method used for linearity study (Section 3.3.2) relative to the working concentration of 100 µg/ml. Each solution was injected in triplicate. The experimental results were analyzed, using MS Excel® (version 2003) to calculate the relative standard deviation (RSD) from each sample.

The requirement for reproducibility precision for this method is that percentage RSD due to lamivudine concentration for the five samples must be less than or equal to 2 %.

Lastly, the repeatability precision was studied whereby the samples were prepared according to the same method as in the reproducibility precision study and examining the variability that takes place when the same analyst works on different days. The experimental results were analyzed, using MS Excel® (version 2003) to calculate RSD from each sample.

Requirements for repeatability precision for this method are that the % RSD for the five lamivudine sample must be less than or equal to 2 %. The mean results obtained in the repeatability and reproducibility precision studies must not differ by more than 2 %.

3.3.4 Accuracy

Accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found (Ermer, 2005: 3-19).

Five different solutions of lamivudine analyte within the range of 50 % - 150 % were prepared, using the same method as used for the linearity study at the relative working concentration of 100 µg/ml. Each solution was injected in triplicate. The experimental results were analyzed, using MS Excel® (version 2003) to calculate percentage recovery from each sample. The theoretical concentrations were calculated from the linear regression curve, and compared to the actual concentration obtained.

The requirement for accuracy for this method is that the percentage recovery of lamivudine for each solution prepared must be within the 98-102 % limit.

3.3.5 Dissolution method

The dissolution test method that was used to calculate the % released of lamivudine in tablets was validated as follows: A standard solution was prepared and used to calculate the drug release. The standard solution was prepared as follows:
A 17 mg quantity of lamivudine (100 % potency) was transferred to a 100 ml volumetric flask and dissolved in 50 ml of water. The solution was then sonicated (degassed) for 5 minutes and allowed to cool at room temperature .The solution was then made up to 100 ml with water and mixed. A 10 µl of standard solution was injected repeatedly until the retention time of the peak due to lamivudine was reproducible. The standard solution

was injected six times and the average of the peak area and % RSD of the six replicates were calculated using MS Excel® (version 2003).

The dissolution method was validated for its precision the requirements for the validation of this method are that % RSD of the peak areas due to the active for six replicate injections is not more than 1 % and the peaks due to the active should elute at the same time.

3.3.6 Assay and content uniformity methods

The assay and content uniformity tests method used to calculate the assay and content uniformity, respectively, of lamivudine in the tablet dosage form were validated as follows. The standard solution was prepared and used to calculate the assay and content uniformity of the tablets: A 10 mg of lamivudine (100 % potency) was transferred to a 100 ml volumetric flask. A 50 ml aliquot of solvent was then added and sonicated for 5 minutes. After sonication the mixture was allowed to cool to room temperature and made up to 100 ml with solvent and mixed well. A 10 µl of the standard solution was repeatedly injected until the retention time of the peak due to lamivudine was reproducible. The standard solution was injected six times and the average of the peak area and the % RSD of the six replicates were calculated using MS Excel® (version 2003).

The assay and content uniformity methods were validated for precision and the requirements for the validation of these methods are that the % RSD of the peak areas due to the active for six replicate injections is not more than 1 % and the peaks due to the active should elute at the same time.

3.4 RESULTS & DISCUSSION

3.4.1 Specificity

Specificity as described above is the ability to accurately measure the analyte response in the presence of all potential sample components (Ermer, 2005: 3-19).

The following is the list of chromatograms indicating the results obtained for the specificity study under different dilutions and stress conditions.

1. **Solvent**: The chromatogram obtained after injecting an aliquot of mobile phase showed only typical baseline noise and no peaks.

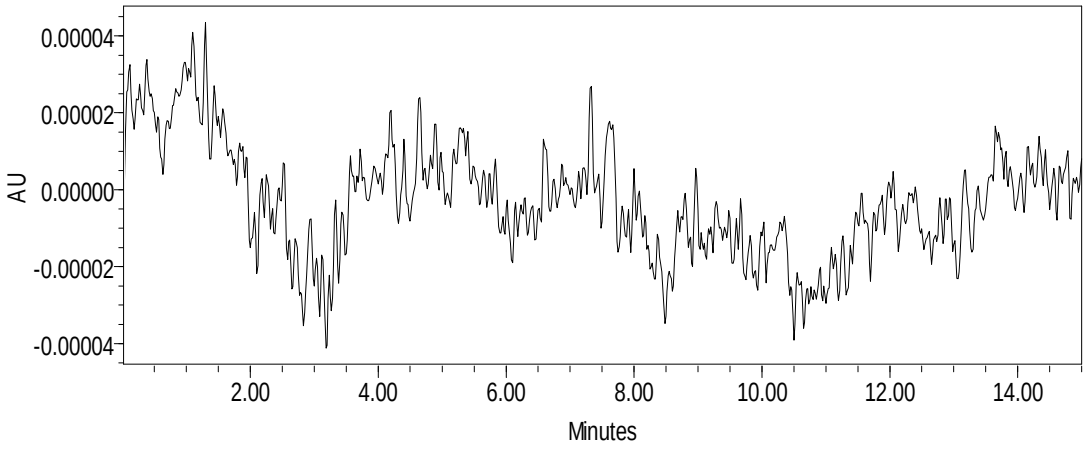


Figure 3.1: Chromatogram obtained for solvent (90 % acetate buffer (pH=3.8): 10% methanol)

2. **Placebo at working concentration:** The chromatogram obtained after injecting a placebo solution showed only typical baseline noise and no peaks.

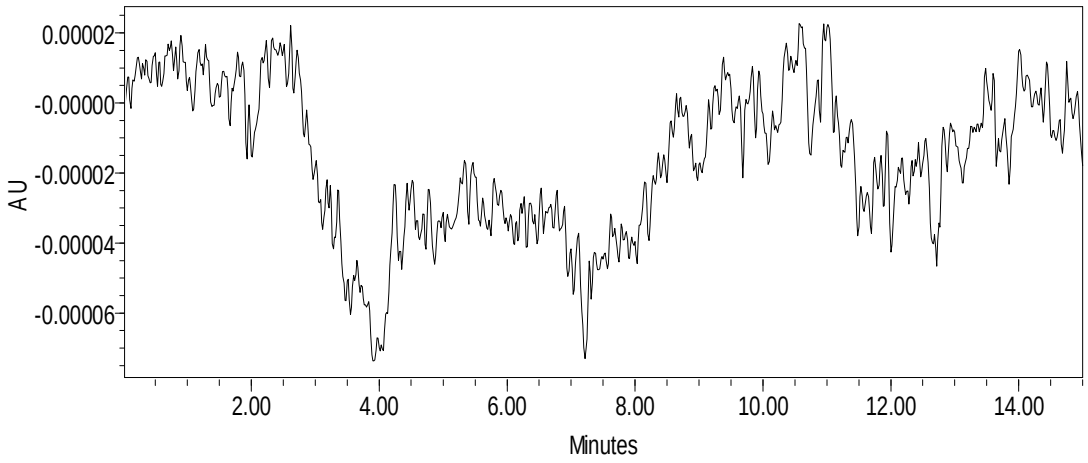


Figure 3.2: Chromatogram obtained for placebo at working concentration

3. **Active at working concentration**: The peak due to lamivudine eluted at **3.249 minutes** with no potential contaminants that co-eluted with the active peak.

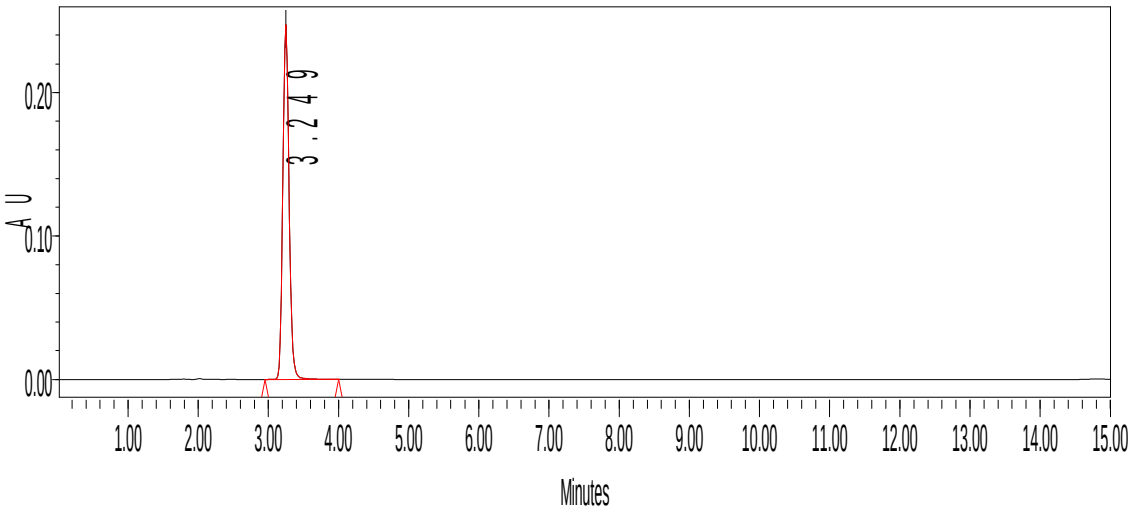


Figure 3.3: Chromatogram obtained for lamivudine at working concentration

4. **Product at working concentration**: Only the peak due to lamivudine which eluted at **3.224 minutes** was observed after injecting the product at working concentration.

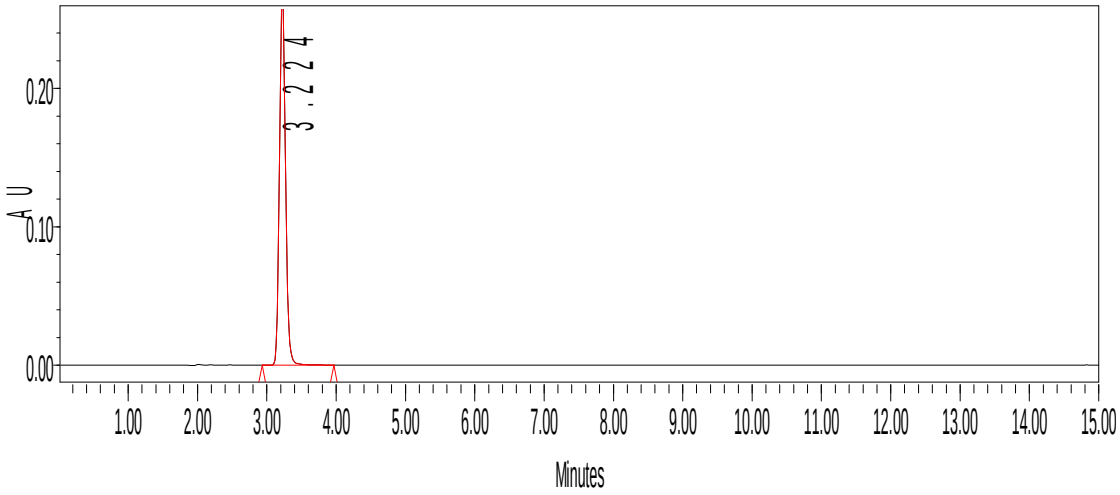


Figure 3.4: Chromatogram obtained for product at working concentration

5. **Product stressed for 24 hours at 60 °C**: Only the peak due to lamivudine which eluted at **3.239 minutes** was observed.

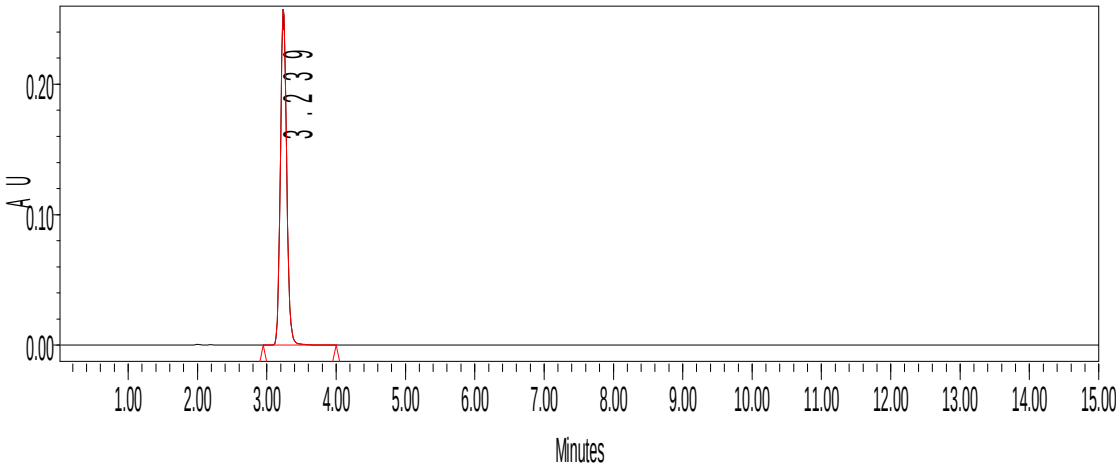


Figure 3.5: Chromatogram obtained for product stressed for 24 hours at 60 °C

6. **Product stressed for 24 hours at 105 °C**: Only the peak due to lamivudine which eluted at **3.237 minutes** was observed.

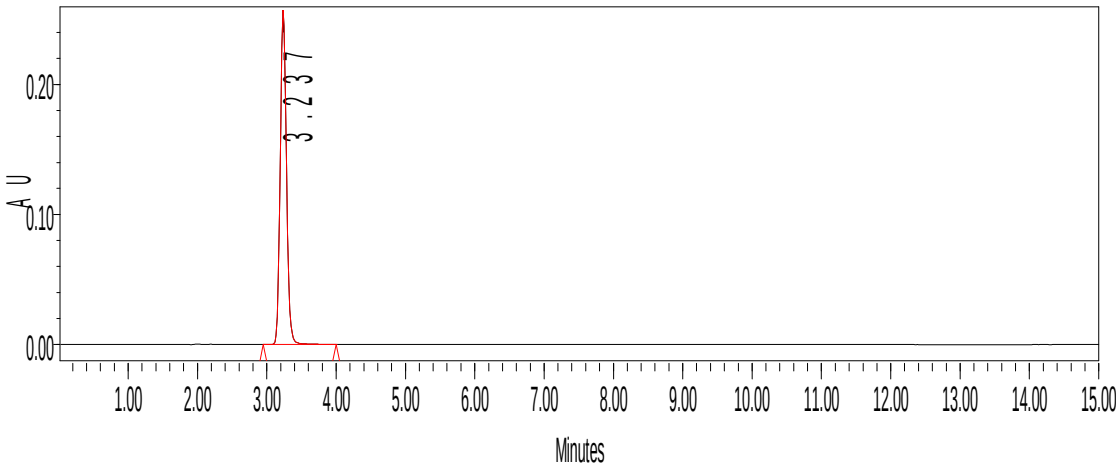


Figure 3.6: Chromatogram obtained for product stressed for 24 hours at 105 °C

7. **Acid hydrolysis (1.0 M HCl hydrolysis, standing-6 hours)**: The peak due to lamivudine eluted at **3.272 minutes** with no other peaks showing co-elution with the active. Four unknown peaks due to degradation products peaks were observed at 2.028, 3.037, 4.042 and 4.564 minutes that do not interfere with the quantification of the active.

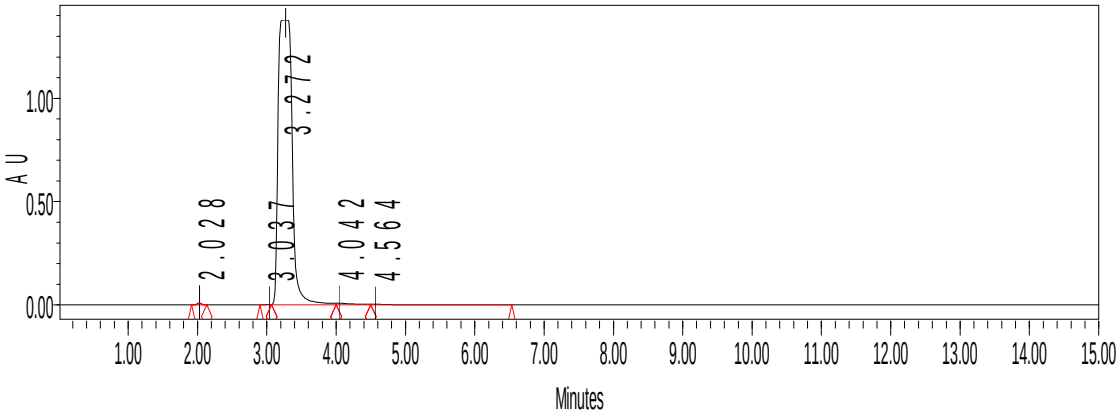


Figure 3.7: Chromatogram obtained for Acid hydrolysis (1M hydrolysis, standing-6 hours)

8. **Base hydrolysis (1.0 M NaOH hydrolysis, standing-6 hours):** The peak due to lamivudine eluted at **3.273 minutes** with no other peaks showing co-elution with the active. Three unknown peaks due to degradation products peaks were observed at 2.032, 4.055 and 4.556 minutes that do not interfere with the quantification of the active.

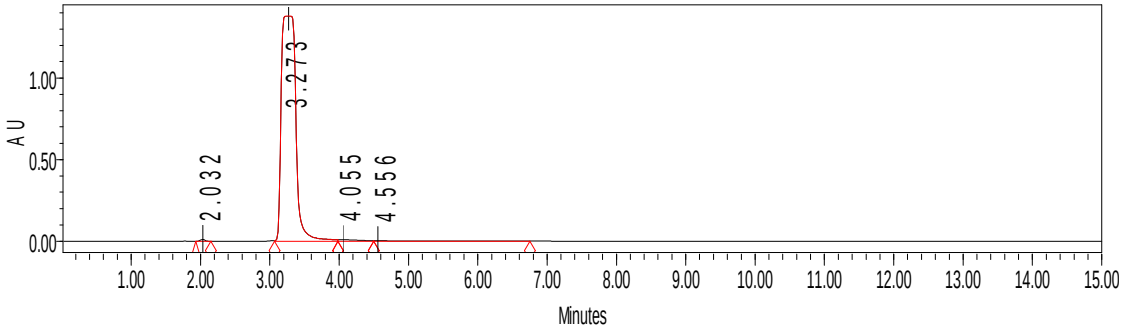


Figure 3.8: Chromatogram obtained for base hydrolysis (1M hydrolysis, standing-6 hours)

10. **Peroxide oxidation (5 % hydrogen peroxide, standing-6 hours)**: The peak due to lamivudine eluted at **3.255 minutes** with no other peaks showing co-elution with the active. Four unknown due to degradation products peaks were observed at 1.764, 2.022, 2.440, 4.015 and 4.525 minutes that do not interfere with the quantification of the active.

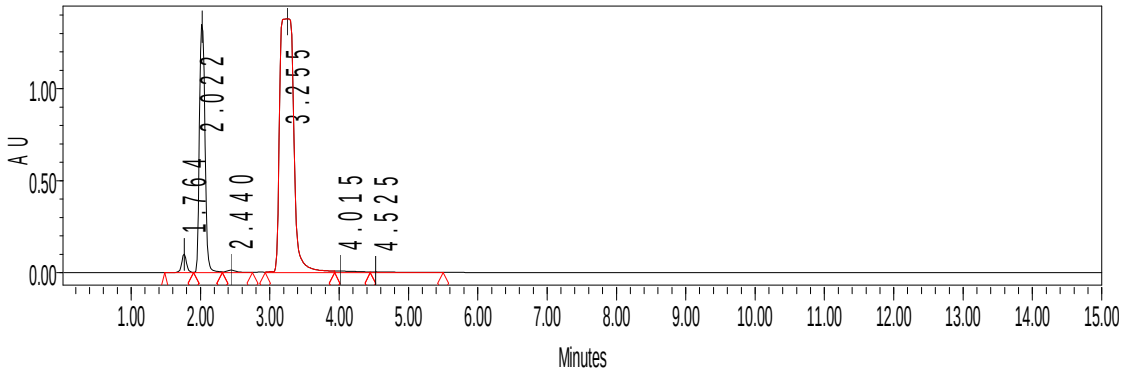


Figure 3.9: Chromatogram obtained for peroxide oxidation (5 % hydrogen peroxide, standing-6 hours)

The results showed that no degradation products co-eluted with the lamivudine peak. The inclusion of excipients did not show any interference with the active elution time as there was consistency with respect to the active elution time. To conclude, it can be stated that none of the peaks that could be generated by the stress treatment interfere with the peak corresponding to the active, therefore showing it was a selective method and suitable for quantitative analysis of lamivudine.

3.4.2 Linearity

Table 3.1 shows the data obtained for linearity study over concentration range 37.50 to 112.50 µg/ml. The plot for the linearity graph, area under curve (AUC) as a function of concentration (µg/ml) was shown to be linear over the concentration range 37.50 to 112.50 µg/ml (Figure 3.10). The linear regression equation for the concentration range 37.50 to 112.50 µg/ml was: $y = 6 \times 10^7 x + 15870$, and a correlation coefficient of the regression line, R^2, was equal to 0.9995. The % RSD of the peak areas due to lamivudine for the triplicate injections was not more than 2 %.

Table 3.1: Linearity data for lamivudine at the concentration range (37.50 to 112.50 µg/ml; n = number of injections per sample)

Concentration (µg/ml)	**Percentage (% m/v)**	**Average response (AUC) (n=3)**	**% RSD**
37.50	50	2237681	0.038
56.25	75	3311151	1.35
75.00	100	4337358	0.066
93.75	125	5535392.67	0.240
112.50	150	6614823.67	0.200

The significance for the y-intercept (z-value) was calculated as described in Section 3.3.2 using working concentration (75.00 µg/ml) as the 100 % response value:

$$z = \frac{y - \text{intercept}}{100\%\text{respose}} \times 100$$

$$z = \frac{15870}{4337358} \times 100$$

$$z = 0.366\%$$

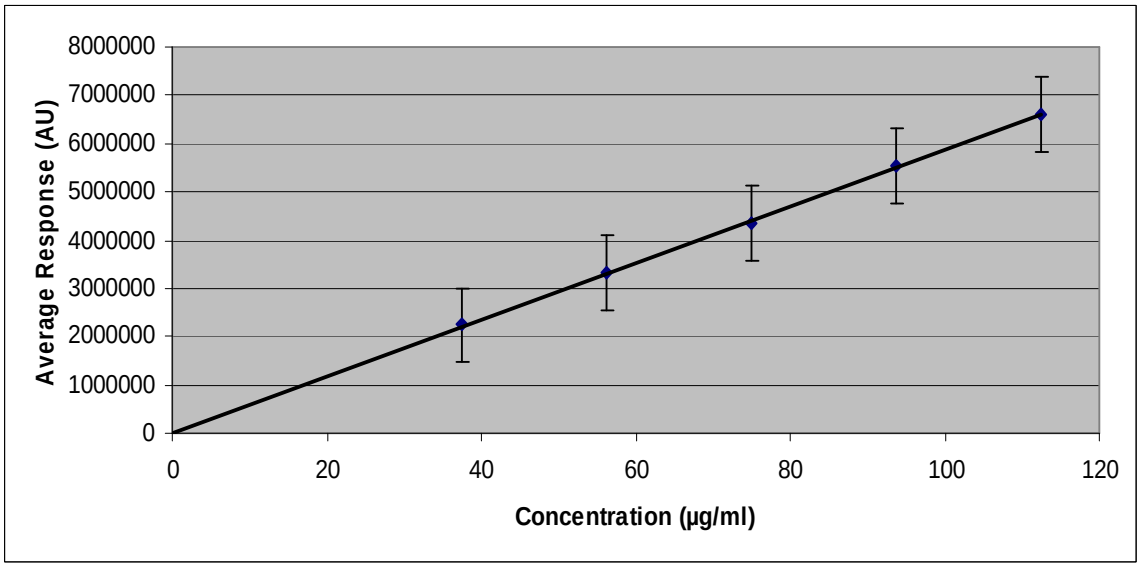


Figure 3.10: Standard curve for the concentration range 37.50 to 112.50 µg/ml (y = 6 $\times 10^7$ x + 15870; R^2 = 0.9995) (n=3 for each point)

The z-value was less that 2 % but greater than -2 % of the 100 % response. In conclusion, the requirements for linearity were met, the correlation coefficient of the regression line was greater than 0.999 and the y-intercept was not significantly different from zero (2>z>-2). The method for lamivudine was therefore linear within the specified concentration range.

3.4.3 Precision

3.4.3.1 Reproducibility precision

The data obtained for the reproducibility precision study over concentration range 37.5 to 112.50 µg/ml is shown in Table 3.2

Table 3.2: Precision data for lamivudine at the concentration range 37.50 to 112.50 µg/ml (n = number of injections per sample)

Concentration (µg/ml)	**Percentage (% m/v)**	**Average response (AUC) (n=3)**	**RSD (%)**
37.50	50	2271454	1.10
56.25	75	3385099	1.83
75.00	100	4452278	1.44
93.75	125	5552078	1.26
112.50	150	6692828	0.90

The percentage relative standard deviations calculated for all the specified concentration ranges were less than 2 % for the area (AUC) obtained. The requirement for precision is that the % RSD for the five lamivudine sample must be less than or equal to 2 %, therefore method for lamivudine was precise within the specified concentration range.

3.4.3.2 Repeatability precision

Table 3.3 shows the data obtained for repeatability precision study over concentration range 37.50 to 112.50 µg/ml. The % RSD calculated for all the specified concentration ranges were less than 2 % for the area obtained as shown in Table 3.3

Table 3.3: Repeatability precision data for lamivudine at the concentration range 37.50 to 112.50 µg/ml (n = number of injections per sample)

Concentration (µg/ml)	Percentage (% m/v)	Average response (AUC) (n=3)	RSD (%)
37.50	50	2310687	0.19
56.25	75	3367160	0.22
75.00	100	4433897	1.26
93.75	125	5584180	1.14
112.50	150	6637602	1.18

Table 3.4 shows the data extracted from the repeatability and reproducibility precision studies to study the consistency of the system over concentration range 37.50 to 112.50 µg/ml.

Table 3.4: Repeatability and reproducibility precision data to compare the average responses

Concentration (µg/ml)	Percentage (% m/v)	Average response (Precision) (n=3)	Average response (Repeatability precision) (n=3)	Average	RSD (%)
37.50	50	2310687	2271454	2291070.5	1.21
56.25	75	3367160	3385099	3376129.5	0.38
75.00	100	4433897	4452278	4443087.5	0.29
93.75	125	5584180	5552078	5568129	0.41
112.50	150	6637602	6692828	6665215	0.59

In both precision studies the % RSD obtained was below 2 %, the limit percentage set for the precision study of the instrumental system, and the mean results obtained in the repeatability and precision studied were not different by more than 2 %, thus showing that the equipment used for the study worked correctly for the developed analytical method.

3.4.4 Accuracy

The results obtained for the accuracy study over the concentration range 37.50 to 112.50 µg/ml are shown in Table 3.3. The linear regression equation from the linearity study ($y = 6 \times 10^7 x + 15870$) was used to calculate the percentage recovery and is calculated as follows:

$$y = 6 \times 10^7 x + 15870$$

$$\% \text{ recovery} = (2254063 - 15870)/6 \times 10^7 / 0.0375 \times 100$$

$$\% \text{ recovery} = 99.47524$$

Table 3.5: Accuracy data for lamivudine at the concentration range 37.50 to 112.50 µg/ml (n = number of injections per sample)

Concentration (µg/ml)	**Percentage (% m/v)**	**Average response (AUC) (n=3)**	**RSD (%)**	**% RECOVERY**
37.50	50	2254063	0.11	**99.48**
56.25	75	3331355	0.53	**98.24**
75.00	100	4448649	1.10	**98.51**
93.75	125	5531663	1.49	**98.06**
112.50	150	6646176	0.46	**98.23**

The percentage recovery for all specified concentration ranges were within the limits of 98 % to 102 %, satisfying the acceptance criteria for the study thus the analytical method for lamivudine quantification proved to be accurate within the specified concentration range.

3.4.5 Dissolution

The peak due to lamivudine for all six injections eluted at **3.25 minutes** with no potential contaminants that co-eluting with the active peak. The % RSD calculated for the peak areas due to the active for six replicate injections was less than 1 % as shown in Table

3.6. The average peak area obtained was used to calculate the % released of lamivudine during tablet dissolution testing

Table 3.6: Summary of the results obtained for dissolution method validation for 170 µg/ml (standard solution)

Lamivudine retention time	Average response (AUC) (n=6)	% RSD
3.25	7117134	0.52

3.4.6 Assay and content uniformity tests

The peak due to lamivudine for all six injections eluted at **3.25 minutes** with no potential contaminants that co-eluted with the active peak. The % RSD calculated for the peak areas due to the active for six replicate injections was less than 1 % as shown in Table 3.7. The average peak area obtained was used to calculate the % released of lamivudine during tablet assay and content uniformity test.

Table 3.7: Summary of the results obtained for dissolution method validation for 100 µg/ml concentration (standard solution)

Lamivudine retention time	Average response (AUC) (n=6)	% RSD
3.25	4511018	0.45

3.5 SUMMARY

An HPLC method for the assay of lamivudine raw material and its tablet formulation was validated in this study. Lamivudine and its degradation products gave chromatograms of very well resolved peaks with no co-elution with the lamivudine peak, which confirms the specificity of the method and the possibility of using it as an indicator of stability.

All the statistical values (correlation co-efficient of the regression line, the y-intercept and the z-value) for linearity study were within the acceptable limits. The percentage RSD for both precision and repeatability precision studies were within the required limits. The results for precision obtained under the same operating condition over a short period of time (repeatability precision) were within the specified limit (RSD < 2 %). The percentage recovery by the assay of known added amount was within the required percentage limit (98-102 %). The method has proved to be linear, accurate and precise between 50 and 150 % of the working concentration (75.00 µg/ml) for lamivudine.

The HPLC method used to determine lamivudine content in tablets has been proven to be linear, precise, accurate and specific, and is therefore suitable for use in routine testing of lamivudine tablets. The developed stability-indicating HPLC method will therefore be used for the quantitative analysis of lamivudine raw material and its tablets dosage form

CHAPTER 4

PREFORMULATION STUDIES

4.1 INTRODUCTION

Preformulation studies are aimed on identifying the physicochemical properties of drug substances and excipients that may influence the formulation design, method of manufacturing and biopharmaceutical properties of the resulting product. These preformulation investigations may merely confirm that there are no significant barriers to the drug development (Fiese & Hagen, 1986: 171-175).

Drug flowability is one of the important aspects of the direct compression method. It is affected mainly by particle size, particle shape and the excipients used. This chapter investigates the lamivudine particle size distribution, morphological features and its compatibility with some of the excipients.

4.2 MATERIALS

Lamivudine fine ($D_{0.9}$ = 100 µm) and coarse grade ($D_{0.9}$ = 350 µm) (Hetero Labs Limited, Gaddapotharam, India), microcrystalline cellulose (Vivapur® PH102), sodium starch glycollate (Vivastar®) (JRS Pharma, Weissenborg, Germany) and magnesium stearate (Liga Magnesium Stearate MF-2-V) (Peter Green, Edisonstraat, Netherlands) were kindly donated by Aspen Pharmacare, Port Elizabeth, South Africa. All the ingredients were used as received. The D-values mentioned for both lamivudine grades were obtained from the supplier's certificate of analysis.

4.3 METHODS OF ANALYSIS

Lamivudine raw material was evaluated for its flow properties, compatibility with the excipients and particle size distribution. The following tests were conducted to evaluate the fine and coarse lamivudine grades, namely bulk and tapped density, particle size distribution, sieve analysis, scanning electron microscopy and drug-excipient compatibility.

4.3.1 Particle size distribution

Particle size distribution of the fine and coarse grades lamivudine materials was evaluated by two methods, namely, laser diffraction and sieve analysis tests

4.3.1.1 Laser Diffraction

A Malvern Mastersizer 2000 (Malvern Instruments Ltd, Worcestershire, England) with Mastersizer software (version 5.22) instrument was used to measure particle size distribution. The measuring cell was placed in the optical bench and connected to the Hydro 2000 SM dispersion unit. The measurement window was opened and all the required measurement parameters were set, as follows:

<u>Dispersion unit</u>

Pump speed : 2000 rpm

Ultrasound : none

<u>Measurement settings</u>

Calculation model : General purpose

Sensitivity : Normal

Measurement time : 20 s

Background time : 40 s

Obscuration range : 5-20 %

A 120 ml of liquid light paraffin was added to the dispersion unit and the pump speed was set to 2000 rpm. The pump was then activated and the background was measured after it had stabilized. After measuring background, the pump speed was increased to 3000 rpm. Sufficient lamivudine sample was added directly to dispersion unit, until a suitable obscuration was achieved. The sample was dispersed for 1 min. at 3000 rpm and the pump speed was decreased to 2000 rpm. Measurements were initialized after the dispersion had stabilized. Five measurements were taken for each sample tested and the average of these was reported.

4.3.1.2 Sieve analysis

Madison test sieves (Madison Filter SA (Pty) Ltd, Johannesburg, South Africa) and a Prufsieb Jel 200 sieve shaker (Engelsmann AG, Rhein, Germany) were used for sieves analysis.

The sieves were arranged in the order of decreasing aperture sizes as shown in Table 4.1. The sieves including the sieve tray were weighed before being arranged to produce 'tare' masses in grams. Approximately 100 g (M_1) from each sample was weighed and poured into the top sieve and then closed with a metal lid to ensure that it does not open. The sieve shaker was then set for 15 minutes. After the shaker had stopped, each sieve with a sample was weighed to give the gross masses (g). The net mass (g) was then calculated as follows:

$$\text{Net(g)} = \text{Gross(g)} - \text{Tare(g)} \qquad \textbf{(}\text{Equation}\textbf{: 4.4)}$$

Particle size distribution was then calculated as follows:

$$\%\ \text{Distribution} = \frac{\text{Nett(g)}}{M_1} \times 100 \qquad \textbf{(}\text{Equation}\textbf{: 4.5)}$$

Table 4.1: Arrangement of test sieves

Aperture size (µm)
850 µm
600 µm
425 µm
250 µm
100 µm
75 µm
TRAY

Since this method requires a large quantity of a chemical to be tested and has proven to be inaccurate, particle size distribution testing by sieve analysis was only conducted on lamivudine fine material.

4.3.2 Scanning electron microscopy (SEM)

Dry powders were examined for their morphological features using a Jeol JSM-6380 LV scanning electron microscope (Akishima, Tokyo, Japan). A portion of each lamivudine grade was mounted on different SEM-stubs. The mounted samples were then sputter coated with gold using a Nanotech SemPrep 2 sputter coater. The photomicrographs for lamivudine fine and lamivudine coarse materials were taken at 370 X and 50 X magnifications, respectively, and analyzed for their morphological features.

4.3.3 Bulk and tapped density

4.3.3.1 Bulk density

The USP1 test apparatus (measurement in a graduated cylinder) was used for bulk and tapped density testing (USP, 2004:2271). A sufficient quantity of the material being tested was passed through a 1.00 mm (US Sieve No. 18) sieve to break up the agglomerates that may have formed during storage, into a dry 100 ml glass graduated

cylinder. The powder was then leveled without compacting. The unsettled apparent volume (V_o) was read to the nearest graduated unit. The graduated cylinder with the powder was weighed again and the difference was calculated to get the mass (M) for the sample tested.

The Bulk Density was then calculated in g/ml, using the following equation (USP, 2004:2271):

$$\text{Bulk density} = \frac{M}{V_O} \qquad \textbf{(Equation: 4.1)}$$

4.3.3.2 Tapped density

The cylinder containing the sample (from the bulk density test) was tapped by raising the cylinder and allowing it to drop under its own weight using an Electrolab Automated Tap Density Tester ETD-1020 (Electrolab, Bombay, India) that provided a fixed drop of 14 ± 2 mm at a nominal rate of 300 drops per minute. Initially, the cylinder was tapped 500 times, and the tapped volume, V_a, was then read to the nearest graduated unit. The tapping was then repeated for 750 times and the tapped volume, V_b, was read to the nearest graduated unit. If the difference between the two volumes was less than 2 %, then V_b would then be the final volume. After 750 times (1250 times in total), the difference between two volumes was greater than 2% therefore the tapping was then increased to 1250 times as needed, volume (V_f), was read and the difference between succeeding readings was less than 2%.

The Tapped Density was then calculated in g/ml, using the following equation (USP, 2004:2271):

$$\text{Bulk density} = \frac{M}{V_f} \qquad \textbf{(Equation: 4.2)}$$

Compressibility Index (Carr's Index) was then calculated using the following formula **(**USP**,** 2004:2271**):**

$$\text{Compressibility Index} = \frac{V_f - V_o}{V_f} \times 100 \quad \textbf{(}\text{Equation}\textbf{: 4.3)}$$

4.3.4 Drug-excipients compatibility by differential scanning calorimetry (DSC)

Differential scanning calorimetry was used to investigate the possible interactions between the lamivudine (drug) and the excipients. Samples of individual substances as well as mixed systems of lamivudine and excipients were prepared as follows: The 1:1 (w/w) ratio was used to prepare the drug-excipient mixtures as shown in Table 4.2. The samples were weighed (Mettler Toledo AB 104 balance, Schwerzenbach, Switzerland) directly in 5 ml glass vials and mixed with a spatula for 2 minutes. The samples were then weighed (Mettler Toledo UMX2 microbalance, Schwerzenbach, Switzerland) directly in Standard series DSC sample aluminium pans (Thermal Analysis, New Castle, USA), the exact quantities are shown in Table 4.2. The pans were then sealed with Standard series DSC sample aluminium lid using a Tzero^{TM} sample press (Thermal Analysis, New Castle, DE, US).

Calibration of temperature and heat flow rate was performed with standard zinc samples. To obtain the DSC curves, the samples were then scanned between 35 °C and 220 °C using DSC Q-100 (Thermal Analysis, New Castle, DE, US) apparatus equipped with a DSC Q-100 cell, under nitrogen flow of 50 ml.min^{-1} (to prevent oxidations) with a heating rate of 10 °C min^{-1}. Data acquisition was performed with DSC Q-100 software.

Table 4.2: Composition of individual and drug-excipient samples and the sample size scanned

Samples	Ratio (drug excipient)	Samples size (mg)
Lamivudine	-	4.7840
Magnesium stearate	-	4.9870
Magnesium stearate + Lamivudine	1:1(w/w)	4.8450
MCC	-	4.9730
MCC + Lamivudine	1:1(w/w)	4.8580
Sodium Starch glycollate	-	4.9510
Sodium Starch glycollate + Lamivudine	1:1(w/w)	4.9110

4.4 RESULTS AND DISCUSSIONS

4.4.1 Particle size distribution

4.4.1.1 Laser Diffraction

In the last 10 to 15 years, there has been a significant increase in the use of laser diffraction methods to estimate the particle size distribution (PSD) in dry powders. This is mainly due to the easiness, high reproducibility and flexibility of the method, in comparison with other techniques that had been conventionally adopted. (Guardani et al., 2002:42-50)

In general, the particle size (distribution) and shape of the API can significantly influence the manufacturability (e.g. content uniformity), the stability and the bioavailability of a direct compression tablet (Tinke et al., 2005:80-88**).** It is unusual for particles to be completely monosized, most powders contain particles with a large number of different equivalent diameters. In order to be able to define a size distribution or compare the characteristics of two or more powders consisting of particles with many different diameters, the size distribution can be broken down into different size ranges, which can be presented in the form of a histogram. (Staniforth, 2002:153-165)

The results as shown in Figure 4.1, obtained with the Malvern Mastersizer 2000 for lamivudine fine material indicated that 90 percent of the particles were less than 100.769 µm and only 10 percent were less than 25.584 µm. The frequency distribution curve was more bimodal than negatively skew due to the two peaks being obtained as shown in Figure 4.2. Although bimodal, a majority of material was found to have a mode of 100 µm indicating a large percentage of coarse material.

d (0.1): 25.584 µm d (0.5): 55.170 µm d (0.9): 100.769 µm

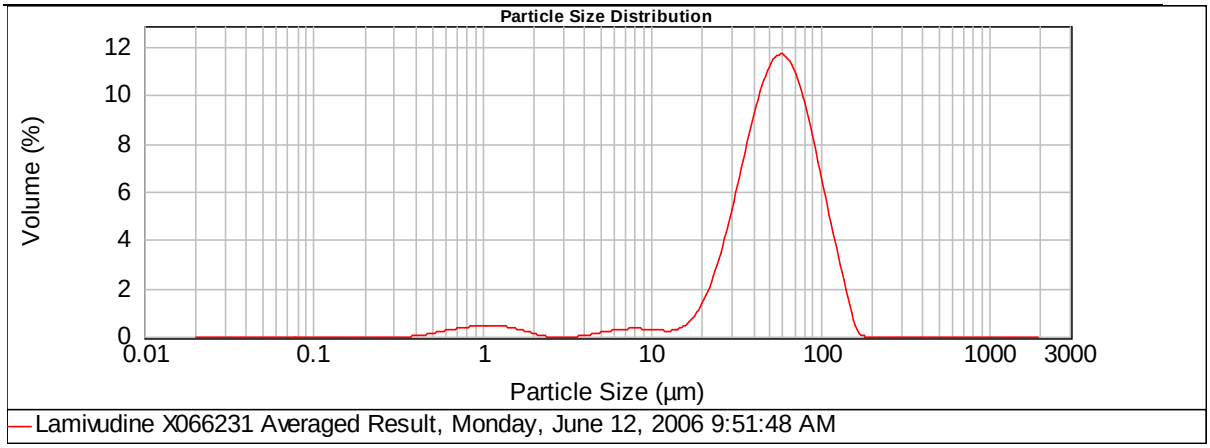


Figure 4.1: Frequency distribution curve obtained with Malvern Mastersizer 2000 for lamivudine fine material

The particle size distribution for lamivudine coarse material shown on Figure 4.2, as expected, contained large proportion of coarse particles as compared to the lamivudine fine materials. The results indicated that 90 percent of the particles were less than 320.94 µm and 10 percent of the particles were less than 32.347 µm. Unlike the lamivudine fine material, the coarse material indicated negative skewness (elongated tail towards smaller size ranges).

d (0.1): 32.347 µm d (0.5): 121.610 µm d (0.9): 320.940 µm

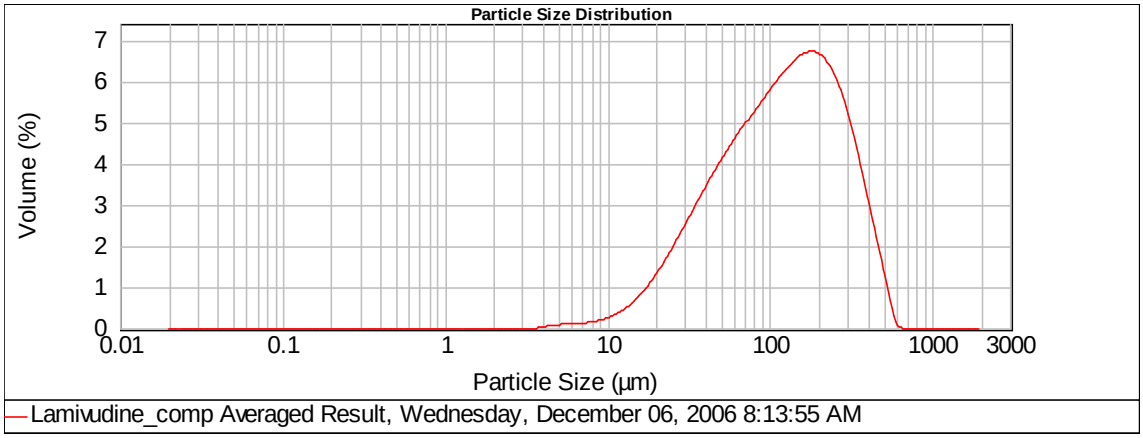


Figure 4.2: Frequency distribution curve obtained with Malvern Mastersizer 2000 for lamivudine coarse material

Laser diffraction may not be accurate since particle size measurement by laser diffraction is based on the assumption that the particles are spherical. For micronised particles, deviation from sphericity is negligible whilst for large crystalline carrier particles, the shape factor should be taken into account in order to obtain an accurate measurement. Nevertheless, laser diffraction may still prove to be a valuable tool for characterizing dry powder formulations under well-controlled conditions. (Marriott, 2006:39-49)

Comparing the two grades of lamivudine, due to wide size distribution range, the flow and physical tablet properties would be expected to vary with the grade used, more especially with direct compression method. More than for wet granulation, direct compression formulations require a strict control of the particle characteristics, i.e. particle size (distribution) and shape, of both the API and the excipients. (Tinke et al., 2005:80-88)

4.4.1.2 Particle size distribution by sieve analysis

The results as shown in Table 4.5 for lamivudine fine material indicated that greater than 61.8% of particles were greater than or equal to 250 µm. There was no similarity between the results obtained with sieve analysis and the results obtained with laser diffraction as

most particles were between the 100µm to 425µm range. Also observed was agglomeration and caking of the powder.

Table 4.3: Results of particle size distribution by sieve analysis for lamivudine fine material (Initial mass used =100.8 g)

Aperture size (µm)	Tare (g)	Gross (g)	Nett (g)	% Distribution
850µm	368.7g	368.7 g	0.0 g	**0.0 %**
600µm	391.7g	391.8 g	0.1 g	**0.1 %**
425µm	342.8 g	343.3 g	0.5 g	**0.5 %**
250µm	308.5 g	370.8 g	62.3 g	**61.8 %**
100µm	262.9 g	296.2 g	33.3 g	**33.0 %**
75µm	280.5 g	282.9 g	2.4 g	**2.4 %**
Tray	419.5 g	421.7 g	2.2 g	**2.2%**

The sieving method is most suitable for particles that are larger than 75 µm. Due to the particles cohesion and adhesion that causes the particles to stick to each other and to the sieve. Therefore the particles that are expected to pass through are retained (USP, 2004:786). Air-jet sieving may be the solution for such materials. From the laser diffraction, it was observed that most particles for lamivudine fine material were less than 100µm. Due to small particles sizes; there was insufficient force to overcome the forces impeding the flow. Due to irregular particles shape, observed from the scanning electron microscopy (Section 4.4.2 below) there could have been an increase in the interlocking forces amongst the particles thereby causing the poor flow and agglomeration.

4.4.2 Scanning electron microscopy

One disadvantage of laser diffraction would be that the particles are assumed to be spherical, thus it is necessary that the morphology of the particles is evaluated. One way for determining the morphological feature of the particles is scanning electron microscopy. Scanning electron microscope is the microscope in which the image is formed by a detector synchronized with a focused electron beam scanning the object.

The morphological features and particle sizes of lamivudine fine raw material were obtained SEM as shown in Figure 4.3. From a morphological point of view, the particle shape seemed to vary as observed from the photomicrograph. Lamivudine fine material consisted of irregular and cylindrical shaped particles. Visually, the diameter from the selected particles appeared to be approximately 30.99 µm, which did not correlate with the results obtained from laser diffraction, whereby most particles were between 50 -100 µm range.

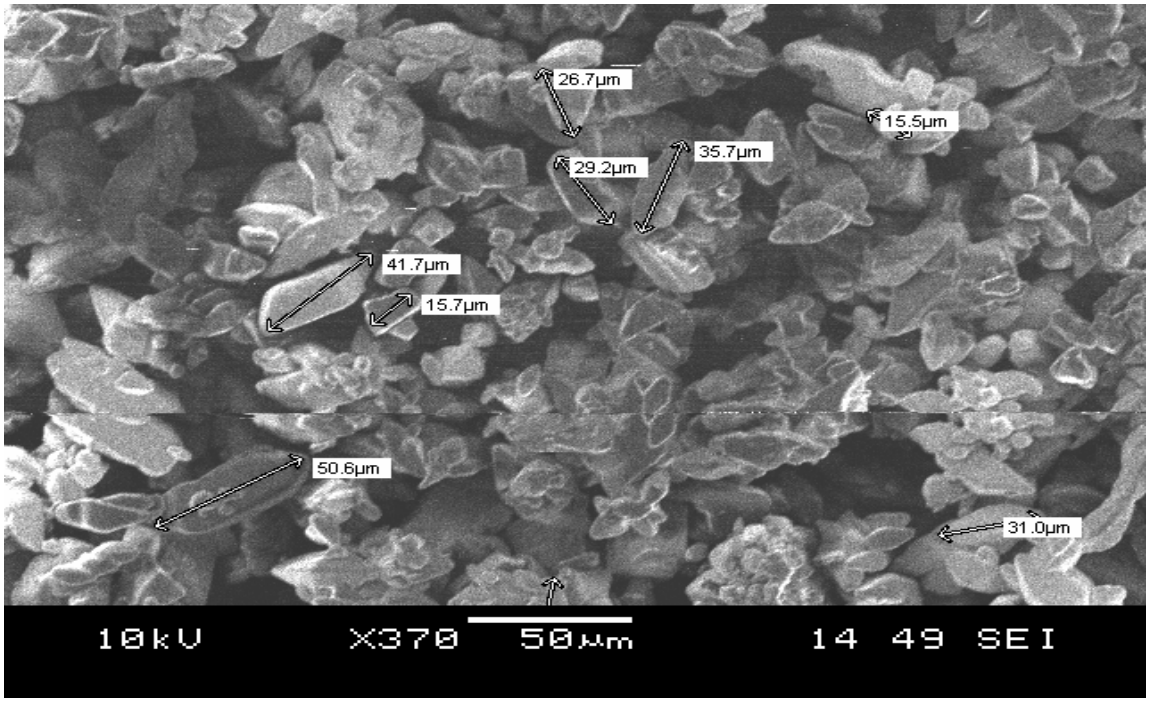


Figure 4.3: SEM photomicrograph of lamivudine fine material obtained with Jeol 6380 at 10kV

Figure 4.4 shows morphological features of lamivudine coarse material obtained with a scanning electron microscope. As expected, most particles were compacted particles mostly dominated by spherical and equant shaped particles and very few particles being discoidal shaped. Visually, the diameter from the selected particles appeared to be approximately 313.5µm, which correlated with the results obtained from laser diffraction.

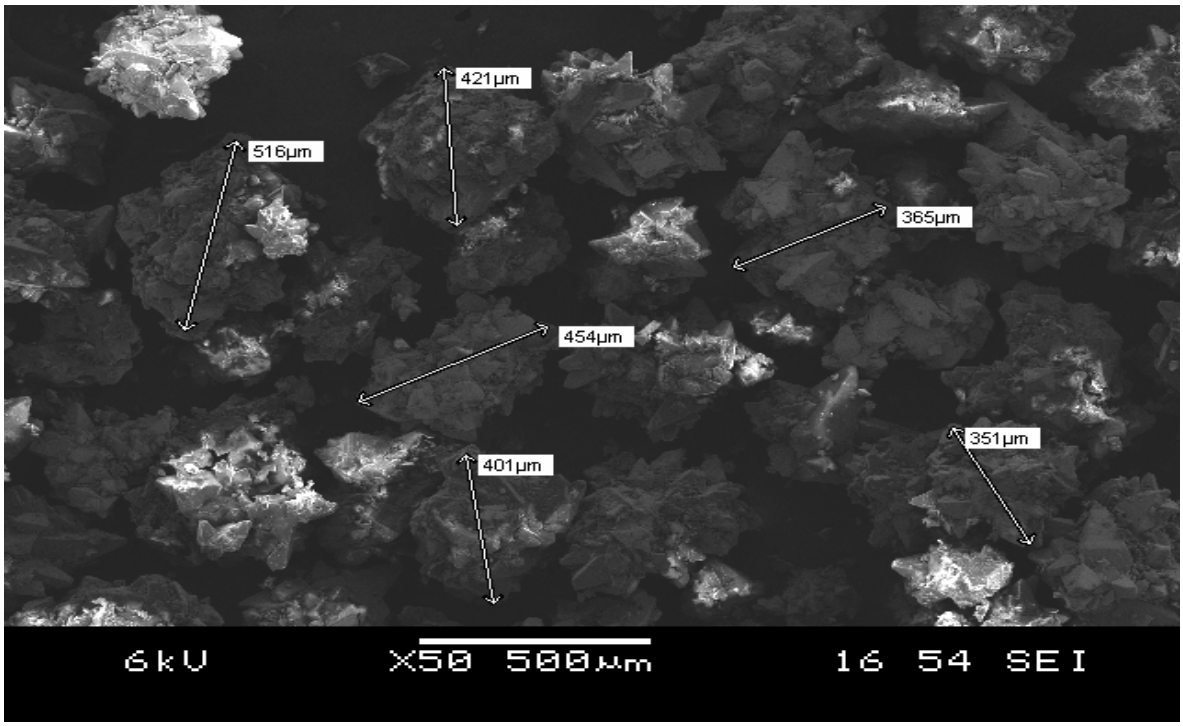


Figure 4.4: SEM photomicrograph of lamivudine coarse material obtained with Jeol 6380 at 6kV

The results from the scanning electron microscopy could not be used to determine particles size, since very few particles were measured for their diameters. Due to its domination by spherically shaped particles, lamivudine coarse material would be expected to have a better flowability than the fine material as shown by the photomicrographs. Due to its increased number of irregular shaped particles resulting on increased interlocking forces, poor flowability would then expected from lamivudine fine material.

4.4.3 Bulk and tapped density test

The bulk density is the density of the powder as poured or as passively filled into a measuring cylinder. The tapped density is a limiting density attained after tapping, a volumetric measuring cylinder containing the powder through a fixed distance. An expression used to predict powder flowability is the Carr's or compressibility index, which is the ratio of the difference between the tap and bulk densities to the tap density,

expressed as a percentage (Equation 4.3).The computed results for bulk, tapped density and compressibility index are shown in Table 4.3.

Table 4.4: Test results showing bulk density, tap density and compressibility index

Test	Lamivudine-Fine	Lamivudine-Coarse
Tapped density **(M/V_f)**	32.30g/49ml **= 0.659 g/ml**	62.63g/75ml **= 0.835 g/ml**
Bulk Density **(M/V_o)**	32.30g/97ml **= 0.332 g/ml**	62.63g/99ml **= 0.632 g/ml**
Compressibility Index **[(V_f-V_o)/V_o*100]**	[(0.659-0.332)/0.659]*100 **= 49.62 %**	[(0.835-0.632)/0.835]*100 **= 24.24 %**

The results shown in Table 4.4 indicated compressibility index for lamivudine fine material and lamivudine coarse material as 49.2 % and 24.24 % respectively. From Table 2.2 (Chapter 2), it can be clearly seen that Carr's index greater than 40 % represents extremely poor flowing material and 23-25 % would be poor flowing material. From the Carr's index scale, lamivudine fine material would be expected to have extremely poor flowability. With respect to flowability, the results shown in Table 4.4 correlates with the results obtained from laser diffraction and scanning electron microscopy. With fine powders (<150 µm), the magnitude of the van der Waals predominate over frictional forces. For larger particles (>150 µm) such as granules frictional forces normally predominate over van der Waals forces, thus they tend to flow better (Banker & Anderson,1986:295-329)

Also particle shape might have had an impact on the poor flowability as described in Section 4.4.2. Lamivudine coarse material has more spherical particles than the fine grade, therefore it flows better due to reduced interlocking forces caused by irregular particles. The bulk densities were comparable for both materials. Bulk density largely depends on particle shape. As the particles become more spherical in shape, the bulk density is increased due to decreased porosity % (Banker & Anderson, 1986:295-329) and that explained the high bulk density value observed for lamivudine coarse material.

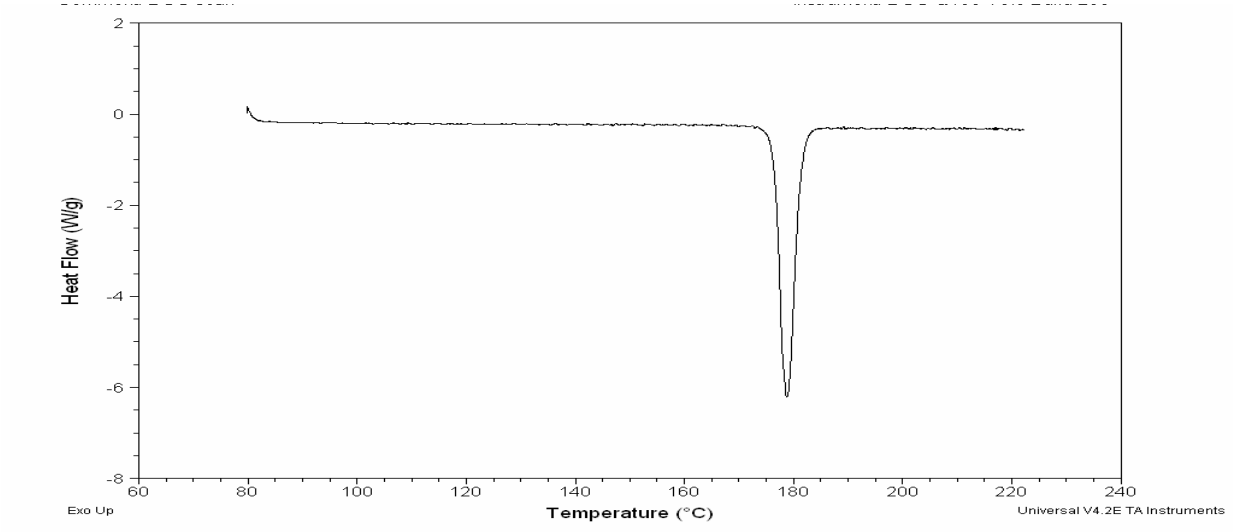
4.4.4 Drug-excipients compatibility by differential scanning calorimetry (DSC)

Studies of drug–excipient compatibility represent an important phase in the preformulation stage for the development of all dosage forms. In fact, potential physical and chemical interactions between drugs and excipients can affect the stability and bioavailability of drugs and, consequently, the drug's therapeutic efficacy. (Corvi et al., 2006:3–10) Excipients are considered pharmaceutically inert, but physical and chemical interactions with an active pharmaceutical ingredient are possible. Differential scanning calorimetry (DSC) is a rapid analytical technique commonly used for evaluating drug–excipient interactions through the appearance, shift, or disappearance of endo- or exothermal effects and/or variations in the relevant enthalpy values. (Corvi et al., 2006:3–10)

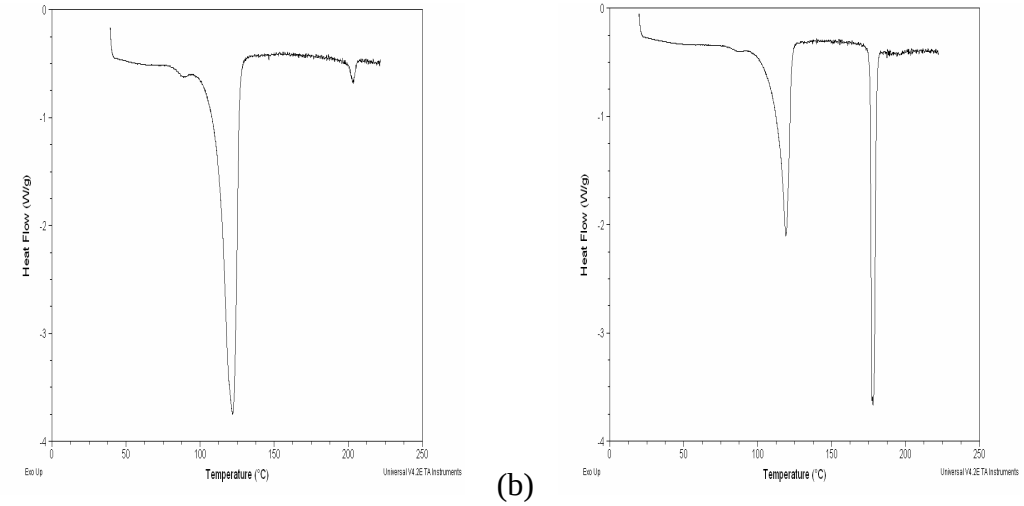
The DSC thermograms of pure substances and mixed systems of lamivudine and excipients (1:1 w/w) are shown in Figure 4.5 below. The thermal behaviour of pure drug, respective excipient, and the combination of drug and excipients are compared in the DSC thermograms. Peak transition temperatures of pure samples as individual samples and lamivudine in various excipient mixtures are shown in Table 4.5 below.

The DSC trace of lamivudine indicated its crystalline structure, showing a single sharp endothermic peak at 179.20 °C, corresponding to its melting point (176 °C) as shown in Figure 4.7 (a). The melting endotherm of the lubricant, magnesium stearate, was followed by a small shoulder at higher temperature as shown in Figure 4.5 (b). The small shoulder was reported by Corvi et al., 2006 as the presence of the corresponding palmitate salt impurity. Wadke et al.,1989, indicated that the analysis of sorption isotherms of excipients such as cellulose and starch derivatives indicates that water may exist in at least two forms, "bound" ("solidlike") and "free". In contrast to sharp peaks produced by magnesium stearate and lamivudine, the thermal curves of microcrystalline and sodium starch glycollate were characterised by a shallow, broad endothermic band at 81.43 and 82.32 °C respectively due to water evaporation as shown in Figure 4.5 (c) and (d). The drug-excipient mixtures showed an endothermic peak of lamivudine at temperature

corresponding to the pure sample's thermogram, indicating that there were no drug-excipient incompatibilities.

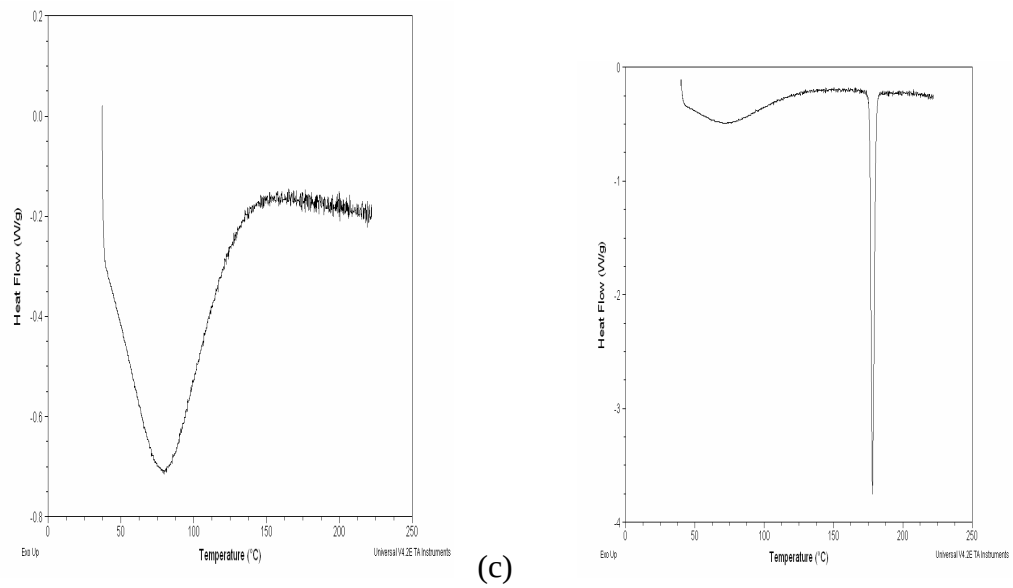


(a)

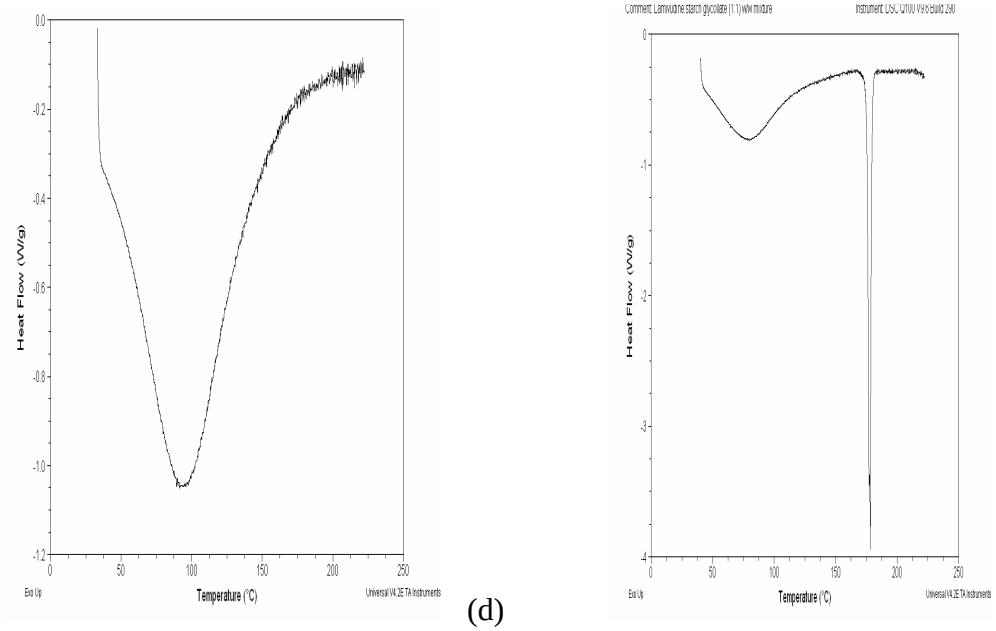


(b)

Figure 4.5.1: DSC curves of pure components and their 1:1 (w/w) physical mixtures (a) lamivudine and (b) magnesium stearate



(c)



(d)

Figure 4.5.2: DSC curves of pure components and their 1:1(w/w) physical mixtures (c) microcrystalline cellulose and (d) sodium starch glycollate

Table 4.5: Peak transition temperatures for pure and mixed samples

Samples	T_{peak} (°C)	
	Drug	Excipient
Lamivudine	179.20	-
Magnesium stearate	-	122.31
Magnesium stearate + Lamivudine	178.30	118.76
MCC	-	81.43
MCC + Lamivudine	178.30	72.55
Sodium starch glycollate	-	82.32
Sodium starch glycollate + Lamivudine	178.00	78.18

The 1:1 w/w ratio was chosen in order to maximise the likelihood of observing any interaction. In many cases, with drug-excipient mixtures, the melting endotherm of drug had slight changes in terms of narrowing or shifting towards the lower temperature.
The quantity of material used, especially in drug–excipient mixtures, affects the peak shape and enthalpy, thus, these minor changes in the melting exotherm of drug could be due to the mixing of drug and excipient, which affects (lowers) the purity of each component in the mixture and may not necessarily indicate potential incompatibility (Mura et al., 2004:65–71).

DSC has certain advantages, such as fast results and small sample sizes. There are also some limitations. This is because of exposure of drug–excipient mixture to high temperatures, which may not always be relevant at ambient conditions, therefore, the DSC results should be interpreted carefully, as the conclusions based on the DSC results alone can often be misleading and inconclusive. (Verma & Garg, 2005:633–44)

4.5 SUMMARY AND RECOMMENDATIONS

Particle size distribution by laser diffraction indicated a smaller particle size for lamivudine fine grade and an increased particle size for lamivudine coarse material. Most cylindrical and irregular shaped particles were observed for lamivudine fine material as indicated by scanning electron microscopy whilst lamivudine coarse material was mostly

dominated by spherical particles. Bulk and tapped density test indicated a high compressibility index (> 40 %) for lamivudine fine material, whilst lamivudine coarse material had the lowest compressibility index indicating a better flow than the fine material. No conclusion could be drawn from the sieve analysis as the method proved to be inaccurate. From the above results it would be expected that lamivudine coarse material to have a better flowability than the fine material. The flowability from the two grades could become a critical factor during direct compression as the technological limitation revolves mainly about the flow. Poor flowing material may cause spasmodic filling of the die cavity and results in non-uniform distribution of the drug.

From the above results it was clear that particle size, density and particle shape resulting in poor flow would definitely have negative impact on direct compression formulation. Thus the solution would the critical selection of excipients in formulating direct compression tablets. This could be achieved by the use of design of experiments (DoE), whereby the process and formulation factors that influence the tablet formulation are critical selected and optimized. The use of a glidant would be recommended to reduce the interparticulate friction and therefore improve flowability. Small particles have a tendency to agglomerate as a result of increased van der Waals forces and cause non uniform distribution of the actives. It would then be recommended that all the ingredients are screened to a uniform size to prevent agglomeration. The other recommendation would the granulation process where particles are modified to a uniform size and spherical shape with better flow. The SEM and Carr's index indicated the coarse material as a material for direct compression. The studies have shown that the dissolution rate can be affected by the particle size of the active ingredient. The decreased (small) particles have shown to have improved dissolution profile than granules (Jinno et al., 2006: 61). Often a decision has to be made as to whether to granulate small particle size, which may result in longer dissolution time. In either case the decision should be based on in vivo blood studies as well as in vitro dissolution tests (Shangraw, 1989:1990).

The thermograms obtained indicated that there were no incompatibilities between the drug and the excipients but the 1:1 (w/w) ratio obtained from DSC studies may be

misleading, since it does not reflect the actual ratios involved in the tablet dosage form. Therefore interactions shown using DSC would still require further investigations using standard stability protocol to assess practically significant interactions between tablet components.

CHAPTER 5

FORMULATION OPTIMIZATION

5.1 INTRODUCTION

The design of experiments (DoE) and multivariate statistical analysis has proven to be efficient and effective in formulation and process development. The major advantage of using DoE to develop formulations is that it facilitates the screening process which allows a systematic evaluation of a large number of excipients simultaneously with a limited number of experiments. (Gabrielsson et al., 2003:1053-1075) Using DoE, one can identify critical factors and evaluate the main and interaction effects of these factors on dosage form response based on statistical analysis. Once these critical factors are identified, the final formulation can be defined by optimizing the levels of all critical factors (Hwang et al., 1998: 48-64).

This chapter presents a systematic approach to optimizing an immediate release lamivudine 150 mg oral tablet formulation using DoE and analysis of variances (ANOVA) for a pharmaceutical product.

The formulation objective of this study was to formulate a solid dosage form containing lamivudine 150 mg that could meet the required specifications for disintegration, dissolution, friability, uniformity of mass, assay and content uniformity. All the other basic tablet criteria had to be met including blend homogeneity, lubricity and compressibility. Another objective was to apply statistical multivariate analysis methodology to study the effect that the considered critical process and formulation factors have on tablet responses.

5.2 METHODOLOGY

The formulation was developed by using a three step approach namely; preliminary formulation development, design of experiments and the formulation optimization study. Since the tablet press used was a conventional eccentric (single punch) tablet press with no compressional force sensor, the preliminary study was designed to define the tablet hardness that would be used throughout the experimentation and to test the viability of the formulation. Together with analysis of variances (ANOVA), the design of experiments (DoE) was used to study the main and interaction effects of the critical process and formulation variables on the tablets responses. After ANOVA had revealed the main and interaction effect of the critical factors on the responses, the final formulation was defined by optimizing the level of each factor.

5.2.1 Preliminary formulation

The preliminary study formulation was established by considering the innovator's product and the literature. Based on literature and results from preformulation studies, the direct compression method was chosen as the method of manufacture (details are discussed in Section 5.3.1 below).

5.2.2 Materials

The following ingredients that were used for tablet formulation were kindly donated by Aspen Pharmacare, Port Elizabeth, South Africa. Lamivudine fine ($D_{0.9}$ = 100 µm) and coarse grade ($D_{0.9}$ = 350 µm) (Hetero Labs Limited, Gaddapotharam, India), Microcrystalline cellulose (Vivapur® PH102), Sodium starch glycollate (Vivastar®) (JRS Pharma, Weissenborg, Germany) and Magnesium stearate (Liga Magnesium Stearate MF-2-V) (Peter Green, Edisonstraat, Netherlands) All the ingredients were used as received

The raw materials used in the preliminary study were selected based on the following properties and their functions: A fine grade of lamivudine was used as an API. Microcrystalline cellulose pH 102, the diluent (bulking agent), improves flow and has good lubrication and disintegration properties (Wheatley, TA, 2000: 1-12; Jivraj, et al., 2000; 58-63). Due to its super disintegration properties, its ability to reduce water solubility while it swells and absorbs water causing the tablet to break and its effectiveness at low levels sodium starch glycollate was chosen as a disintegrant (Wheatley, TA, 2000: 1-12). Magnesium stearate, a lubricant, prevents the adhesion of tablet material to the surface of the dies and punches and facilitates ejection of the tablet from the die. It is the most commonly used and effective lubricant (Wheatley, TA, 2000: 1-12). The composition of the formulation used for the preformulation is shown in Table 5.1.

Table 5.1: Ingredients and quantities used for preliminary the study formulations

Ingredients	**m/m Composition**		
	Percent (%)	**mg/tablet**	**g/batch**
Lamivudine(fine material)	42.86	150.00	75.00
Sodium starch glycollate	3.00	10.50	5.25
Magnesium stearate	0.50	1.75	0.875
Microcrystalline cellulose pH 102	53.64	187.75	93.875
Total	**100.00**	**350.00**	**175.00**

5.2.3 Method of manufacture

All the ingredients were weighed (Mettler Toledo® AB 104 balance, Schwerzenbach, Switzerland) according to the quantities shown in Table 5.1. To reduce agglomeration of particles lamivudine and microcrystalline cellulose were screened using a 710 µm sieve (Laboratory Test Sieve, Endecotts Ltd, London, England) and magnesium stearate and starch glycollate were screened with a 425 µm sieve. All the chemicals, with the exception of magnesium stearate were blended for 5 minutes in an Erweka® AR 400-

Rotating Cube mixer (Erweka® GmbH, Heusenstamm, Germany). Magnesium stearate was added and blended for an additional 3 minutes to lubricate the mixture.

The above mixture was then transferred to a Korsch Fe246 sRC single tablet press machine (Korsch®, Wangen, Switzerland) equipped with shoe hopper for die feed and 11 mm circular biconcave compression tooling. To prevent dust contamination into the mixture, the shoe hopper was sealed with aluminium foil. The tablet press's compressional force was set to produce four different batches from the same powder mixture to produce tablets with hardness of 30 N, 50 N, 75 N and 85 N hardness with ±10 N difference and also tablet mass of 350 mg ± 5 %. The first 10 and last 10 tablets of each batch were rejected so as to prevent weight variation due to initial flow and insufficient powder feed to the hopper.

5.2.4 Evaluation of tablets

During the compression cycle, the tablets were continuously evaluated for any deviations on their hardness and mass uniformity. After completion of each batch, the tablets were evaluated for friability, disintegration and dissolution. The preformulation study was not aimed at optimizing the formulation as a result the tablets were only evaluated for hardness, uniformity of mass, friability and disintegration times.

5.2.4.1 Tablet hardness

A Pharma-Test® PTB 311(Pharma Test ® GmbH, Hainburg, Germany) hardness tester was used to determine the crushing strength of the tablets. The instrument also automatically records the tablet diameter. Five tablets were randomly selected at regular intervals during the compression and were tested. The mean of 20 determinations was taken as the tablet hardness (Newtons) for each batch of tablets and represented as Mean (N) ± % RSD.

5.2.4.2 Uniformity of mass

At least 20 tablets (randomly selected) were weighed individually for uniformity of mass determination. The results were then represented as Mean (mg) ± % RSD.

5.2.4.3 Friability

To evaluate the tablets' friability, the USP 27th (2004) edition method was utilized. 10 tablets were randomly selected, dedusted and weighed and the mass was recorded as the initial mass represented by M_i. The tablets were then transferred to an Erweka TA3 friabilator (Erweka® GmbH, Heusenstamm, Germany) for friability testing. The friabilator was then allowed to run at 25 rpm for 4 minutes (100 revolutions). After 4 minutes had elapsed the tablets were carefully removed, dedusted and weighed and the mass was recorded as the final mass represented by M_f. Friability was then calculated using Equation 5.1, and represented as percentage mass loss (% loss).

$$\%\ \text{loss} = \frac{M_i - M_f}{M_i} \times 100 \qquad \text{(Equation: } \mathbf{5.1}\text{)}$$

5.2.4.4 Disintegration

To test for disintegration times, a Vankel 10-911-71X disintegration tester (VanKeL®, Edison, USA) was used. One tablet was introduced on each of cylindrical glass tubes containing fluted discs. Distilled water was used as the medium at 37 ± 0.5 ºC, with the apparatus suspended so that when it was in the highest position the wire mesh was at least 15 mm below the surface of the medium and 25 mm above the bottom of the beaker when suspended in the lowest position. The upper open ends of the tubes remained above the surface of the water. The time it took for each tablet to disintegrate such that all particles had passed through the mesh screen was recorded as the disintegration time and represented in minutes (min.).

5.2.4.5 Dissolution profile

Dissolution studies were performed using a United States Pharmacopoeia (USP) II dissolution apparatus (Hanson SR II 6-Flask Dissolution Test Station, Hanson Research Corporation, Chatsworth) equipped with six vessels and filled each 900 ml of water as a solvent at 37 ºC with the paddles rotating at 50 revolutions per minute (rpm). One tablet was transferred to each of the six vessels and allowed to sink to the bottom before switching on the paddles. 3 ml aliquots were withdrawn (Terumo® syringe, Terumo® Corporation, Laguna, Philippines) at 5 minute intervals and filtered using Millex®-HV Hydrophilic PVDF 0.45µm filter units (Millipore, Billerica, USA) into separate test tubes. The HPLC method described in Section 3.3.5 was used for the analysis of the samples. A 10µl of each sampled aliquot was injected in triplicates to produce the peak area results. The MS Excel® (version 2003) was used to calculate the tablets % released within 5 minutes interval using Formula 5.2.:

$$\%\ \text{lamivudine released within } 5\,\text{min interval} = \frac{P2 \times \text{mass std} \times \text{vol diss med} \times C \times 100}{P1 \times \text{vol std} \times \text{label claim } 100}$$

(Equation: **5.2**)

Table 5.2: Parameters used in the formula for dissolution test

P1	Area of the lamivudine peak in the standard solution
P2	Area of the lamivudine peak in the sample solution.
Mass std.	Mass of lamivudine RS taken to prepare the standard solution, expressed in mg **(17mg)**
Vol std.	Volume to which the standard solution is made up, expressed in ml **(100ml)**
Vol diss. Med	Volume of dissolution medium in the dissolution vessel, expressed in ml **(900ml)**
Label claim	Amount of lamivudine present in a dosage unit, expressed in mg **(150mg)**
C	Potency of the lamivudine standard, expressed as a percentage **(100%)**

The values in the brackets represent the mass weighed, volume of the standard solution, volume of the dissolution medium, label claim and the potency of lamivudine.

5.2.4.6 Lamivudine assay

To evaluate the tablets assay, 20 tablets were randomly selected and ground to a fine powder using a mortar and pestle. Approximately 216 mg of the sample (mass accurately known) was transferred to a 100 ml volumetric flask. About 50 ml of the solvent was added to the powder and the sonicated for 10 minutes. After sonication the mixture was allowed to cool to room temperature and made up to volume with solvent and mixed well. The solution was then filtered, discarding the first 5 ml. A 5 ml of the filtrate was accurately measured and diluted to 50 ml with the solvent. The HPLC method described in Section 3.3.6 was used for the analysis of the samples. About 10µl of the sample solution was injected in triplicates to produce the required peak area and the assay was calculated with MS Excel® (version 2003) using Formula 5.3 and expressed as % m/m of lamivudine

$$\%\ \ m/m\ \ \text{lamivudine} = \frac{P2 \times \text{mass}\ \ \text{std} \times \text{vol}\ \ \text{sample} \times 50 \times C \times 100}{P1 \times \text{vol}\ \ \text{std} \times \text{label}\ \ \text{mass}\ \ \text{sample} \times 5 \times 100}$$

(Equation: **5.3**)

Table 5.3: Parameters used in the formula for assay determination

P1	area of the lamivudine peak in the standard solution
P2	area of the lamivudine peak in the sample solution
Mass std	mass of lamivudine RS taken to prepare the standard solution (**10 mg**)
Mass sam.	mass of sample taken to prepare the sample solution, expressed in mg
Vol sam.	volume to which the sample solution is made up **(100 ml)**
Vol std	volume to which the standard solution is made up **(100 ml)**
C	potency of the lamivudine standard **(100 %)**

5.2.4.7 Content uniformity

To ensure uniformity of content, 30 tablets were randomly selected. One tablet was transferred to each of the 10 separate 200 ml volumetric flask. About 150 ml of the solvent (same as mobile phase) was added and then sonicated for 15 minutes. After

sonication, the mixture was allowed to cool to room temperature and made up to volume with solvent and mixed well. The solution was then filtered and 10 ml of the filtrate was then diluted to 100 ml with the solvent. The HPLC method described in Section 3.3.6 above was used for the analysis of the samples. Approximately10 µl of each sample solution was injected in triplicate to produce the peak area. MS excel® (version 2003) was used to calculate the assay for each sample using Formula 5.4 and the results were expressed as average % m/m of lamivudine ± % RSD.

$$\%\text{m/m lamivudine} = \frac{\text{P2} \times \text{mass std} \times 10 \times 200 \times 100 \times \text{C} \times 100}{\text{P1} \times 100 \times 100 \times \text{label claim} \times 10 \times 100} \quad \text{(Equation: } \mathbf{5.4}\text{)}$$

Table 5.4: Parameters used in the formula for content uniformity

P1	area of the lamivudine peak in the standard solution
P2	area of the lamivudine peak in the sample solution
Mass std	mass of lamivudine RS taken to prepare the standard solution
C	potency of lamivudine RS, expressed as a percentage
Label claim	label claim of lamivudine, expressed in mg

5.2.5 Design of experiments

Based on literature, four critical formulation and process factors (also called independent factors) that are known to affect the tablet formulation were selected as shown in Table 5.5. Formulation factors included active pharmaceutical ingredient (API) particle size, disintegrant concentration and magnesium stearate concentration and only lubrication blending time was selected as a process variable. Normally the magnitude of van der Waals forces predominates over frictional forces in fine material causing an increased in cohesiveness of the powder and resulting in poor powder flow (Gordon et al., 1989:298). Many drugs are commonly micronised as to improve dissolution and bioavailability (Shangraw, 1989: 199-201). Starch glycollate is an insoluble disintegrant, when added in appropriate amount will result in rapid disintegration of the tablet (Lo´pez-Solı´s & Villafuerte-Robles, 2001:127). High magnesium stearate concentration and longer

lubrication blending times result in coverage of all drug particles surfaces and due to magnesium stearate hydrophobicity, it retards the dissolution process. (Makoto et al., 2001:1-11)

After critical factors were selected, the levels at which the experimentation would occur were defined. All four factors were evaluated at high and low levels.

Table 5.5: Formulation and process factors and their levels for the design of experiments

Independent variables (factors)		**Levels**	
		Low	**High**
1	API particle size	Fine ($D_{0.9}$ = 100µm)	Compact ($D_{0.9}$ = 350µm)
2	Disintegrant content	3%	0%
3	Lubricant content	0.25%	1.5%
4	Lubrication blending time	1 minute	10 minutes

The main purpose of this study was to study the main and interaction effects of the already defined factors on respective responses using multivariate analysis. Therefore the dependent variables (responses) that were measured to study the main and interaction effect of these independent variables were uniformity of (measured as % RSD), tablet friability, tablet disintegration and tablet dissolution profile. Although tablet hardness was not considered as variable in multivariate analysis, the tablets were required to be in the required range of hardness. All the responses (dependent variables) and their specifications are listed in Table 5.6.

Table 5.6: Responses used to evaluate the tablets quality

Dependent variables (responses)	**Specifications**
Uniformity of mass	Not more than 2 out of 20 tablets may deviate from the average tablet mass by more than 5.0 %
Tablet friability	Less than 0.5 % is lost after 100 revolutions
Tablet disintegration time	Less than 15 minutes
Tablet dissolution rate	Not less than 85 % released in 30 minutes (Q = 80 %) Not less than 75% released in 15 minutes
Assay	150mg (332.5-367.5 mg) or 95%-105% of 150 mg
Content uniformity	Nine of the ten tablets must contain not less than 85% or not more than 115% of the label claim, Relative Standard Deviation must be less than or equal to 6.0%.

After four independent factors were defined as critical factors at high and low levels, a 2^4 full factorial experimental design was used for the design of experiments (numericals two and four representing the levels and the number of critical factors involved respectively). This experimental design generated sixteen experiments (2^4) as shown in Table 5.7, the (+) and (-) representing the high and low levels respectively. The actual values and levels used for the design of experiment represented by the (+) and (-) signs are also shown in Table 5.7. To minimize bias, the experiments were executed in random order and as shown by the sequence column in Table 5.7. The second column (sequence) represents the execution or sequence order used for experimentation and the first column represents the formulation corresponding to the sequence/order used.

Table 5.7: Full factorial experimental design (seq. denotes the execution sequence)

Formulation	Seq.	Factor 1 API Particle Size	Factor 2 Disintegrant content (%)	Factor 3 Lubricant content (%)	Factor 4 Lubrication Blending Time (min)
1	16	Compact (+)	3% (+)	1.50% (+)	10 mins (+)
2	7	Compact (+)	3% (+)	1.50% (+)	1 min (-)
3	12	Compact (+)	3% (+)	0.25% (-)	10 mins (+)
4	9	Compact (+)	3% (+)	0.25% (-)	1 min (-)
5	14	Compact (+)	0% (-)	1.50% (+)	10 mins (+)
6	11	Compact (+)	0% (-)	1.50% (+)	1 min (-)
7	4	Compact (+)	0% (-)	0.25% (-)	10 mins (+)
8	13	Compact (+)	0% (-)	0.25% (-)	1 min (-)
9	2	Fine (-)	3% (+)	1.50% (+)	10 mins (+)
10	6	Fine (-)	3% (+)	1.50% (+)	1 min (-)
11	15	Fine (-)	3% (+)	0.25% (-)	10 mins (+)
12	10	Fine (-)	3% (+)	0.25% (-)	1 min (-)
13	3	Fine (-)	0% (-)	1.50% (+)	10 mins (+)
14	8	Fine (-)	0% (-)	1.50% (+)	1 min (-)
15	1	Fine (-)	0% (-)	0.25% (-)	10 mins (+)
16	5	Fine (-)	0% (-)	0.25% (-)	1min (-)

The data generated by the design of experiments indicated only the levels used for the four critical factors. The actual composition of the tablets was computed based on DoE to give the actual values used for the ingredients and is shown in Table 5.10. The lamivudine content was the same for all 16 formulations, namely 150 mg and the values were calculated for a tablet mass of 350 mg. The lubrication blending time and the lamivudine grade varied depending on the formulation as shown in Table 5.7 above.

Table 5.8: Tablet composition (mg)

Sequence	Formulation	MCC 102 (mg)	Sodium starch glycollate (mg)	Magnesium stearate (mg)	Total Mass (mg)
1	**15**	199.125	0.00	0.875	350
2	**9**	184.250	10.5	5.250	350
3	**13**	194.750	0.00	5.250	350
4	**7**	199.125	0.00	0.875	350
5	**16**	199.125	0.00	0.875	350
6	**10**	184.250	10.5	5.250	350
7	**2**	184.250	10.5	5.250	350
8	**14**	194.750	0.00	5.250	350
9	**4**	188.625	10.5	0.875	350
10	**12**	188.625	10.5	0.875	350
11	**6**	194.750	0.00	5.250	350
12	**3**	188.625	10.5	0.875	350
13	**8**	199.125	0.00	0.875	350
14	**5**	194.750	0.00	5.25	350
15	**11**	188.625	10.5	0.875	350
16	**1**	184.250	10.5	5.250	350

The method of manufacture, ingredients, the equipment and evaluation of tablets were the same as described in Sections 5.2.2 -5.2.4 above. The only differences were the lubrication blending times and the quantities used as generated by the DoE. The tablet hardness and tablet mass responses were kept constant and the experimentation was executed in the random order.

5.2.6 Statistical analysis

The software Statistica (version 7.1) (StatSoft®, Oklahoma, U.S) was used for the analysis of the main and the interactions effects of the considered factors on the

responses. The software utilizes analysis of variances (ANOVA) which uses the probability value (p-value) to determine the main effect and the statistical significance of the considered factors on the responses at the 95 % confidence interval. The tools also uses vertical error bars to illustrate the change in the mean values when there has been a change on level settings. The interaction plots were generated for each response so as to determine the extent of the interactions between the factors.

5.3 RESULTS AND DISCUSSION

5.3.1 Choice of manufacturing method

Physicochemical properties of lamivudine (Section 2.2.1) indicated that approximately 70 mg dissolves in 1 ml of water at 20 °C, which makes the chemical very soluble in water. From the preformulation studies of lamivudine fine material, it was shown to have irregular particle shape and poor flowability (Sections 4.4.2 & 4.4.3). Although direct compression would be more efficient than wet granulation, the poor flowability and irregular particle shape of the API could prove to be a major problem with direct compression. Drug-excipient compatibility studies (Section 4.4.4) also indicated no incompatibilities between the active ingredient and the excipients. According to the European Pharmacopoeia, the moisture content of MCC should not exceed 7.0% (w/w). (Heidarian et al., 2006:139–145). It was indicated by the suppliers of the raw material, that the MCC used conform to the above mentioned specification (acceptable amount of water), indicating its suitability to be used as a direct compression diluent or bulking agent.

The main advantage of wet granulation is that the poor compressional and flow properties exhibited by many drug substances, like lamivudine fine material can be masked as a result of their incorporation into a granule. However, with the right selection excipients combined with the drug substance, the reward for establishing a direct compression process can be substantial. Jivraj et al., (2000) viewed direct compression as the technique of choice for the manufacture of tablets containing thermolabile and moisture-

sensitive drugs and although it affords many advantages it is still not as popular as wet granulation. Direct compression was then chosen as the method of formulation but if not successful then wet granulation would be attempted. Not only the moisture sensitivity factor was considered for the selection of direct compression as a method of manufacturing but also, direct compression is the fastest, most direct method of tablet production and it is the cheapest approach, involving only mixing and compressing. (Shangraw, 1989:195-203)

5.3.2 Preliminary study

The main purpose of the preliminary study was to identify and define the tablet hardness that would be used throughout the formulation and to test the viability of the formulation. The results from the preliminary study are summarized in Table 5.11. For all four batches the target tablet mass was 350mg and the compressional force was varied for each batch to give the desired hardness as explained in Section 5.3.2 above

Table 5.9: Summary of the major tablet responses for preformulation study

Trial No.	Hardness ± (% RSD) (N) (n=20)	Mass ± (%RSD) (mg) (n=20)	Disintegration time (s) (n=6)	Friability (% loss) (n=10)
1	**29.9 ± 3.1 N**	356.02 ± 1.04 mg	12.20 s	2.09 %
2	**51.9 ± 2.2 N**	350.59 ± 1.09 mg	13.61 s	0.42 %
3	**75.4 ± 2.3 N**	357.31 ± 0.95 mg	13.02 s	0.30 %
4	**87.2 ± 2.8 N**	355.46 ± 0.94 mg	13.65 s	0.11 %

There was a very little difference with respect to disintegration times and uniformity of mass (% RSD) as shown in Table 5.11. The results indicated low friability for trial batch number four and high friability for trial batch number one. Since the difference was only notable on friability, the hardness was then selected based on the trial batch with lowest friability.

From the literature, hardness is defined as the function of applied compressional force. The increase in pressure (compressional force) beyond the maximum value does not cause an increase in tablet hardness but causes the tablets to laminate or to cap. (Rosanske et al., 1989:327-329) Since trial batch number four had a low friability at 87 N and physical properties of the tablets were acceptable, there was therefore no need to increase the compressional force further.

There are many factors that affect tablet hardness during production, like machine speed, particle size distribution and particle density. These factors were not considered for the preliminary study as the machine speed was constant for all four batches and the particles were screened to obtain a uniform particle size. Also observed from the formulations were low disintegration times and low % RSD for hardness and uniformity of mass. The low % RSD values could be attributed to the lubrication properties of microcrystalline cellulose (Shangraw, 1989:211) and magnesium stearate causing the powder particles not to adhere to the die walls ensuring proper filling of the die. The low disintegration time could be attributed to the use sodium starch glycollate, which is a super-disintegrant and microcrystalline cellulose that has disintegrating effect. When used together, the two ingredients could act synergistically to reduce disintegration times (Carter, 2002).The focus of this preliminary study was not to optimize the formulation but to define the hardness thus further discussion about the other parameters are discussed on the design of experiments.

From the results it was shown that trial batch number four had lowest friability therefore 87 N was defined as the target hardness which was used throughout the experimentation. Due to change in particle size and other parameters, it would not be possible to obtain 87 N in all tablets therefore acceptable hardness values was 10 N more or less of the defined value (87 N ± 10 N).

5.3.3 Evaluation of tablets

5.3.3.1 Appearance

Appearance of the tablets is a highly important quality as viewed by the consumer. All the tablets should have an aesthetic appearance that is free of any kind of defect (Rosanske et al., 1989:321). In all 16 tablets formulations, typical tablet defects such as capping, filming, chipping and picking, were not observed. These defects may be due to damaged tooling and sometimes the use of deep concave punches. Binding, capping, lamination and sticking may be caused by decreased lubrication and increased moisture content of the powders (Bandelin, 1989:188-190).

5.3.3.2 Tablet hardness

Tablet hardness becomes an important consideration to a formulator as it can have significant influence on tablet quality parameters such as disintegration and dissolution properties (Rosanske et al., 1989:327). Since the tablet press used did not allow the set up of the compressional force, the tablet hardness was kept constant for all formulations. The tablet hardness target was between 77-97 N as explained in the preliminary study (Section 5.3.2). The results obtained indicated that for all formulations, the average tablet hardness was within the specified range as shown in Table 5.10. Since the hardness was kept constant so the other specified responses were used to evaluate and compare the quality of the tablets.

As it was described in the preliminary study, hardness is a function of the applied compressional force and depends on the factors that cause pressure to vary. Machine speed, dirty worn track and changes in the particle size distribution of the powder mix during the compression run may alter the tablet hardness. Particle density is also known to affect tablet hardness with dies having a light fill (large particles and low density) produce soft tablets. Lubricants can have a significant effect on tablet hardness when used in too high concentration or mixed for too long. The lubricant coats the particles and interferes with tablet bonding causing softening of tablets (Rosanske et al. 1989:327;

Banker & Anderson, 1986:297-299). The lubrication blending times and lubricant concentration, were varied as generated by the DoE (Table 5.7) but due to the fact that the hardness for all formulations was almost the same, it was the not possible to study the effect of lubrication, as well as other formulation and process parameters on tablet hardness.

Table 5.10: Summary of the major tablet responses

Form.	Hardness (N) (n=20)	Mass (mg) (n=20)	Uniformity of mass (%RSD) (n=20)	Disintegration Time (min) × 60sec. (n=6)	Friability (% loss) (n=10)	Assay content (%m/m) (n=20)
15	80.20	353.65	1.3248	1.2980	0.1244	111.65
9	82.00	353.49	0.2269	0.2978	0.08803	94.31
13	87.15	355.39	0.3761	1.2193	0.1747	98.30
7	85.00	356.80	0.2355	1.0934	0.03362	100.15
16	85.05	365.27	1.7046	0.3546	0.03546	101.68
10	77.35	347.98	0.9732	0.2458	0.2118	99.54
2	92.25	352.54	0.4997	0.3065	0.08810	98.65
14	88.15	345.95	0.7792	1.4692	0.07244	104.35
4	90.75	349.34	0.3864	0.1366	0.08864	99.55
12	79.55	356.94	1.1302	0.1707	0.06378	102.04
6	89.35	353.98	0.3637	2.7608	0.07060	108.81
3	82.15	354.19	0.2696	0.1717	0.05364	101.91
8	86.90	355.65	0.4960	1.1572	0.09518	103.73
5	77.94	349.28	0.2487	6.6533	0.1006	100.42
11	88.65	344.68	1.3472	0.1925	0.07455	99.37
1	80.85	345.9	0.3621	0.9238	0.2862	100.05

5.3.3.3 Uniformity of mass

The uniformity of mass can be considered as an indication of dosage uniformity, provided that the major component of the tablet is an active ingredient or the tablet being

tested contains 50 mg or more of a single active ingredient (Rosanske et al., 1989:325). The uniformity of mass test is still not sufficient to assure uniformity of content of low-dose drugs in which the excipients make up the bulk of the tablet weight, as a result content uniformity test is applied (Banker & Anderson, 1986:300-301). According to the USP 27th edition (2004), the maximum official uniformity of mass for tablets heavier than 250 mg is 5 % of the tablet mass, therefore all formulations met the required specification for uniformity of mass, all indicating % RSDs of less than 5 %, as shown in Table 5.10.

5.3.3.4 Friability

As mentioned earlier in the literature review chapter (Section 2.5.4.5), another measure of a tablet's strength (in addition to hardness) is its friability. According to the USP 27th edition (2004), tablets that lose less than 0.9 % in mass are considered acceptable. Regardless of the percentage loss, when capping is observed during friability testing the tablets should not be considered acceptable. All the formulations indicated to be less friable when tested by friabilator at 100 revolutions showing low friability of less than 0.3 % as shown in Table 5.10. Also, there was no capping observed therefore the formulations met the USP 27th edition specification of friability.

5.3.3.5 Disintegration

For a drug to be readily available to the body it must be in solution and the first important step toward solution is the breakdown of a tablet into smaller particles or granules, a process known as disintegration (Banker & Anderson, 1986:301; Alderborn, 2002:417-418). The limit for an uncoated immediate release tablet to disintegrate is 15 minutes. Most formulations presented fast disintegration times of less than 2 minutes except formulation 6 and 5 that presented the tablet disintegration of 2.76 and 6.65 minutes respectively, but were still within the required limit of 15 minutes as shown in Table 5.10.

5.3.3.6 Assay determination

The potency of tablets is expressed in terms of grams, milligrams or micrograms of drug per tablet and is given as the label strength of the product. Compendial or other standards provide an acceptable range for potency around the label potency. For large-dose drugs in tablet form, the official potency range is not less than 95 % and not more than 105 % of the labeled amount. (Rosanske et al., 1989:321) All formulations were within the specified range (95-105 %) except for formulations 9, 15 and 6 with assay outside the specified range of the labeled amount (150 mg) as shown in Table 5.10.

Limited data is available on the consequences of ingestion of over- and underdose in humans for lamivudine tablets (Medsafe, 2005). It is then recommended that both overdose and underdose are avoided. Formulations 6 and 15 presented higher than the specified limit assay content, with formulation 9 presenting underdose assay value and this can result in inaccurate dosing by patients. Exceeding the official potency range is not only undesirable but could be dangerous. Sometimes, even though the average assay result looks acceptable, it could be the result of a wide variation in individual tablet potency (Rosanske et al., 1989:325-326), with the result that a patient could be underdosed or overdosed.

5.3.3.7 Dissolution

The disintegration test simply identifies the time required for the tablet to break up under conditions of the test. The test offers no assurance that the resultant particles will release the drug in solution at an appropriate rate. For this reason the dissolution tests and specifications have now been developed for tablet products (Rosanske et al., 1989:332-333; Banker & Anderson, 1986:297-299). The requirements for the dissolution were that not less than 85 % of the API is released in 30 minutes and not less than 75 % is released in 15 minutes. All formulations met the required specifications at both 15 and 30 minutes intervals as shown in Table 5.11. Also interesting to note was the active released after 40 minutes interval for formulation 9, indicating an assay of 95.58 % which was above the

'underdose' value obtained in assay content test. Factors that could have resulted in this 'non-correlation' could have been accuracy in preparing the mixtures and wide variation in individual tablet potency.

Table 5.11: Tablets dissolution profiles (% released of lamivudine at minutes intervals)

Formulation	**Lamivudine released (% m/v) (n=6)**					
	5 min	10 min	**15 min**	20 min	**30 min**	40 min
15	62.33	85.95	**93.96**	95.17	**95.30**	94.44
9	85.22	95.39	**97.03**	96.46	**96.30**	95.58
13	49.33	66.25	**81.33**	85.09	**94.37**	95.10
7	64.17	85.38	**94.87**	100.37	**103.07**	104.05
16	74.19	97.61	**102.77**	104.04	**103.16**	102.38
10	98.29	96.15	**97.64**	97.26	**96.95**	96.11
2	98.16	104.27	**101.74**	102.16	**103.82**	101.81
14	60.51	69.21	**80.20**	86.95	**92.02**	94.58
4	76.38	92.42	**96.11**	93.99	**93.60**	93.79
12	80.81	94.35	**96.11**	96.25	**96.02**	96.39
6	59.99	66.77	**75.51**	80.22	**87.18**	90.59
3	77.90	93.17	**95.85**	95.11	**94.66**	94.27
8	72.53	84.63	**91.35**	94.46	**96.28**	96.59
5	49.16	65.51	**77.70**	84.52	**91.84**	97.31
11	90.84	91.53	**91.44**	92.07	**91.81**	92.76
1	77.45	85.91	**92.72**	91.34	**91.10**	91.95

(Values in bold indicate dissolution at 15 & 30 mins. intervals for the specifications)

5.3.4 Statistical analysis

The main aim of the design of experiment for this study was to identify the most significant or critical factors affecting the tablets' dissolution profile, disintegration, uniformity of mass and friability responses and establish the best optimal level settings for the formulation. Four factors, namely API particle size, disintegrant concentration, lubricant concentration and lubrication blending time were used as critical factors at high and low levels and their influence on the responses was studied. In order to evaluate the effect of these factors on the considered responses, analysis of variance (ANOVA) was applied. ANOVA has the ability to identify the main and interaction effects of independent factors on the responses. ANOVA uses a calculated probability value (p-value) to determine if the main effect of the independent value on dependent value (response) is statistically significance. A p-value equal or less than 0.05 % is considered significant for statistical analysis. ANOVA does not only help to determine the main effect of independent factors on the responses during multivariate analysis but also evaluates the interactions amongst these factors. Sometimes a statistically significant value would indicate that the effect of one factor on the response is not statistically significant as indicated by p value greater than 0.05. Therefore a change in the level of that factor may not affect the response and interact with the other factors since it is not statistically significant.

Two factors are said to interact with each other if the effect of one factor in the response is different at different levels of the other factor. In order to interpret the interactions, the interaction plots are constructed where one factor is plotted in the x-axis as a function of another factor. When lines are non-parallel to each other, an interaction exists between the factors as shown in Figure 5.3 below. This means that the change in the mean response from low to high level of a factor depends on the level of the other factor. The greater the degree of departure from being parallel, the stronger the interactive effect is. (Gonz´alez-Rodr´ıguez, 2007:341–342).

5.3.4.1 Effects on uniformity of mass

The statistical significance of the main effect of the four factors on uniformity of mass is shown in Table 5.14. The ANOVA tool showed that only the factors API particle size and magnesium stearate concentration were statistically significant for uniformity of mass response (% RSD) at the 95 % confidence range as shown in Table 5.14

Table 5.12: Probability value summary; U mass = uniformity of mass; D. time = disintegration time (Bold numericals denotes p value < 0.05)

Factors	U. mass (% RSD)	Friability (% loss)	D. time (min)	% released at 5 minutes	% released at 15 minutes
API	**0.001639**	0.4000	0.1404	0.4768	0.5975
Disintegrant	0.7211	0.8606	**0.02035**	**0.0002**	**0.0231**
Magnesium Stearate	**0.0292**	0.6176	0.0901	0.5626	0.05210
Lubrication blending time	0.1129	0.7881	0.3170	0.0939	0.5515

The ANOVA tool revealed that API particle size and magnesium stearate concentration considerably affected tablet uniformity of mass. When the particle size was changed from fine to coarse material, a decrease in % RSD was obtained as shown in Table 5.13 and illustrated in Figure 5.1 below.

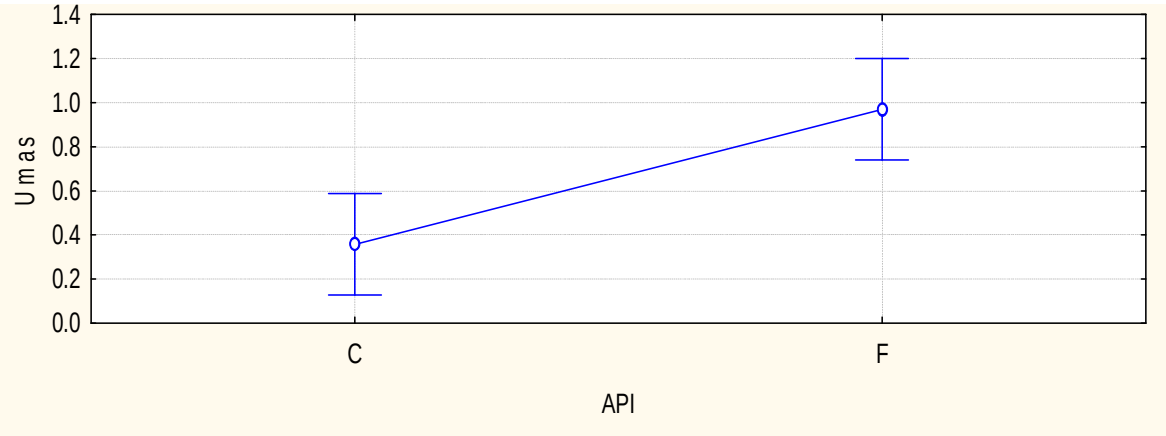


Figure 5.1: API grades vertical bar on uniformity of mass (% RSD) (C=coarse grade, F= fine material)

Table 5.13: Effect summary of the four critical factors on the responses

Factors	**Levels**	**U mass** (% RSD)	**Friability** (% loss)	**D. time** (min)	**% released at 5 min.**	**% released at 15 min.**
API	Coarse	0.3577	0.1021	1.6504	71.96	90.73
	Fine	0.9703	0.1455	0.6560	75.19	92.56
Disintegrant content	3 %	0.6369	0.1193	0.3057	85.62	96.08
	0 %	0.6911	0.1282	2.0007	61.53	87.21
Lubricant content	1.5 %	0.4787	0.1366	1.7346	72.26	87.98
	0.25 %	0.8493	0.1110	0.5718	74.88	95.31
Lubrication blending time	10 min.	0.5363	0.1170	1.4812	69.55	90.61
	1 min.	0.7916	0.1306	0.8252	77.60	92.68

The % RSD for the uniformity of mass can also be used as an indication of weight variation. If everything is working well mechanically weight can be caused to vary by a poorly flowing powder material, which causes spasmodic filling of the dies (Rosanske et al., 1989:325). The high % RSD for the uniformity of mass is sometimes an indication of poor flowing powder. The results obtained from the preformulation studies indicated a low Carr's index for the coarse material, indicating better flow as compared to fine material (Section 4.4.3). This was justified by the results from scanning electron microscopy which showed that the coarse material was mainly dominated by spherically shaped particles with fine material presenting irregularly shaped particles (Section 4.4.2). Therefore, due to fine material irregular shaped particles that could have resulted on increased interlocking forces causing overall poor flowability of the powder mix and resulted in an increased % RSD indicating decreased in tablet uniformity of mass.

Not only was shape a critical factor between the two API grades but also the average size of the two grades. The results from SEM and laser diffraction indicated that the particle size distribution for lamivudine coarse material contained a large proportion of coarse particles as compared to the lamivudine fine materials. Normally the magnitude of Van der Waals forces predominates over frictional forces in fine material causing an increased in cohesiveness of the powder and resulting in poor powder flow (Rosanske et al.,

1989:298). This was indicated by the high Carr's index of the fine material indicating poor powder flow which was reflected by the decreased uniformity of mass as indicated by high % RSD for the fine material powder mix.

As it was predicted from the preformulation studies that the flowability of the two grades could prove to be a critical factor during direct compression as the technological limitation revolves mainly about the flow thus the difference was significant between the two API grades (Section 4.5).

When the magnesium stearate concentration size was changed from a low level (0.25 %) to a high level (1.5 %), a decrease in % RSD for the uniformity of mass was obtained as shown in Table 5.13. and illustrated in Figure 5.2.

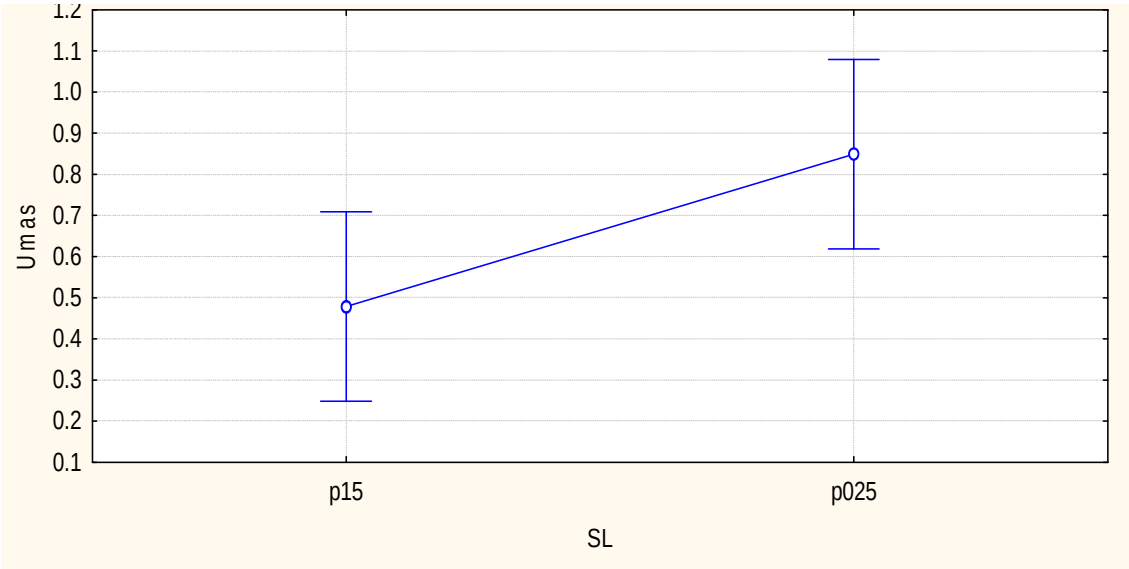


Figure 5.2: Magnesium stearate concentration vertical bar on uniformity of mass (% RSD) (p1.5 = high lubricant level, p = 0.25 low lubricant level)

Lubricants are necessary in tablet formulations to reduce adherence of the powder material into the die walls and the punches. Magnesium stearate is one of the most commonly used lubricants. (Wheatley, TA, 2000: 1-12). It is well known that the concentration of magnesium stearate and the mixing time can have significant effects on the compaction properties of pharmaceutical powders. With a well-defined particle size

distribution, magnesium stearate improves powder flow, ensuring a high content uniformity, even in low-dosage formulations. It covers or coats the powder particles and provides extra lubrication whilst it also interferes with tablet bonding. Bandelin, 1989 reported that magnesium stearate can also serve as a glidant (improves powder mix flow). Increased concentration of magnesium stearate presents more lubrication and reduces the interparticulate friction resulting in improved powder flowability but adversely affect hardness and dissolution (Bandelin, 1989 169-179). This was indicated from the formulations by the decreased uniformity of mass RSD with high magnesium stearate levels indicating improved powder flowability.

From the ANOVA tool, it could be then concluded that API particle size and magnesium stearate levels significantly affected the uniformity of mass. Increase in levels of both factors resulted in decreased RSD for the uniformity of mass. Therefore, to obtain low RSD value for this response the higher levels (coarse API particles and 1.5 % lubricant) of these factors must be used. There were four factors involved in design of experiments but ANOVA revealed that only API particle size and magnesium stearate level were only significant for uniformity of mass at the 95 % confidence interval. As it was explained above that two factors are said to interact with each other if the effect of one factor in the response is different at different levels of the other factor. The interaction plots were constructed to study the interaction of all four factors on the uniformity of mass. When lines are non-parallel to each other, an interaction exists between the factors. The ANOVA can only reveal an interaction between two variables at a time with the other variables being kept constant. There were then twenty four interaction graphs that were generated by the software and some of the examples are shown in Figures 5.3 & 5.4.

The interaction plots showed non-parallel lines between the four factors indicating interactions with each other. Although the main effects of lubrication blending time and starch glycollate levels on the uniformity of mass response were not significant at the 95% confidence interval, the interactions with the other factors (API grade and magnesium stearate level) produced significant effects on the uniformity of mass response. Due to interactions amongst the considered factors, meant that to affect the

uniformity of mass (% RSD) response, the level of one factor could not be changed independently of the level of the other factor.

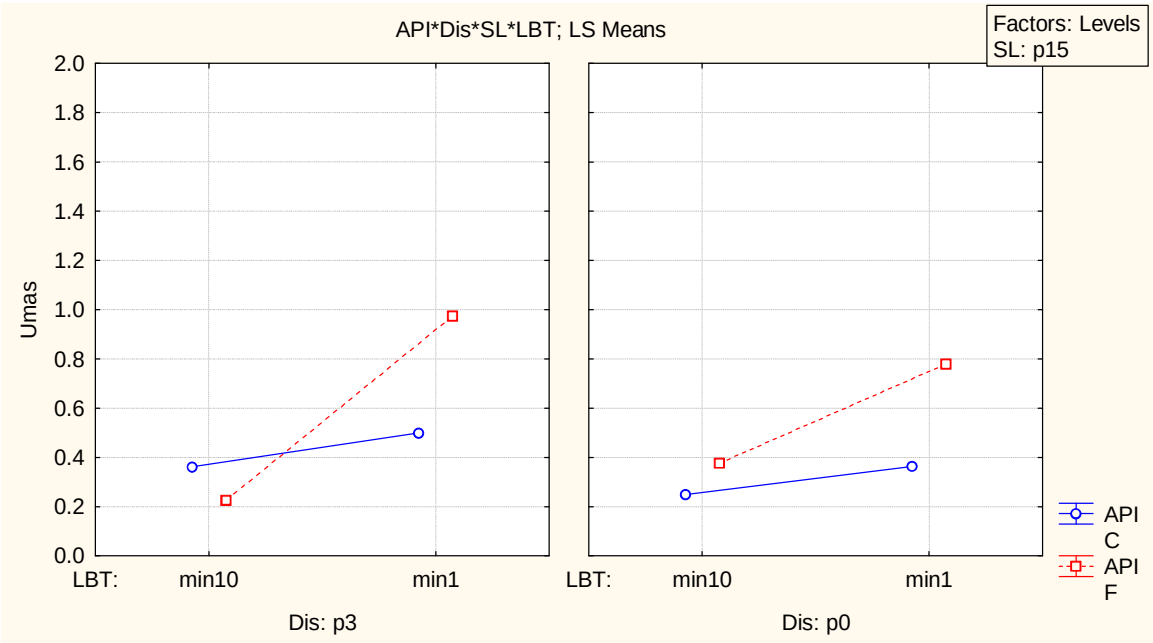


Figure 5.3: Interaction plots between magnesium stearate concentration and lubrication blending times (LBT = lubrication blending time, API = lamivudine grade, SL = lubricant content, Dis = disintegrant content)

Besides the factors mentioned above, uniformity of mass is also known to be affected by non-uniformity of particle density within the powder mix. Non-uniform densities cause varying amounts of powder to fill the dies, resulting in variation in tablet mass. Even with a good flowing powder mix, mechanical problems can cause the mass to vary. A lower punch with non-uniform length and cupped lower punch that is filled in with a sticking powder material will cause mass to vary. (Rosanske et al., 1989:325)

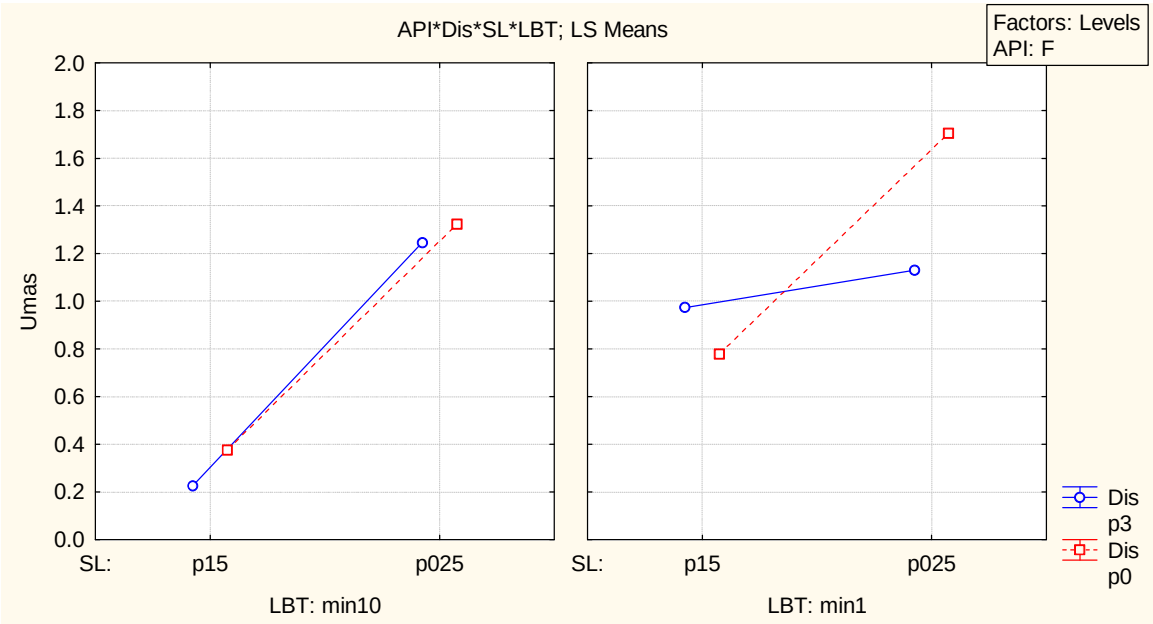


Figure 5.4: Interaction plots between magnesium stearate concentration and disintegrant concentration (LBT = lubrication blending time, API = lamivudine grade, SL = lubricant content, Dis = disintegrant content)

5.3.4.2 Effects on friability

Friability can be defined as a measure of the tablet's ability to withstand both shock and abrasion without crumbling during handling of manufacturing, packaging, shipping and consumer use (Rosanske et al., 1989:330). The ANOVA tool revealed that none of the four factors considerably affected the tablets friability as indicated in Table 5.12 by probability values of more than 0.05 for all four factors.

The punches used and the moisture content of the powder mix are known to affect the tablet friability. When the deep concave punches are used in tabletting, especially if the punches are in poor condition, the tablets produced could exhibit chipping at the tablet edge. Tablet friability may also be influenced by moisture content of the tablet powder mix. A low but acceptable moisture level frequently serves as a binder (Rosanske et al., 1989:330).

The low friability percentage values could be attributed to the use of MCC. The MCC was the major component in all tablet formulations making more than 50 % m/m of the tablet mass. MCC is effective as a binder in direct compression, and its ability to act as a binder depends on moisture level. Its effectiveness decreases with increased moisture level thus it is important that the MCC moisture content is kept below 7.0 % as explained in Section 5.3.1. Also, another important property of MCC is its ability to deform plastically when compressed and the water it contains, maintains its ability to deform plastically. (Shangraw, 1989: 210-213) These two properties of the MCC ensure that tablets particles remain intact with reduced friability during manufacturing, packaging, shipping and consumer use.

The tablets hardness was optimized to find the hardness that would be used throughout experimentation (Section 5.6.2). The hardness for all formulations was kept constant, between (77 -97N) as shown in Table 5.10. The low friability % for all formulations could also be attributed to the hardness. The hardness is function of compressional force and related to tablet strength and an increase in pressure will not always increase the hardness thus increasing the strength but it will hold up to a maximum value to which the increase will cause the tablet to cap.

The interaction plots showed non-parallel lines between the four factors indicating interactions with each other as shown by the example in Figure 5.5. The figure shows the interaction between lubrication blending time and lubricant concentration with disintegrant and API particle size kept constant. Due to interactions amongst these factors, meant that to affect the friability response, the level of one factor could not be changed independently on the level of the other factor.

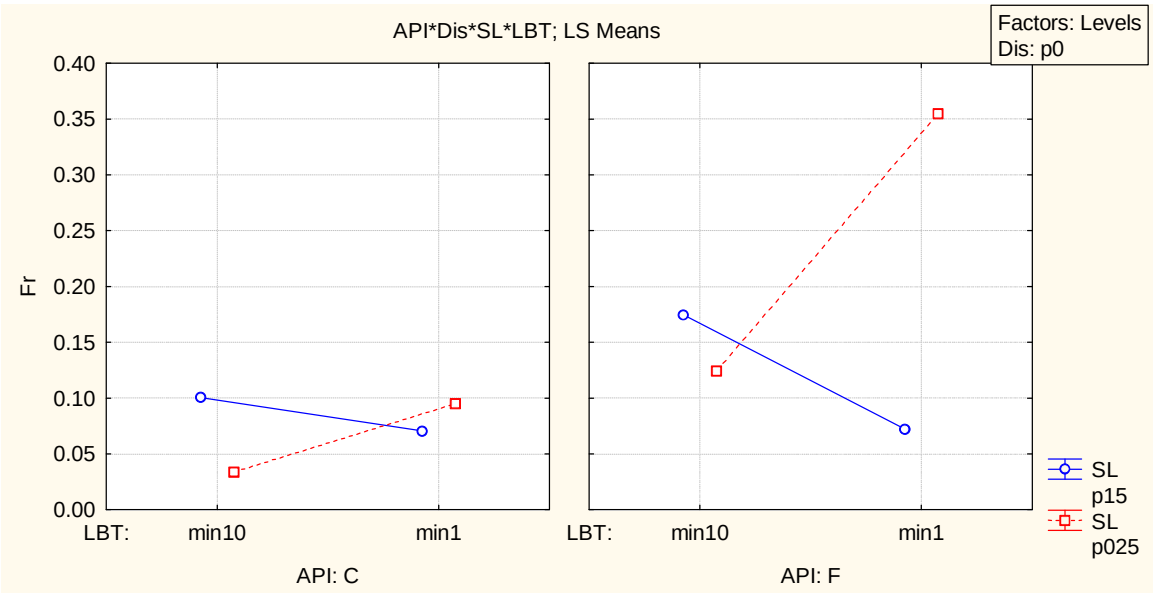


Figure 5.5: Interaction plots between lubrication blending time and magnesium stearate concentration (LBT = lubrication blending time, API = lamivudine grade, SL = lubricant content, Dis = disintegrant content)

5.3.4.3 Effects on disintegration

Tablet disintegration has received considerable attention as an essential step in obtaining fast drug release. The emphasis on the availability of drug highlights the importance of the relatively rapid disintegration of a tablet as a criterion for ensuring uninhibited drug dissolution behavior.

The ANOVA tool showed that only disintegrant (sodium starch glycollate) concentration was shown to significantly affect disintegration at the 95 % confidence range as shown in Table 5.13. The ANOVA tool revealed that disintegrant concentration considerably affected the tablets' disintegration time. When disintegrant concentration was changed from a low to a high level, a decrease in disintegration times was obtained as shown in Table 5.13 and illustrated in Figure 5.3.

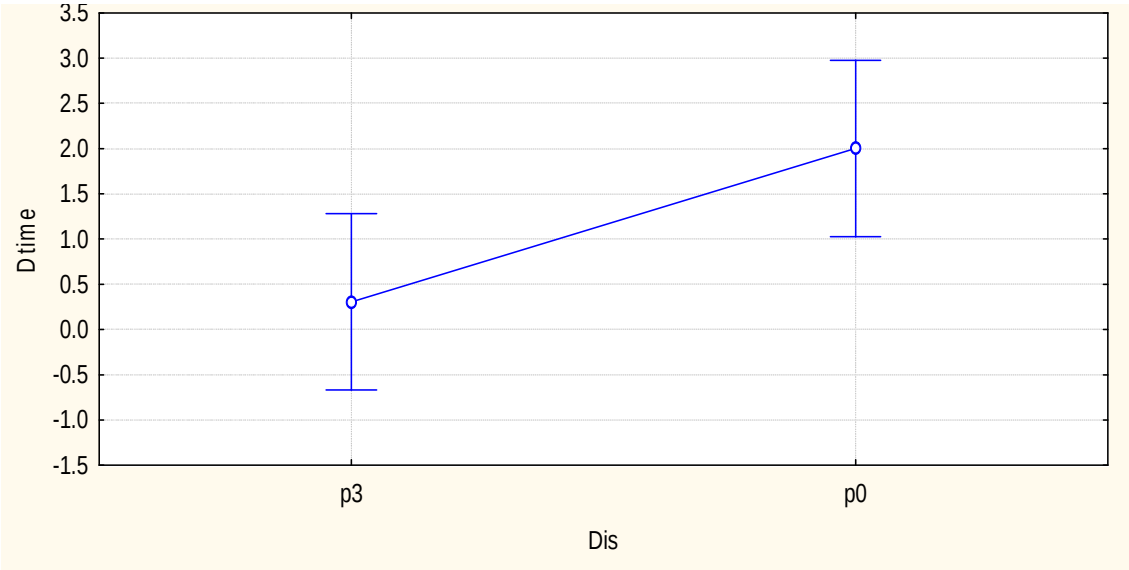


Figure 5.6: Sodium starch glycollate vertical bar on disintegration times (p3 = high disintegrant level, p 0 = low disintegrant level).

Disintegrants are agents added to tablet formulations to promote the breakup of the tablet into smaller fragments in an aqueous environment thereby increasing the available surface area and promoting a more rapid release of the drug substance (Uddhav et al., 2006). Because of the increased demands for faster dissolution requirements, super-disintegrants were developed to improve disintegration times, resulting in faster disintegration of the tablets than with a normal disintegrant. Sodium starch glycollate is a known super-disintegrant, which is effective even at low concentration. When in contact with water, it causes swelling of the tablet and due to swelling pressure is exerted in the outer direction or radial direction and causes the tablet to burst (Carter, 2002). Unlike in wet granulation whereby the disintegrant is incorporated in a granule, in direct compression all of the disintegrant is able to perform optimally and cause the tablet to disintegrate rapidly (Shangraw, 1989:198). This results in faster disintegration times, which is shown in Table 5.10.

The interaction plots showed non-parallel lines between the four factors indicating interactions with each other as shown by the example in Figure 5.7. The interaction plot of lubricant content as function of disintegrant at 10 minutes lubrication blending time

when the lamivudine fine grade was used presented rather parallel lines for the two levels as shown in Figure 5.7, indicating less interaction effect between the two factors. Although the main effects of the other three factors were not significant, the interaction with each other and with the disintegrant's concentration factor produced a significant effect on this response. Due to interactions amongst these factors, meant that to affect the disintegration, the level of a disintegrant could not be changed independently on the level of the other factors.

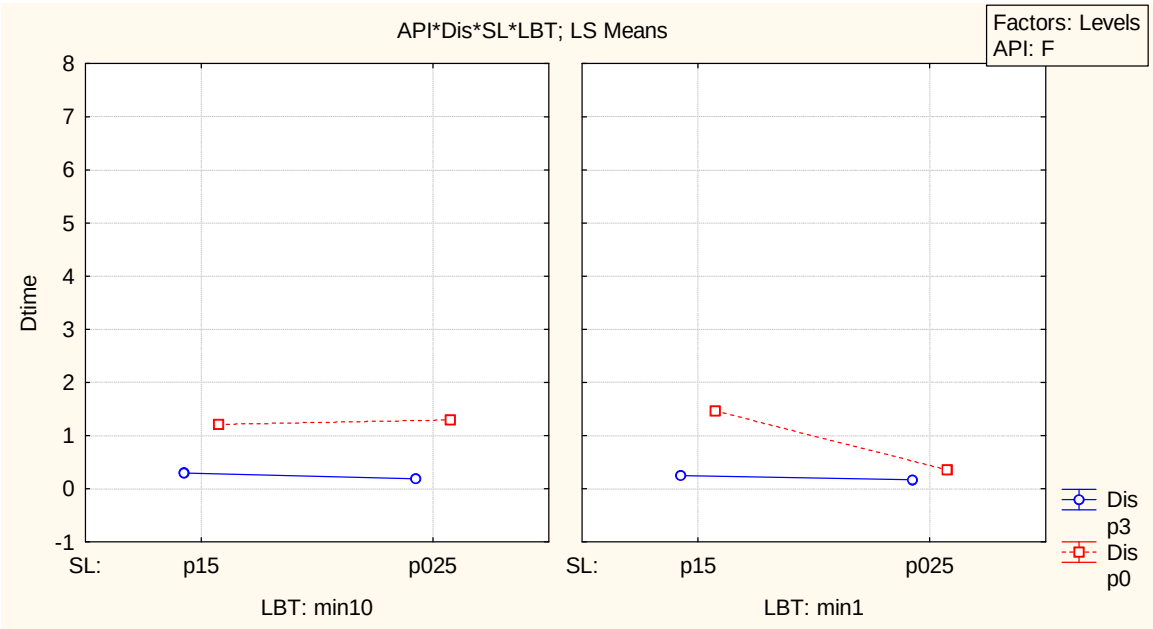


Figure 5.7: Interaction plots between magnesium stearate concentration and sodium starch glycollate concentration (LBT = lubrication blending time, API = lamivudine grade, SL = lubricant content, Dis = disintegrant content)

Most lubricants including magnesium stearate are hydrophobic substances and during blending, the lubricant's particles may adhere to the surface of the other particles. This hydrophobic coating inhibits the wetting and consequently tablet disintegration (Banker & Anderson, 1986:301). Therefore interaction between the disintegrant concentration and lubrication concentration would be antagonistic in nature, with increased lubricant concentration causing increased disintegration time as shown by the interaction plot in Figure 5.7. Formulation number 5 indicated the effect of excluding a disintegrant and the

presence of high lubrication on disintegration time and that resulted on slower disintegration as shown in Table 5.10.

Faster disintegrations times for formulations without disintegrant could be attributed to the presence of microcrystalline cellulose. MCC has an ability to act as a disintegrant. It functions by allowing water to enter the tablet matrix by means of capillary pores, and breaks the hydrogen bonding between adjacent bundles of cellulose microcrystals (Uddhav et al., 2006). Unlike water-soluble diluents that tend to dissolve rather than disintegrate, microcrystalline cellulose, which is insoluble in water, produces rapid disintegration. It has also been shown that super-disintegrants have a greater effect on disintegration time in an insoluble system than in a soluble or partially soluble system, thus starch glycollate functions optimally in the presence of MCC. (Chebli & Cartilier: 1998:101)

5.3.4.4 Effects on dissolution profiles

Since a drug must be in solution before absorption can take place, orally administered tablets must dissolve in the contents of the gastrointestinal tract before systemic absorption can occur (Rosanske et al., 1989:332-335). The main objective in the development of the in vitro dissolution test is to show that the release of the tablets is as close as possible to 100 % (Banker& Anderson, 1986:297-299). All formulation met the dissolution test specification as shown in Table 5.10 and discussed in Section 5.3.3.7. The dissolution profile may determine the total amount of drug absorbed as well as the rate of absorption. Thus, the rate of dissolution may be directly related to the efficacy of the tablets produced but the most direct assessment of drug release would be in vivo bioavailability studies (Banker& Anderson, 1986:297-299). The dissolution profiles for all formulation is shown in Figure 5.8, the y-axis represents the % release of the API with x-axis representing the time interval. Lamivudine is soluble in water (Section 2.2.1) and unlike a hydrophobic drug, in which drug release is regulated by erosion of the tablet matrix and produces a linear dissolution curve, with water soluble drug like lamivudine, release is regulated by diffusion through the gel layer resulting in inconsistency in drug

release, more especially in the first 30 minutes. This was observed in all formulations as shown in Figure 5.8. The 5 and 15 minutes intervals were chosen to study the effect of the considered factors on the dissolution profile.

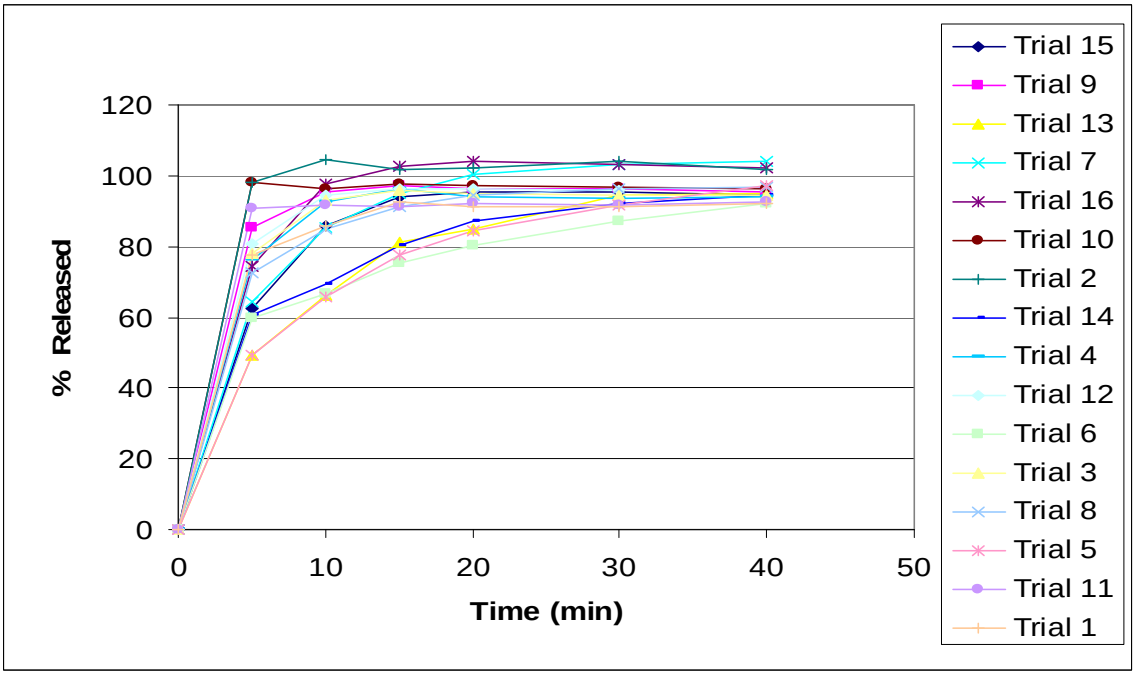


Figure 5.8: Formulations dissolution profiles

The ANOVA tool showed that only disintegrant concentration was shown to significantly affect dissolution at the 95 % confidence range as shown in Table 5.12. The ANOVA tool revealed that disintegrant concentration considerably affected the dissolution profile at 5 and 15 minutes intervals. When disintegrant concentration was changed from a low to a high level, an increased in % released was obtained at both intervals as shown in Table 5.13.

As mentioned in Section 5.3.3.7, the disintegration tests offer no assurance that the formulation will release the drug, even in the form of small particles. Sodium starch glycollate is a super-disintegrant which exerts its effect on the tablet by causing the tablet to swell when in contact with water causing the tablet to break up into particles. Disintegrant causes the break up of a tablet into smaller particles causing an increased

surface area and resulting on increased dissolution rate. Unlike water-soluble disintegrants that tend to dissolve rather than disintegrate, starch glycollate is an insoluble disintegrant and when added in an appropriate amount will result in rapid disintegration of the tablet (Lo´pez-Solı´s & Villafuerte-Robles, 2001:127). Water was used as a medium for dissolution testing and super-disintegrants tend to promote faster dissolution in a neutral pH medium than in an acidic medium (Lo´pez-Solı´s & Villafuerte-Robles, 2001:127)

The interaction plots showed non-parallel lines between the four factors indicating interactions with each other as shown by the example in Figure 5.9. This meant that at both 5 and 15 minutes intervals the dissolution profile could not be affected by changing the disintegrant level only and independently of the levels other factors. Figure 5.9 shows an antagonistic interaction between magnesium stearate level and disintegrants level at constant lubrication blending times.

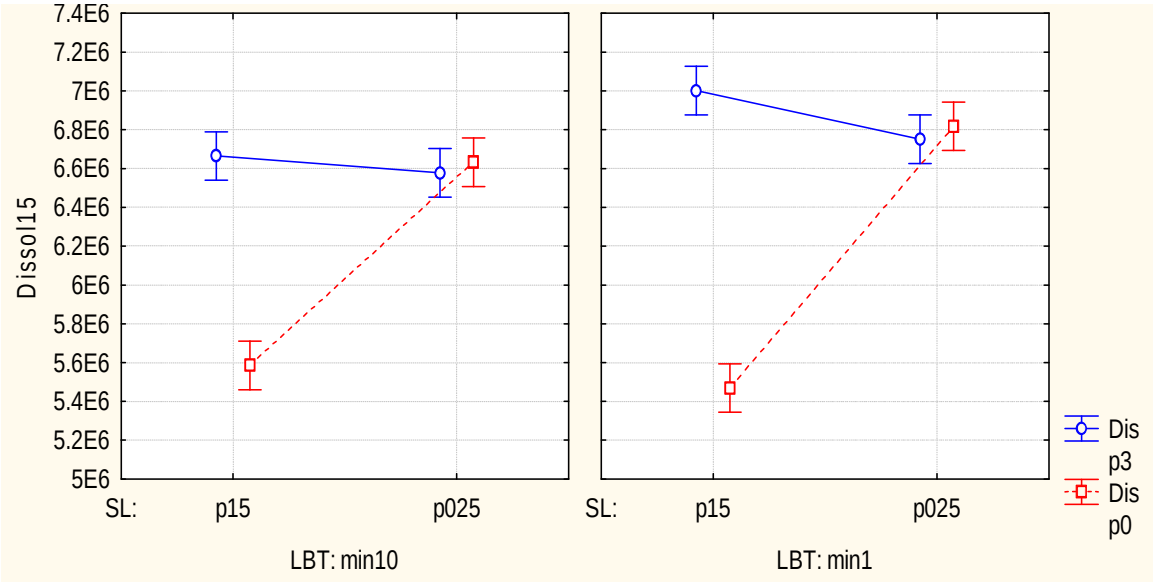


Figure 5.9: Interaction plots between magnesium stearate concentration and sodium starch glycollate concentration (LBT = lubrication blending time, API = lamivudine grade, SL = lubricant content, Dis = disintegrant content)

Although the effect of magnesium stearate levels and API particle size were not significant, studies have shown that both factors affect the dissolution profile of a drug.

The drug-excipient studies did not indicate any interaction between the magnesium stearate and lamivudine (Section 4.3.5) but Makoto et al.(2001) have reported that magnesium stearate interacts with drug powders when thoroughly mixed before tablet compression and reduces tablet hardness and prolongs drug release. High magnesium stearate concentration and longer lubrication blending times result in coverage of all drug particle surfaces and as a result of its hydrophobicity, it retards the dissolution process. Thus the antagonistic interaction was observed between magnesium stearate levels and disintegrants levels as shown in Figure 5.9. This effect is even more prominent in direct compression than in wet granulation since all drug particles are exposed in direct compression. Therefore length and concentration of lubrication becomes much more critical in direct compression. It is advisable that in all direct compression powder blending should not include the lubricant during the majority of the blending time.

Due to increased emphasis on dissolution and bioavailability, many drugs are commonly micronised. Small drug particles lead to interparticulate friction and decreased powder fluidity, and may also result in poor compressibility but the studies have shown that the dissolution rate increases with decreased particle size probably due to increased surface area. Very often a decision has to be made as to whether to granulate a micronized drug, which may result in reduced dissolution profile or to directly compress larger particles with better flowability. (Shangraw, 1989: 199-201)

5.3.5 Optimization

All the formulations met the required specifications, except in the assay determination test whereby formulations 9, 6 and 15 were outside the required assay content range. Although most of the formulations met the required specifications, the formulation presenting the optimum responses could not be selected by visual inspection since one formulation for example had the lowest friability but the highest uniformity of mass RSD. The main aim of the optimization study was to optimize both the process and formulation factors so as to obtain a formulation with low uniformity of mass RSD, low friability, low disintegration times and good dissolution profile. All the basic tablet criteria had to be

met, including assay content of between 95-105 % and uniformity of mass with at least 9 out of 10 tablets should contain not less than 85 % or not more than 115 % of the label claim and the RSD must be equal or less than 6.0 %. The ANOVA revealed the main and interaction effect of the chosen factors on the responses. Since there were interactions amongst the considered factors, the responses could not be affected by changing the level of a significant factor independently of the other factors. The main and interaction effects of the considered factors on the responses that were revealed by the ANOVA formed the basis for the formulation optimization.

Multivariate multiple regression (MMR) analysis was used for formulation optimization. The tool could only optimize the levels at which the experiments had been evaluated and tested as a two level system cannot determine if a linear relationship exists. Therefore there were only two levels for each factor that were considered for optimization. The ANOVA revealed that the main effect of some of the factors was not significant with respect to tablet responses. The MMR analysis tool considered the interactions and statistical significance revealed by the ANOVA for formulation optimization. This tool uses the regression co-efficient to predict the change in the dependent variable (response) when there is a unit change in a factor. The predictions by MMR are shown in Table 5.16. The negative sign indicates a decrease in the tablet response when the independent variable has been increased by a unit. This tool uses the data created by the ANOVA to predict statistical significance of the unit increase in a factor. A p-value equal or less than 0.05 is considered significant for statistical analysis as explained in Section 5.3.4 above. Like with the ANOVA, the same factors that were statistically significant with respect to the tablet responses would still produced considerable effect on tablet responses.

Table 5.14: Regression co-efficient of multivariate multiple analysis (Values in bold are statistically significant)

Factors	U. mass (% RSD)	Friability (% loss)	D. time (min)	% released at 5 min	% released at 15 min
API	**-0.6125**	-0.04347	0.9944	-3.2325	-1.8288
Disintegrant	-0.01805	-0.002975	**-0.5650**	**8.0317**	**2.9563**
Magnesium Stearate	**-0.2965**	0.02041	0.9302	-2.0960	-2.8590
Lubrication blending time	-0.02836	-0.001519	0.07289	-0.8942	-0.2296

The statistical significance of the change in the dependent variable (response) when there was a unit change in a factor was considered for the selection of the levels for the optimized formulation. Only the API grade factor would considerably affect the uniformity of mass RSD. When the particle size would be increased by a unit the uniformity of mass RSD would decrease by 0.6125. Since the MMR indicated the API grade factor as only significant factor for the uniformity of mass response and the use of coarse grade of the API would result on low uniformity of mass RSD %. It was then decided that the coarse grade would be selected for the optimized formulation. Like with the ANOVA, the predicted change of the dependent variable when disintegrant unit level would be changed would only be significant for disintegration and dissolution profiles. When the level of a disintegrant would be increased by a unit, the disintegration time would decrease by 0.5650 with dissolution at 5 and 15 minutes increasing by 8.0317 and 2.9563 respectively. It was therefore decided that the high level of a disintegrant would be selected. The predicted change of a dependent variable when the magnesium stearate unit level would be changed was only significant for the uniformity of mass RSD response. Like with API grade factor, when the magnesium stearate concentration would be increased by a unit level then uniformity of mass would decrease as indicated by the negative sign in Table 5.16. Although the increased level of magnesium stearate are known to negatively affect the disintegration and dissolution responses as discussed in Section 5.3.4.3 above, its effect on these formulations would not be considerable.

Therefore the high level of magnesium stearate was selected for the optimized formula. When the lubrication blending time would be increased or decreased by a unit level no change in any of the dependent variables would be statistically significant. Since longer lubrication blending times are known to retard tablets disintegration and dissolution (Banker & Anderson, 1986:301), it was therefore decided that the low level of lubrication blending time would be selected. Table 5.15 shows the optimum factor settings for the lamivudine 150 mg tablets.

Table 5.15: The optimized values for the considered factors

Factor	**API**	**Disintegrant**	**Magnesium Stearate**	**Lubrication blending time**
Optimized variables	Coarse grade	3 %	1.5 %	1 min.
Levels	+	+	+	-

The optimum factor settings corresponded with formulation number 2 from the design of experiments (Table 5.7). Therefore the conditions presented by formulation number 2 were considered as optimum factor settings. The tablet composition for the optimized formula for a tablet mass of 350 mg is shown in Table 5.16.

Table 5.16: Tablet composition for optimal factor settings (mg)

API grade	MCC 102 (mg)	Sodium starch glycollate (mg)	Magnesium stearate (mg)	Total Mass (mg)
Coarse grade	**184.250**	**10.5**	**5.250**	**350**

The optimized formulation met all the required specifications as shown in Table 5.19. The average assay for the formulation was 98.65. Even though the average assay result looks acceptable, it could mask a wide variation in potency, with the result that a patient could be variably under-dosed or over-dosed (Banker & Anderson, 1986:300). Although the uniformity of mass can be considered as an indication of dosage uniformity for the tablets containing 50 mg or more of the API, the uniformity of mass test is still not

sufficient to assure uniformity of content, and as a result, the content uniformity test is applied. Non-uniform distribution of the active ingredient throughout the powder mixture, segregation of the powder mixture and tablet mass variation are the known factors that contribute directly to content uniformity problems (Rosanske et al.,1989:322). The method of this test is described in Section 5.2.4.7. The formulation indicated an average assay of 101.12 % with % RSD of 2.9740 for the ten tablets that were randomly selected for this test. All ten tablets contained the API between 85 % and 115 % as required. Therefore the formulation met the required specification for content uniformity. The tablet responses from the optimized tablet formulation are shown in Table 5.17.

Table 5.17: Summary of the dependent variable for the optimized formulation

Test	Results
Hardness (N)	**92.25**
Mass (mg)	**352.54**
Uniformity of mass (% RSD)	**0.4997**
Disintegration Time (min.)	**0.3065**
Friability (% loss)	**0.08810**
Dissolution profile (% dissolved @15 &30 mins.)	**101.74 & 103.82**
Assay content (%m/m)	**98.65**
Content uniformity (%m/m ± %RSD)	**101.12 ± 2.9740**

The optimized formulation was considered as acceptable and could be justified by the constituents of the formulation. In the preformulation study the coarse material was shown to have better flow than the fine material and thus presented low uniformity of mass RSD. Although the fine material is known to improve the drug dissolution, its effect on dissolution was not statistically significant for this product. Sodium starch glycollate is a super-disintegrant which is very effective in reducing disintegration times and improving the drug dissolution profiles. Although it sometimes retards disintegration and dissolution, magnesium stearate is a good lubricant and has glidant properties. Since the high level of magnesium stearate was selected, the low level of lubrication blending time

was selected to decrease the exposure of the API particles onto the magnesium stearate. An optimum formulation generated by the use of statistical tools was produced and analyzed. The composition of this optimized tablet is shown in Table 5.16

5.4 SUMMARY

The formulation was developed by three step approach namely; preliminary formulation development, design of experiments and the formulation optimization study. The purpose of the preliminary study was to identify and define the tablet hardness that would be used throughout the experimentation. Together with analysis of variances (ANOVA), the design of experiments (DoE) was used to study the main and interaction effects of the critical process and formulation variables on the tablets responses. There were four factors that were considered as critical which were evaluated at high and low levels. The 2^4 full factorial experimental design was used for the design of experiments and generated 16 experiments. To minimize bias, the experiments were executed in random order. The ANOVA tool revealed that the API particle size and magnesium stearate concentration considerably affected tablets uniformity of mass. When the particle size was changed from fine to coarse material and the magnesium stearate concentration was changed from low level (0.25 %) to high level (1.5 %) a decrease in uniformity of mass RSD was obtained. The ANOVA tool revealed that none of the four factors considerably affected the tablets friability. The ANOVA tool showed that only disintegrant (sodium starch glycollate) concentration had statistically significant effect on the tablet disintegration and dissolution responses. When disintegrant concentration was changed from low to high level, a decrease in disintegration times and increase in the dissolution profile were obtained. The interaction plots showed non-parallel lines between the four factors indicating interactions with each other for all the considered responses. This meant that to affect the uniformity of mass RSD response, the level of one factor could not be changed independently of the level of the other factor. After ANOVA had revealed the main and interaction effect of the critical factors on the responses, the final formulation was defined by optimizing the level of each factor using multivariate multiple regression (MMR) analysis.

MMR predicted that the coarse grade, high disintegrant concentration, high lubricant concentration and low lubrication blending time would produce the optimal factor settings for a lamivudine 150 mg tablets. The optimized formulation was considered acceptable, meeting all the required specifications. An optimum formulation generated by the use of statistical tools was produced and analyzed. The DoE, ANOVA and MMR analyses were used as tools to obtain the optimal formulation, which met all the required pharmaceutical specifications. This study has demonstrated the efficiency and effectiveness of using DoE and statistical analysis to develop a tablet formulation.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSION

This study presented a systematic approach to optimizing an immediate release oral tablet formulation containing 150 mg lamivudine using DoE and ANOVA. The preformulation studies indicated a high compressibility index for both lamivudine grade materials indicating a poor flow for the active ingredient. No incompatibilities between the drug and the excipients were detected.

The formulation was developed by using a three step approach namely; preliminary formulation development, design of experiments and the formulation optimization study. The preliminary study was designed to test the viability of the formula and to define the tablet hardness that would be used throughout the experimentation. Together with analysis of variances (ANOVA), the design of experiments (DoE) was used to study the main and interaction effects of the critical process and formulation variables on the tablets responses. After ANOVA had revealed the main and interaction effect of the critical factors on the responses, the final formulation was defined by optimizing the level of each factor.

Based on the literature, four independent factors that are known to affect the tablet formulation were selected. All four factors were evaluated at high and low levels. A 2^4 full factorial experimental design was used for the design of experiments and generated sixteen experiments. To minimize bias, the experiments were executed in a randomized order.

All formulations met the required specification for uniformity of mass, friability, disintegration and dissolution. The ANOVA revealed that only the API particle size and magnesium stearate concentration considerably affected tablet uniformity of mass. The

disintegration and dissolution were considerable affected by the disintegrant content. None of the four factors considerably affected the tablets' friability. The ANOVA revealed interactions amongst these factors therefore, to affect (change) any of the responses, the level of one factor could not be changed independently on the level of the other factors.

MMR analysis was used for formulation optimization. The tool could only optimize the levels at which the experiments had been evaluated and tested. This tool uses the regression co-efficient to predict the change in the dependent variable (response) when there is a unit change in a factor. The coarse grade lamivudine material, 3.0 % of sodium starch glycollate, 1.5 % of magnesium stearate and 1 minute lubrication blending time were selected as the optimum factor settings for this formulation. An optimum formulation generated by the use of statistical tools was then produced and analyzed.

Since there are so many formulation and process variables that one must consider when developing a pharmaceutical formulation. The use of statistical tools allows one to efficiently evaluate multiple variables, while minimizing the experimentation. Although most formulations met the required specifications, this study has demonstrated and described the efficiency and effectiveness of using a statistically designed methodology to develop the tablet formulation.

The efficiency of using direct compression method in tablet formulation was also demonstrated. The wet granulation includes other steps like granulation, screening and drying of granules that are not involved in direct compression. There were therefore reduced processing time and thus reduced labour costs, fewer manufacturing steps and pieces of equipment in direct compression. Also observed with the sixteen formulations generated by the DoE were the low disintegration times. Shangraw (1989) reported that disintegrant added prior to wet granulation are known to be less effective than those added just prior to direct compression. In direct compression all of the disintegrant is able to perform optimally since it is not incorporated in the granules.

6.2 RECOMMENDATIONS

The use of full factorial experimental design is recommended more than fractional factorial design when one deals with less than five factors. Unlike fractional factorial design, the full factorial experimental design includes all factors and all these factors and their defined levels are included in the DoE. It does not only cover the entire area of interest with as few experiments as possible, it also makes it possible to examine the interaction effects. The number of experiments in full factorial design grows rapidly with an increase in number of variables and therefore when dealing with more than five factors, a full factorial design is not the best option. The drawback with the fractional factorial design is loss of information caused by confounding of interaction effects. Also the use of more than two levels for the factors involved is recommended. The more levels that are used for the design of experiments, the closer one gets to the optimum level settings. Although the use of more than two levels results in increased number of experiments, it provides better optimal factor settings than two levels.

After the optimum formulation has been identified, it is recommended that the ICH stability studies (ICH, 2003: Q1A) are performed on this formulation to asses its stability under stressed storage conditions. It is up to the applicant to decide whether the long term, intermediate or accelerated stability studies are performed on the product. The parameters for the stability testing are shown in Table 6.1

Table 6.1: ICH stability testing parameters

Study	**Storage condition**	**Minimum time period covered by data at submission**
Long term	25°C ± 2°C/60% RH ± 5% RH or 30°C ± 2°C/65% RH ± 5% RH	12 months
Intermediate	30°C ± 2°C/65% RH ± 5% RH	6 months
Accelerated	40°C ± 2°C/75% RH ± 5% RH	6 months

With a critical approach to the selection of raw materials (DoE and statistical analysis), wet granulation can sometimes be simplified using direct compression approach.

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APPENDIX A

CONCEPT ARTICLE

The development of an antiretroviral solid dosage form using multivariate analysis

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ABSTRACT

The development of a new and generic formulation is still based on a large number of experiments. Statistics provides many tools for studying the conditions of formulations and processes and enables us to optimize the same while being able to minimize our experimentation. The purpose of this study was to apply experimental design methodology (DoE) and multivariate analysis to the development and optimization of tablet formulations containing lamivudine 150 mg manufactured by direct compression. Following the trial formulation, both formulation and process factors to be used were identified. Sixteen formulations involving four factors evaluated at two levels (2^4) were generated by full factorial design with different proportion of API particle size, lubrication blending times, magnesium stearate levels and starch glycollate. Standard pharmacopoeial methods were used to test for physical properties of the tablets. The analysis of variances (ANOVA) showed the main and interaction effects of the defined factors on the responses that were statistical significant at 99% confidence interval. An optimum formulation generated by the use of statistical tools was produced and analyzed. This study proved the efficiency of using DoE and statistical analysis for formulation development

Keywords: Analysis of variances (ANOVA), Design of experiments (DoE), multivariate analysis, pharmaceutical development, lamivudine

1. Introduction

After an initially slow start, the development of new antiviral agents and their generics has now entered an accelerated growth phase. Lamivudine and several other compounds have been approved for treatment of the hepatitis B and HIV virus infections. Lamivudine is a nucleoside reverse transcriptase inhibitor (NRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1) and hepatitis B virus (HBV) [11].

The aim of pharmaceutical development is to design a quality product and its manufacturing process to consistently deliver the intended performance of the product [7]. Direct compression is the preferred method of manufacture due to fewer steps involved in manufacturing, resulting in lower production costs, and causing fewer problems with bioavailability and stability [9]. Formulation of medical products (e.g., tablets) was previously performed mainly on the basis of the experience of the formulator, often in combination with the approach of changing one separate factor at a time, but the use of statistical experimental design in connection with commercial statistical software has found widespread use [4]. DoE is a tool that allows the formulator to evaluate a multitude of factors concurrently while minimizing experimentation. Another tool available is Analysis of Variance (ANOVA), which allows one to test for significant differences between means and uncovers the main and interaction effects of independent variables on dependent variables. [5] The advantage of experimenting with multiple variables concurrently is that we learn about interaction effects that would be hidden if we only observed one variable at a time [5, 13].

The formulation objective of this study was to formulate a solid dosage form containing lamivudine 150 mg that could meet the required specifications for disintegration, dissolution, friability, uniformity of mass, assay and content uniformity. All the other basic tablet criteria had to be met including blend homogeneity, lubricity and

compressibility. Another objective was to apply statistical multivariate analysis methodology to study the effect that the considered critical process and formulation factors have on tablets responses.

2. Materials and method

2.1 Materials

The following ingredients that were used for tablet formulation were kindly donated by Aspen Pharmacare, Port Elizabeth, South Africa. Lamivudine fine ($D_{0.9}$ = 100 µm) and coarse grade ($D_{0.9}$ = 350 µm) (Hetero Labs Limited, Gaddapotharam, India), Microcrystalline cellulose (Vivapur® PH102), Sodium starch glycollate (Vivastar®) (JRS Pharma, Weissenborg, Germany) and Magnesium stearate (Liga Magnesium Stearate MF-2-V) (Peter Green, Edisonstraat, Netherlands) All the ingredients were used as received

2.2 Experimental Methods

2.2.1 Method of manufacture

All the ingredients were weighed (Mettler Toledo® AB 104 balance) according to the quantities generated by the DoE. To reduce agglomeration of particles lamivudine and microcrystalline cellulose were screened using a 710 µm sieve (Laboratory Test Sieve) and magnesium stearate and starch glycollate were screened with a 425 µm sieve. All the chemicals, with the exception of magnesium stearate were blended for 5 minutes in an Erweka® AR 400-Rotating Cube mixer. Magnesium stearate was added and blended for an additional time as determined by DoE to lubricate the mixture. The above mixture was then transferred to a Korsch Fe246 sRC single tablet press machine equipped with shoe hopper for die feed and 11 mm circular biconcave compression tooling.

2.2.2 Formulation optimization

The formulation was developed by using a three step approach namely; preliminary formulation development, design of experiments and the formulation optimization study. The preliminary study was designed to test the viability of the formula and to define the tablet hardness that would be used throughout the experimentation. Together with analysis of variances (ANOVA), the design of experiments (DoE) was used to study the main and interaction effects of the critical process and formulation variables on the tablets responses. After ANOVA had revealed the main and interaction effect of the critical factors on the responses, the final formulation was defined by optimizing the level of each factor.

2.2.2.1 Preliminary formulation

The preliminary study formulation was established by considering the innovator's product and the literature. Four trial batches that were compressed at different forces were prepared.

2.2.2.2 Design of experiment

Based on the literature, four independent factors that are known to affect the tablet formulation were selected as shown in Table 1. All four factors were evaluated at high and low levels. All the responses (dependent variables) and their specifications are listed in Table 2. A 2^4 full factorial experimental design was used for the design of experiments and generated sixteen experiments as shown in Table 3. To minimize bias, the experiments were executed in a randomized order.

2.2.3 Statistical analysis

The software Statistica (version 7.1) was used for the analysis of the main and the interactions effects of the considered factors on the responses. The software utilizes

analysis of variances (ANOVA) which uses the probability value (p-value) to determine the main effect and the statistical significance of the considered factors on the responses at the 95 % confidence interval. The interaction plots were generated for each response so as to determine the extent of the interactions between the factors.

3. Results and Discussions

3.1 Preliminary study

The main purpose of the preliminary study was to test the viability of the formula and identify and define the tablet hardness that would be used throughout the formulation. The results from the preliminary study are summarized in Table 4. The results indicated low friability for trial batch number four and high friability for trial batch number one. Since the difference was only notable on friability, the hardness was then selected based on the trial batch with lowest friability. From the results it was shown that trial batch number four had lowest friability therefore 87 ± 10 N was defined as the target hardness which was used throughout the experimentation.

3.2 Evaluation of tablets

In all 16 tablets formulations, typical tablet defects such as capping, chipping and picking, were not observed. All formulations met the required specification for uniformity of mass, all indicating % RSDs of less than 5 %, as shown in Table 5. All the formulations indicated to be less friable when tested by friabilator at 100 revolutions showing low friability of less than 0.3 % as shown in Table 5. Also, there was no capping observed therefore the formulations met the USP 27th edition (2004) specification of friability. The limit for an uncoated immediate release tablet to disintegrate is 15 minutes. Most formulations presented fast disintegration times of less than 2 minutes except formulation 6 and 5 that presented the tablet disintegration of 2.76 and 6.65 minutes respectively, but were still within the required limit of 15 minutes as shown in Table 5.10.

All formulations were within the specified range (95-105 %) except for formulations 9, 15 and 6 with assay outside the specified range of the labeled amount (150 mg) as shown in Table 5. The requirements for the dissolution were that not less than 85 % of the API is released in 30 minutes and not less than 75 % is released in 15 minutes. All formulations met the required specifications at both 15 and 30 minutes intervals.

3.3 Statistical analysis

The main aim of the design of experiment for this study was to identify the most significant or critical factors affecting the tablets' dissolution profile, disintegration, uniformity of mass and friability responses and establish the best optimal level settings for the formulation. ANOVA uses the probability value (p-value) to determine if the main effect of the independent value on dependent value (response) is statistically significance. A p-value equal or less than 0.05 is considered significant for statistical analysis.
Two factors are said to interact with each other if the effect of one factor in the response is different at different levels of the other factor. In order to interpret the interactions, the interaction plots are constructed where one factor is plotted in the x-axis as a function of another factor. When lines are non-parallel to each other, an interaction exists between the factors as shown. This means that the change in the mean response from low to high level of a factor depends on the level of the other factor. [6]

3.3.1 Effects on uniformity of mass

The statistical significance of the main effect of the four factors on uniformity of mass is shown in Table 6, as indicated by the p-values. The ANOVA tool showed that only the factors API particle size and magnesium stearate concentration were statistically significant for uniformity of mass response (% RSD) at the 95 % confidence range as shown in Table 6.

The ANOVA tool revealed that API particle size and magnesium stearate concentration considerably affected tablet uniformity of mass. When the particle size was changed from fine to coarse material, a decrease in % RSD was obtained as shown in Table 7. Normally the magnitude of Van der Waals forces predominates over frictional forces in fine material causing an increased in cohesiveness of the powder and resulting in poor powder flow [10].

When the magnesium stearate concentration size was changed from a low level (0.25 %) to a high level (1.5 %), a decrease in % RSD for the uniformity of mass was obtained as shown in Table 7. Magnesium stearate is one of the most commonly used lubricants. [16] With a well-defined particle size distribution, magnesium stearate improves powder flow, ensuring a high content uniformity, even in low-dosage formulations. Bandelin (1989) reported that magnesium stearate can also serve as a glidant (improves powder mix flow). Increased concentration of magnesium stearate presents more lubrication and reduces the interparticulate friction resulting in improved powder flowability. This was indicated from the formulations by the decreased uniformity of mass RSD with high magnesium stearate levels indicating improved powder flowability.

There were four factors involved in design of experiments but ANOVA revealed that only API particle size and magnesium stearate level were only significant for uniformity of mass at the 95 % confidence interval. The interaction plots were constructed to study the interaction of all four factors on the uniformity of mass. The interaction plots showed non-parallel lines between the four factors indicating interactions with each other as shown in Figure 1. Due to interactions amongst the considered factors, meant that to affect the uniformity of mass (% RSD) response, the level of one factor could not be changed independently of the level of the other factor.

3.3.2 Effects on friability

The ANOVA tool revealed that none of the four factors considerably affected the tablets friability as indicated in Table 6 by the probability values of more than 0.05 for all four

factors. The low friability percentage values (Table 5) could be attributed to the use of microcrystalline cellulose. MCC has an ability to deform plastically when compressed and act as a binder in direct compression [12]. Also, another important property of MCC is its ability to and the water it contains, maintains its ability to deform plastically. The interaction plots showed non-parallel lines between the four factors indicating interactions with each other.

3.3.3 Effects on disintegration

The ANOVA tool showed that only disintegrant (sodium starch glycollate) concentration was shown to significantly affect disintegration at the 95 % confidence range When disintegrant concentration was changed from a low to a high level, a decrease in disintegration times was obtained as shown in Table 7. Sodium starch glycollate is a known super-disintegrant, which is effective even at low concentration [2]. Unlike in wet granulation whereby the disintegrant is incorporated in a granule, in direct compression all of the disintegrant is able to perform optimally and cause the tablet to disintegrate rapidly [12]. This results in faster disintegration times, as shown in Table 5. The interaction plots showed non-parallel lines between the four factors indicating interactions with each other. Faster disintegrations times for formulations without disintegrant could be attributed to the presence of microcrystalline cellulose [14], which has ability to a disintegrant. It has also been shown that super-disintegrants have a greater effect on disintegration time in an insoluble system than in a soluble or partially soluble system, thus starch glycollate functions optimally in the presence of MCC [3].

3.3.4 Effects on dissolution profiles

The ANOVA tool showed that only disintegrant concentration was shown to significantly affect dissolution at the 95 % confidence range The ANOVA tool revealed that disintegrant concentration considerably affected the dissolution profile at 5 and 15 minutes intervals. When disintegrant concentration was changed from a low to a high level, an increased in % released was obtained at both intervals as shown in Table 7.

Although the effect of magnesium stearate levels and API particle size were not significant, studies have shown that both factors affect the dissolution profile of a drug [8]. Small drug particles lead to interparticulate friction and decreased powder fluidity, and may also result in poor compressibility but the studies have shown that the dissolution rate increases with decreased particle size probably due to increased surface area. [12]

3.4 Optimization

Although most of the formulations met the required specifications, the formulation presenting the optimum responses could not be selected by visual inspection since one formulation for example had the lowest friability but the highest uniformity of mass RSD. The MMR analysis was used for formulation optimization. The tool could only optimize the levels at which the experiments had been evaluated and tested. This tool uses the regression co-efficient to predict the change in the dependent variable (response) when there is a unit change in a factor. The predictions by MMR are shown in Table 8. The coarse grade lamivudine material, 3.0 % of sodium starch glycollate, 1.5 % of magnesium stearate and 1 minute lubrication blending time were selected as the optimum factor settings for this formulation as shown in Table 9. An optimum formulation generated by the use of statistical tools was then produced and analyzed.

The optimum factor settings corresponded with formulation number 2 from the design of experiments (Table 3). Therefore the conditions presented by formulation number 2 were considered as optimum factor settings. The optimized formulation met all the required specifications (Table 2) as shown in Table 10. The average assay for the formulation was 98.65. The tablet responses from the optimized tablet formulation are shown in Table 10.

The optimized formulation was considered as acceptable and could be justified by the constituents of the formulation. An optimum formulation generated by the use of statistical tools was produced and analyzed.

4. Conclusion

Since there are so many formulation and process variables that one must consider when developing a pharmaceutical formulation. The use of statistical tools allows one to efficiently evaluate multiple variables, while minimizing the experimentation. Although most formulation met the required specifications, this study has demonstrated and described the efficiency and effectiveness of using a statistically designed methodology to develop the tablet formulation.

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Table 1: Formulation and process factors and their levels for the design of experiments

Independent variables (factors)		**Levels**	
		Low	**High**
1	API particle size	Fine($D_{0.9}$ = 100µm)	Compact ($D_{0.9}$ = 350µm)
2	Disintegrant content	3%	0%
3	Lubricant content	0.25%	1.5%
4	Lubrication blending time	1 minute	10 minutes

Table 2: Responses used to evaluate the tablets quality

Dependent variables (responses)	**Specifications**
Uniformity of mass	Not more than 2 out of 20 tablets may deviate from the average tablet mass by more than 5.0 %
Tablet friability	Less than 0.5 % is lost after 100 revolutions
Tablet disintegration time	Less than 15 minutes
Tablet dissolution rate	Not less than 85 % released in 30 minutes (Q = 80 %) Not less than 75% released in 15 minutes
Assay	150mg (332.5-367.5 mg) or 95%-105% of 150 mg
Content uniformity	Nine of the ten tablets must contain not less than 85% or not more than 115% of the label claim, Relative Standard Deviation must be less than or equal to 6.0%.

Table 3: Full factorial experimental design (seq. denotes the execution sequence)

Formulation	Seq.	Factor 1 API Particle Size	Factor 2 Disintegrant content (%)	Factor 3 Lubricant content (%)	Factor 4 Lubrication Blending Time (min)
1	16	Compact (+)	3% (+)	1.50% (+)	10 mins (+)
2	7	Compact (+)	3% (+)	1.50% (+)	1 min (-)
3	12	Compact (+)	3% (+)	0.25% (-)	10 mins (+)
4	9	Compact (+)	3% (+)	0.25% (-)	1 min (-)
5	14	Compact (+)	0% (-)	1.50% (+)	10 mins (+)
6	11	Compact (+)	0% (-)	1.50% (+)	1 min (-)
7	4	Compact (+)	0% (-)	0.25% (-)	10 mins (+)
8	13	Compact (+)	0% (-)	0.25% (-)	1 min (-)
9	2	Fine (-)	3% (+)	1.50% (+)	10 mins (+)
10	6	Fine (-)	3% (+)	1.50% (+)	1 min (-)
11	15	Fine (-)	3% (+)	0.25% (-)	10 mins (+)
12	10	Fine (-)	3% (+)	0.25% (-)	1 min (-)
13	3	Fine (-)	0% (-)	1.50% (+)	10 mins (+)
14	8	Fine (-)	0% (-)	1.50% (+)	1 min (-)
15	1	Fine (-)	0% (-)	0.25% (-)	10 mins (+)
16	5	Fine (-)	0% (-)	0.25% (-)	1min (-)

Table 4: Summary of the major tablet responses for preformulation study

Trial No.	Hardness ± (% RSD) (N)	Mass ± (%RSD) (mg)	Disintegration time (s)	Friability (% loss)
1	**29.9 ± 3.1 N**	356.02 ± 1.04 mg	12.20 s	2.09 %
2	**51.9 ± 2.2 N**	350.59 ± 1.09 mg	13.61 s	0.42 %
3	**75.4 ± 2.3 N**	357.31 ± 0.95 mg	13.02 s	0.30 %
4	**87.2 ± 2.8 N**	355.46 ± 0.94 mg	13.65 s	0.11 %

Table 5: Summary of the major tablet responses

Form.	Hardness (N)	Mass (mg)	Uniformity of mass (%RSD)	Disintegration Time (min) × 60sec.	Friability (% loss)	Assay content (%m/m**)**	% Released at 15 min.	% Released at 30 min.
15	80.20	353.65	1.3248	1.2980	0.1244	111.65	93.96	95.30
9	82.00	353.49	0.2269	0.2978	0.08803	94.31	97.03	96.30
13	87.15	355.39	0.3761	1.2193	0.1747	98.30	81.33	94.37
7	85.00	356.80	0.2355	1.0934	0.03362	100.15	94.87	103.07
16	85.05	365.27	1.7046	0.3546	0.3546	101.68	102.77	103.16
10	77.35	347.98	0.9732	0.2458	0.2118	99.54	97.64	96.95
2	92.25	352.54	0.4997	0.3065	0.08810	98.65	101.74	103.82
14	88.15	345.95	0.7792	1.4692	0.07244	104.35	80.20	92.02
4	90.75	349.34	0.3864	0.1366	0.08864	99.55	96.11	93.60
12	79.55	356.94	1.1302	0.1707	0.06378	102.04	96.11	96.02
6	89.35	353.98	0.3637	2.7608	0.07060	108.81	75.51	87.18
3	82.15	354.19	0.2696	0.1717	0.05364	101.91	95.85	94.66
8	86.90	355.65	0.4960	1.1572	0.09518	103.73	91.35	96.28
5	77.94	349.28	0.2487	6.6533	0.1006	100.42	77.70	91.84
11	88.65	344.68	1.3472	0.1925	0.07455	99.37	91.44	91.81
1	80.85	345.9	0.3621	0.9238	0.2862	100.05	92.72	91.10

Table 6: Probability value summary; U mass = uniformity of mass; D. time = disintegration time (Bold numericals denotes p value < 0.05)

Factors	U. mass (% RSD)	Friability (% loss)	D. time (min)	% released at 5 minutes	% released at 15 minutes
API	**0.001639**	0.4000	0.1404	0.4768	0.5975
Disintegrant	0.7211	0.8606	**0.02035**	**0.0002**	**0.0231**
Magnesium Stearate	**0.0292**	0.6176	0.0901	0.5626	0.05210
Lubrication blending time	0.1129	0.7881	0.3170	0.0939	0.5515

Table 7: Effect summary of the four critical factors on the responses

Factors	**Levels**	**U mass** (% RSD)	**Friability** (% loss)	**D. time** (min)	**% released at 5 min.**	**% released at 15 min.**
API	Coarse	0.3577	0.1021	1.6504	71.96	90.73
	Fine	0.9703	0.1455	0.6560	75.19	92.56
Disintegrant content	3 %	0.6369	0.1193	0.3057	85.62	96.08
	0 %	0.6911	0.1282	2.0007	61.53	87.21
Lubricant content	1.5 %	0.4787	0.1366	1.7346	72.26	87.98
	0.25 %	0.8493	0.1110	0.5718	74.88	95.31
Lubrication blending time	10 min.	0.5363	0.1170	1.4812	69.55	90.61
	1 min.	0.7916	0.1306	0.8252	77.60	92.68

Table 8: Regression co-efficient of multivariate multiple analysis (Values in bold are statistically significant)

Factors	U. mass (% RSD)	Friability (% loss)	D. time (min)	% released at 5 min	% released at 15 min
API	**-0.6125**	-0.04347	0.9944	-3.2325	-1.8288
Disintegrant	-0.01805	-0.002975	**-0.5650**	**8.0317**	**2.9563**
Magnesium Stearate	**-0.2965**	0.02041	0.9302	-2.0960	-2.8590
Lubrication blending time	-0.02836	-0.001519	0.07289	-0.8942	-0.2296

Table 9: The optimized values for the considered factors

Factor	**API**	**Disintegrant**	**Magnesium Stearate**	**Lubrication blending time**
Optimized variables	Coarse grade	3 %	1.5 %	1 min.
Levels	+	+	+	-

Table 10: Summary of the dependent variable for the optimized formulation

Test	**Values**
Hardness (N)	**92.25**
Mass (mg)	**352.54**
Uniformity of mass (% RSD)	**0.4997**
Disintegration Time (min.)	**0.3065**
Friability (% loss)	**0.08810**
Dissolution profile (% dissolved @15 &30 mins.)	**101.74 & 103.82**
Assay content (%m/m)	**98.65**
Content uniformity (%m/m ± %RSD)	**101.12 ± 2.9740**

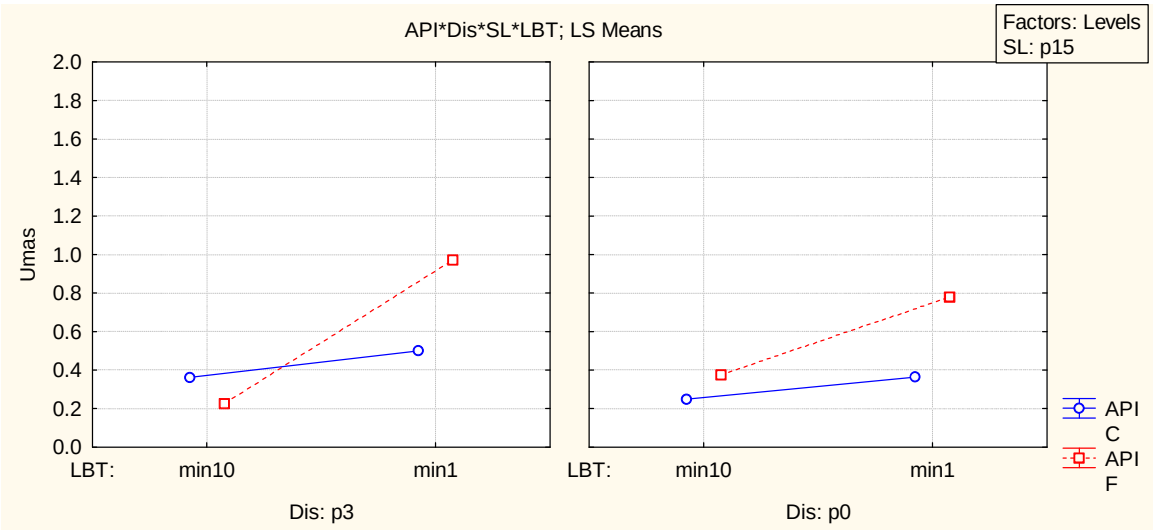

Figure 1: Interaction plots between magnesium stearate concentration and lubrication blending times (LBT = lubrication blending time, API = lamivudine grade, SL = lubricant content, Dis = disintegrant content)

APPENDIX B

ABSTRACT SUBMITTED FOR THE ACADEMY PHARMACEUTICAL SOCIETY OF SOUTH AFRICA (APSSA) CONFERENCE

Entry: The young scientist award

Held at Club Mykonos in Langebaan, 4th-7th September 2007

Development of antiretroviral solid dosage form using design of experiments and multivariate analysis

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Purpose:

The aim of pharmaceutical development is to design a quality product and the manufacturing process to deliver the product in a reproducible manner. The development of a new and generic formulation is still based on a large number of experiments. Statistics provides many tools for studying the conditions of formulations and processes and enables us to optimize the same while being able to minimize our experimentation. The purpose of this study was to apply experimental design methodology (DOE) and multivariate analysis to the development and optimization of tablet formulations containing Lamivudine 150 mg manufactured by direct compression.

Methods:

Following the trial formulation, both formulation and process factors to be used were identified. Sixteen formulations involving four factors evaluated at two levels (2^4) were generated by full factorial design with different proportion of API particle size, lubrication blending times, magnesium stearate levels and starch glycollate. Standard pharmacopoeial methods were used to test for physical properties of the tablets.

Results:

The analysis of variances (ANOVA) showed the main and interaction effects of the defined factors on the responses that were statistical significant at 99% confidence interval. This study proved the efficiency of using DoE and statistical analysis for formulation development