Study to design stable lansoprazole pellets

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Professor Dr. H-P Hauri
Dekan

To my loved ones

My parents Habiba and Mensur

My husband Mehmed

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Symbols and Abbreviations

BCS	Biopharmaceutical Classification System
DoE	Design of experiments
DSC	Differential scanning calorimeter
FDA	Food and Drug Administration
GIT	Gastro-intestinal tract
GMD	Geometric mean diameter
HSM	Hot stage microscopy
LSP	Lansoprazole
MC	Moisture content
MCC	Microcrystalline cellulose
MFT	Minimum film-forming temperature (°C)
PAT	Process Analytical Technology
PPI	Proton pump inhibitor
RH	Relative humidity
rpm	Round per minute
RSD	Relative standard deviation (%)
SEM	Scanning electron microscopy
STAVEX:	Statistische Versuchsplanung mit Expertensystem
Tg	Glass transition temperature
TGA	Thermogravimetric analysis
$t_{0.5}$	Half-life
$t_{0.9}$	Shelf-life
USP:	United States Pharmacopoeia
v/v	Percent by volume
w/w	Percent by weight

1. Summary

Pharmaceutical product development is a complex and creative design process, that involves many factors, many unknowns, many disciplines and has a multiple iterations and a long life-cycle. In the development of pharmaceutical dosage forms, one of persistent challenges is getting an early stability assessment providing an understanding of critical formulation and process parameters. In depth and science based knowledge, whether to use one excipient or another, or to apply one process before the other, could help shortening the process time and as a consequence save the money which is one of the goals of pharmaceutical industry.

Pelletization processes are usually lengthy and expensive. Processing of a single batch may sometimes require hours or even days to be completed, and it can result in a non-robust process. Formulation of a stable delivery system for lansoprazole is extremely difficult. Lansoprazole belongs to class II drugs of the Biopharmaceutical Classification System (BCS), characterized by low solubility and high permeability. Furthermore, lansoprazole degrades in a highly acidic and highly basic environment, and it is also unstable under conditions of high temperature and also high humidity, with a decrease in the amount of lansoprazole and discoloration of the material being noted on storage under such conditions Tetsuro et al., 1992. Additionally, a strong pH-dependent solubility of the drug was observed. There is therefore a need for a pharmaceutical delivery system which protects the active substance both during storage as well as the passage through the stomach.

The aim of this study was on the one hand the multifactorial investigation of crucial parameters involved in the stability of lansoprazole pellets focusing on the formulation parameters and preparation technique and on the other hand application of Arrhenius equation as a comparative technique in stability of pellets as a solid dosage form.
Firstly, thermal characterization of lansoprazole has been conducted in order to clarify the differences reported in the literature and elucidate the reason of the uncommon behaviour when different heating rates were applied. Combining a differential scanning calorimeter (DSC), thermogravimetric analysis (TGA) and hot-stage microscopy (HSM) technique, the results confirmed that the melting point depression at low heating rates was due to eutectic behavior of the drug with its decomposition products formed at low heating rates. Even when the high heating rates (30 and 40°C/min) were applied melting point of lansoprazole did not show independence on the heating rate and difference in the melting point was 1°C. Combination of different techniques and highly dynamic and standardized methods for determination of thermal properties of decomposable substances should be used.

Series of experiments were devised to study the effects of various formulation and processing variables on preparation and the stability of lansoprazole in order to examine some of the precautions which can be taken to minimize the loss of activity. Lansoprazole pellets were prepared using two different pelletizing techniques, solution suspension layering in bottom spraying fluidized bed and direct pelletization in rotor processor. Firstly, in a solution suspension layering, influence of type of neutral pellet (sugar based and microcrystalline cellulose based), type of stabilizing agent (influence of neutral and weak basic microenvironmental pH), presence of protective HPMC coating, type of aqueous enteric polymer based on shellac or methacrylic acid copolymer (Marcoat 125® or Eudragit L30 D-55®) and the coating levels on surface morphology, porosity, dissolution and stability of enteric coated pellets containing acid-liable drug, was evaluated.

Furthermore, the aim was to investigate the feasibility of rotary processor for preparing lansoprazole loaded pellets based on Balocel®, which is a pre-mixed excipient blend containing microcrystalline cellulose, lactose and sodium carboxymethyl cellulose. Since pelletization in fluidized bed rotary processor is a multivariable process and the final characteristics of produced pellets are affected by several factors, in order to achieve a controlled, robust process and to optimize desired pellet properties, experimental design has been applied using expert design system STAVEX. The most important process variables related to the geometric mean diameter of lansoprazole pellets and the moisture content at the end of liquid addition phase, according to the pre-experiments, included spray rate and rotor speed, while the most influential formulation variable was a level of drug load. The study revealed that even though the process has been optimized to obtain pellets of optimum size and shape, another crucial property of pellets, dissolution, was disregarded and was confounded by another factor which could not be controlled (inlet air humidity) and which was not included in the design. This led to a conclusion, that no matter how comprehensive pre-experimental part of the design is, screening design should be applied.

Accelerated degradation, studying the temperature effects in the presence of moisture on the degradation rate constant of lansoprazole in pellets prepared using different pelletization techniques, has been applied. In order to obtain rapid degradation with science based screening approach, Arrhenius equation has been used as a screening and comparative technique to describe a breakdown of lansoprazole in a solid dosage form and it has proved to be helpful tool in obtaining information on the most important formulation parameters and the optimum formulation of lansoprazole pellets for stability.

Solution suspension layering technique proved to be more controllable process and more advantageous in terms of pellets size, shape and stability, but more time consuming in comparison to the direct pelletization. Study has confirmed that the key mechanism in obtaining a stabile lansoprazole delivery system is not only suppression of proton attacks but also a

limitation of its solubility in the moisture layer, since it was found that in the pellets, lansoprazole degrades following apparent zero-order kinetics. With weak basic microenvironmental pH in the pellets it was possible to keep the degradation and solubility of lansoprazole on a low level. Sugar core stabilized lansoprazole in a way of incorporating the drug in the core forming a less porous active layer on the surface, disabling a contact of water and the active substance. Presence of the protective layer has been justified since it increased the stability of lansoprazole acting as a physical barrier between the drug and the free carboxyl groups of enteric coating polymer. Predicted shelf-lives of pellets on room temperature should be confirmed with the data obtained in a real time stability testing under the same conditions of relative humidity.

2. Theoretical section

2.1. PAT and quality by design

The goal of Process Analytical Technology (PAT) initiative, as Food and Drug Administrations (FDA's) new concept of quality assurance in the 21st century, is to understand and control the manufacturing process and formulation. Nowadays it is generally acknowledged that "quality can not be tested into products; it should be built-in or should be by design" according to the ICH Guideline Q8 – Pharmaceutical Development, released on 10th November 2005. The main goal of PAT is to ensure product quality applying systems for designing, analyzing and controlling manufacturing through timely measurement (i.e. during processing) of critical quality and performance attributes of raw and in-process materials and processes.

Formulations nowadays are usually developed under high-time pressure on the basis of "trial and error" experiments which often result in non-robust product (Leuenberger and Lanz, 2005). They are variable and complex systems influenced not only by formulation parameters, meaning the properties of active substance and excipients, but also in the large number of processes involved in manufacturing.

The benefits claimed by the FDA for the industry introducing the PAT concept are better understanding of the process, an introduction of real time release, a reduction of cycle times, less batch failure, a better management of change controls and regulatory relief (Orelli, 2005).

2.1.1. Experimental design techniques

Experimental design techniques such as factorial design and optimization are useful tools in the characterization of pharmaceutical processes by studying the effects of variables affecting them and their possible interactions (Paterakis et al., 2002).

Statistical experimental design provides an economical way to efficiently gain the most information while expanding the least amount of experimental effort (Stesko, 1986). Application of statistical design of experiments using modern software tools helps in understanding of how formulation and process factors impact product performance, and at the same time shortens the time and costs required for development of the new product. Specially designed expert systems enable us to gain the necessary process knowledge and this way high quality of pharmaceutical products can be achieved (Feiler and Solot, 2006).

The choice of statistical design of experiments (DoE) leads in general to a linear (factorial) or quadratic (central composite design) regression model, which permits the prediction of system properties (response surface methodology) within a variable space (Leuenberger and Lanz, 2005).

In the experiment we deliberately change one or more of process factors in order to observe the effect the changes have on the chosen responses. The final goal is to obtain data which can be analyzed to yield valid and objective conclusion in order to find the optimal settings of all factors and responses.

The first step in designing the experiments is to decide on the response variables and the second one is to select the factors and determine their levels which might influence those response variables.

Factors or process variables represent the physical property which can be changed in the experiment in order to influence the response. Levels of factors should be chosen carefully in order to avoid impracticable or impossible combinations (i.e., very thin tablets and very high compression force) and their difference should be neither too big nor too small. The term “level” stands for upper and lower value of the factor. Pre-experimental runs are suggested in order to determine the levels of factors in order to be in the effective range so that the process could be carried out.

Factor can be either quantitative or qualitative. Quantitative factor is described by amount or the size of the factor (i.e., amount of excipient, compression force, spray rate, etc.), while qualitative factor represents presence or absence of excipient, different packaging, different auxiliary materials, etc.

Response variables are the objectives of the study which can be influenced by changing the level of factors. Some of the examples are dissolution time, hardness of tablets, disintegration time, etc.

Sometimes it is necessary to eliminate the influence of extraneous factors when running an experiment and this can be done by blocking.

The combination of an appropriate design of experiments and an adequate statistical evaluation of their results leads to experiments with high significance. The designs are chosen, so that the minimum experiments give maximum of information.

STAVEX (Statistische Versuchsplanung mit Expertensystem) is an expert system developed by Ciba-Geigy AG and AICOS Technologies AG for statistical experimental design and evaluation of a series of experiments (process controls, product optimization or validation, etc) (Aicos, 1999). Stavex differentiates between three phases in experimental design:

- Screening
- Modeling
- Optimization

The type of study carried out will depend on the stage of the project. In particular, experimental design may be carried out in stages, and the experiments of a factor study may be augmented by further studies to a design giving the detailed information needed for true optimization (Lewis, 2006). It is suggested by the experts, to perform each stage of the design, whenever it

is applicable in respect to time and money. According to the response variables and the process factors chosen, STAVEX generates different possible factorial designs, among which the user may choose the most appropriate one.

Screening

Screening represents the first phase of sequential experimental design and it is used when the number of factors exceeds 8. The main purpose of this design is to find the most important factors so that the unimportant factors are eliminated using linear models, without finding the dependence of the response variables, or estimating interactions between the factors.

Modeling

Modeling is performed when the number of experiments is moderate (between 4 and 8) and an attempt is made to estimate the effect of relevant factors using linear models with interactions, so possible interactions and maybe also quadratic effects are considered.

Optimization

Optimization is employed when the number of factors is small (less than 4, possibly 4). It can be employed when the most important factors are already know or determined. Optimization phase tries to find the optimal settings (minimum and maximum) for the identified important factors and prediction power of the model should be validated by confirmatory experiments (Aicos, 1999). In optimization the experimental design analysis determines two optima, the “global” and the one lying in the “experimental area”, so called local optima. The following results are shown:

- Factors with their optimum levels
- Response variables with their optimum values
- 90% confidence intervals for these optimum values

The quality of model can be characterized by different parameters. The coefficient of correlation or so called goodness of fit R^2 gives information about weather the model fits with the collected data. A high R^2 stands for a well fitted model. However, R^2 is very sensitive since each variable or the process run added to the model increases the value of R^2 (Kablitz, 2007). For this reason, corrected goodness of fit, Rc^2 is used to evaluate the model and it is adjusted for the degree of freedom and does not increase automatically with adding variables. Second parameter which is used for evaluation of the model is a normal plot of model deviations. With the use of Shapiro-Wilk test it is possible to determine if there are significant deviations of model from normality or not. Below 5%, deviation from normality is significant (Aicos, 2000). The third parameter used for evaluation is analysis of variance of model deviations, with the F-test for the equality of means (are the mean values similar at different levels, with corresponding p-value) and Bartlett test for equality of variances (are the variances similar at the different values, with corresponding p-value). Furthermore, in the case of optimization phase, one of the ways to determine the prediction power of the model in the 90% confidence interval is to conduct the

confirmatory experiment. A p-value<0.1 indicates a significant effect of the factor on the analyzed response variable (Aicos, 1999).

2.2. Proton-pump inhibitors (PPI's)

2.2.1. Properties of proton-pump inhibitors

Lansoprazole belongs to a class of compounds called proton pump inhibitors (PPI) which inhibits gastric acid secretion regardless of the primary stimulus and is applied in the therapy of gastric and duodenal ulcerative disease, for the treatment of the heartburn and other symptoms associated with Gastroesophageal Reflux Disease (GERD), for the treatment of erosive esophagitis and long term treatment of pathological hypersecretory conditions, including Zollinger-Ellison syndrome. The key action mechanism of the PPI's is inhibition of H+/K+-adenosine triphosphate (also known as acid pump or proton pump), an enzyme present in the gastric parietal cells (Horn, 2000). These drugs are metabolized in the parietal cells to active sulfenamide metabolites that inactivate the sulfhydryl group of the proton pump, thus reducing the hydrogen ion secretion. Absorption of the most PPI's takes place in the proximal small intestine (Horn and Howden, 2005).

Group of proton pump inhibitors includes derivatives of benzimidazole, like omeprazole, lansoprazole, pantoprazole, rabeprazole, etc.

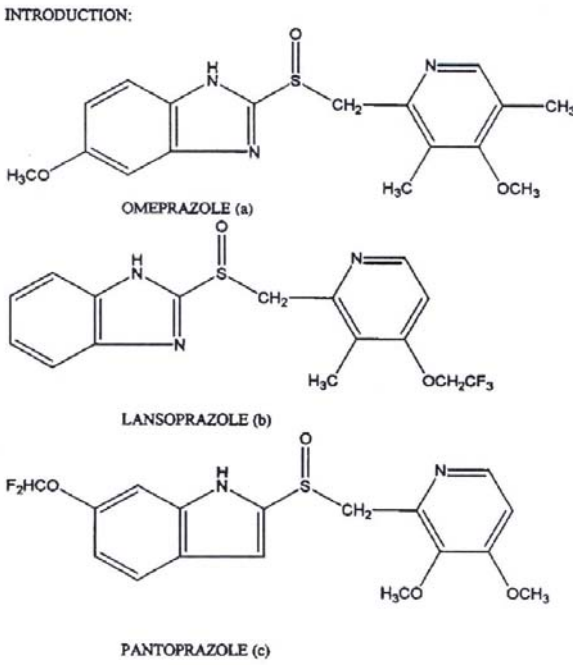

Figure 2.1. Structural formula of omeprazole, lansoprazole and pantoprazole.

All PPI's are extensively protein-bound, and all undergo hepatic metabolism. All of the currently available delayed-release proton pump inhibitors have a short elimination half-life ($t_{1/2}$) of between 1 and 2 hours. Aside from bioavailability in the first few days of oral dosing, there are no substantive differences among currently available delayed release PPI's with respect to pharmacokinetics (Table 2.1) (Horn and Howden, 2005).

Table 2.1. Pharmacokinetics of delayed-release proton-pump inhibitors

	Esomeprazole	Lansoprazole	Omeprazole	Pantoprazole
Absolute bioavailability (%)	64 – 90	>80	40	77
Time to peak plasma level (h)	1.5	1.7	0.5 – 3.5	2 – 4
Plasma half-life (h)	1.0 – 1.5	1.5	0.5 – 1.0	1.0
Plasma protein binding (%)	97	97	95	98
Hepatic metabolism	Yes	Yes	Yes	Yes

Lansoprazole belongs to class II drugs of the BCS, characterized by low solubility and high permeability. It exists in two polymorphic forms designated as form A and form B. Form B is unstable and is completely converted to the stable form A under physical stress (milling) or even after some time at ambient temperature (Kotar et al., 1996).

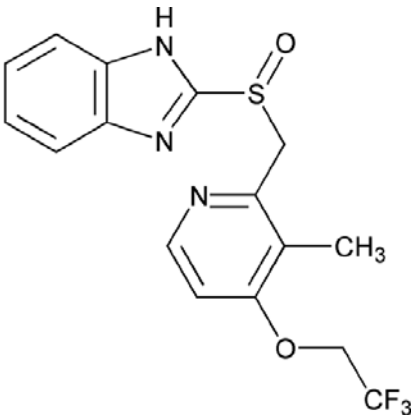

Figure 2.2. Chemical structure of lansoprazole, $C_{16}H_{14}F_3N_3O_2S$, 1*H*-Benzimidazole, 2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridinyl]methyl]sulfinyl]-2-[[[3-Methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]-methyl]sulfinyl]benzimidazole

Lansoprazole has been found to have absolute bioavailability lower than 80%, time to peak plasma level 1.7 hours, plasma half-life 1.5 hours and binding to plasma proteins in 97% (Horn and Howden, 2005).

Lansoprazole is applied as a racemic mixture and converted by acid environment of parietal cells into an achiral active sulfenamide, which reacts with the accessible cysteins of the gastric (H^+, K^+)-ATPase.

The reduction of gastric acid secretion acts as a negative feedback mechanism, resulting in an increase in serum gastrin level. Furthermore, lansoprazole increases stomach pH to reduce stomach pepsin secretion and activity and also increases serum pepsinogen level. In addition lansoprazole has an inhibitory effect against Helicobacter pylori present in patients with stomach and duodenal ulcer. This contributes to increase in concentration and effects of antibiotics used in combination with lansoprazole such as amoxicillin, clarithromycin, by inhibition of gastric acid secretion, or maybe by a direct antibiotic effect of lansoprazole whose mechanism has not yet been established.

Proton-pump inhibitors (PPIs) is a group of drug compounds that has an acidic p*Ka* value at 8-9 and basic p*Ka* at 3-5 and it is known that lansoprazole as well as other derivatives of benzimidazole, is susceptible to degradation in neutral and acid media. Thus the drug degrades as soon as it comes in contact with the gastric contents or if exposed to humidity during storage. As an example, lansoprazoles half-life is 4 minutes in a methanol-phosphate (5/95, v/v) pH 3 buffer (Oernskov et al., 2003). At 25°C the t½ is approximately 0.5 hours at pH 5.0 and approximately 18 hours at pH 7.0. Regarding the structure of lansoprazole, which is a derivative of 2-[[pyridyl]-methyl]sulphynyl]benzimidazole, it can possess three dissociation constants in the range of 1-14, two basic and one acidic, since the p*Ka* of pyridine is 5.2, while the p*Ka* values of benzimidazole is 5.5 as a base, and 12.3 as an acid (Kotar et al., 1996). Lansoprazole has a p*Ka* value of 8.78 and 3.82 determined by capillary electrophoresis (Oernskov et al., 2003).

Ekpe and Jacobsen 1999, have investigated the effect of various pH levels and salts on the stability of lansoprazole, omeprazole and pantoprazole and found out that lansoprazole is the least stable compound. As a pH value increased, the rate of degradation decreased. The stability of investigated PPI compounds in salt solutions were found to be in the following order: phosphate buffer < trisodium citrate ≤ citrate buffer ≤ acetate buffer < citric acid ≤ calcium carbonate etc., but none of the used salt solutions has improved the stability of any of the tested PPIs. Degradation kinetics of the compounds in salt solutions appeared to be second-order reaction. There is therefore a need for a pharmaceutical delivery system which protects the active substance both during storage as well as the passage through the stomach. As it has been reported by Ekpe and Jacobsen 1999, in the study the rate of degradation of omeprazole, lansoprazole and pantoprazole was least accelerated in calcium carbonate followed by sodium bicarbonate, but the buffers used in this study did not improve the stability of any of the drug substances under investigation.

Another study conducted by DellaGreca et al., 2006, on degradation of lansoprazole and omeprazole in the aquatic environment, has revealed that lansoprazole was stable in pH 7 and

pH 9 for 72 hours in the dark. It was determined that 40% of lansoprazole degrades in water after 72 hours in the dark, and when irradiated with a solar simulator more than 80% of drug was degraded. According to DellaGreca et al., 2006, isolated degradation products from drugs in aqueous suspension are presented in the Figure 2.3.

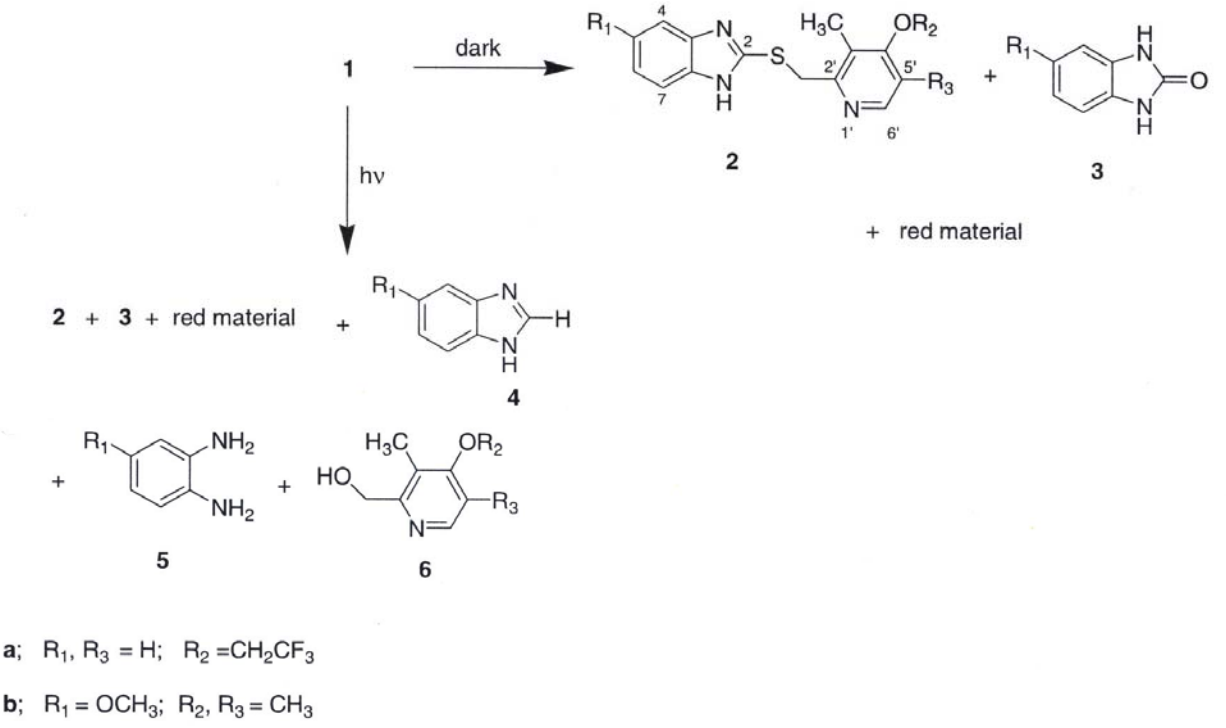

Figure 2.3. Isolated degradation products from lansoprazole and omeprazole in aqueous suspension DellaGreca et al., 2006

Unfortunately, attempts to characterize the red-colored mixture failed, due to its complexity and changeable nature. Probably, this red material consists of a mixture of very liable degradation products, and it has been previously reported by Brandstrom et al. 1989 and DellaGreca et al., 2006.

2.2.2. Employed ways of stabilization of proton-pump inhibitors

Formulation of stable delivery system for lansoprazole is extremely difficult. The numerous available patents all deal with methods and techniques for stabilizing LSP against the stomach environment. For the drug to be therapeutically active after oral administration, it must be protected from contact with the acidic gastric juice and be transferred intact to the neutral or alkaline part of the gastrointestinal tract, where rapid absorption can occur. Lansoprazole is also unstable under conditions of high temperature and also high humidity, with a decrease in the amount of lansoprazole and discoloration of the material being noted on storage under such conditions (Tetsuro et al., 1992). Since as pharmaceutical ingredient, lansoprazole must be

stored and transported, often for long time periods, the issue of the instability of the primary active material must also be addressed.

There exist an extensive number of patents claiming the formulation of a stable dosage form of lansoprazole. There are several methods and techniques used to stabilize lansoprazole. A number of different approaches to stabilize substituted benzimidazoles have been suggested and they all claim to succeed.

Subcoating has been proposed as a method to improve acid resistance for enteric coated dosage forms. Polymer subcoats seal the substrate from the aqueous enteric film coating, thus preventing the migration of water-soluble drugs into polymeric film, as well as preventing drug-polymer interactions (Bruce and J.J., 2003). In the case of substituted benzimidazoles, subcoating or seal coating has been described in a numerous patent literature as a barrier between an enteric coating and acid liable drug to prevent degradation of the compound, since the polymers for enteric film coatings contain free carboxyl groups which can increase degradation of acid liable drug. Direct contact of lansoprazole and enteric coating polymer can lead to solid-solid interactions and degradation of lansoprazole, therefore the presence of subcoting can be justified.

It has been demonstrated that the pH of the diffusion layer at the surface of a dosage form resembles that of a saturated solution of a drug and excipients in a dissolution media and represents the microenvironment pH of the system (Doherty and York, 1989). During dissolution, medium that may eventually penetrate the pellet core, or during storage moisture may penetrate into the core, resulting in a saturated solution of a drug and excipients. In the case of lansoprazole if the micro-environmental pH is too low, and moisture penetrates in the core, the drug will degrade and the initial amount of drug will decrease during storage. Too basic pH (lansoprazole degrades even at the high basic pH) will create saturated diffusion layer at the surface which can cause ionization of the carboxylic groups of the enteric polymer. Presence of subcoating can be essentially important in this case.

In addition to subcoating, necessity of presence of pH adjusters in the core of pellets containing substituted benzimidazoles, was described in a number of patent applications. In the strictest sense, the term pH is not defined in a solid system. For it to have a meaning there must be some water mediation (Carstensen, 2000). With addition of pH adjusters it is possible to control the pH of the microenvironment. Incorporation of pH adjusters such as has been utilized to maintain the micro-environmental pH in a range that will increase drug solubility and improve stability during manufacture and storage. If it is desired to control the pH of the microenvironment than citric, tartaric and fumaric acids are the acids of choice. They are, however, all corrosive, and their pharmaceutical handling is far from ideal. In the case of alkali, sodium bicarbonate, sodium carbonate, and magnesium and calcium oxides are common (Carstensen, 2000). Alkaline materials, which are capable of providing pH $\geq$ 7 when present

alone in water and which are pharmaceutically approved include: organic basic salts (Na-stearate); inorganic basic salts (heavy Mg-carbonate, Mg-oxide, precipitated Ca-carbonate and Ca-hydroxide) and others. In addition the core is coated with an enteric coat. This will protect the drug from contact with the gastric juice during the passage through the stomach into the neutral or alkaline part of the GI tract where rapid absorption can occur. The variation in assay and color of solid lansoprazole over time on storage at various temperatures and humidity's was studied in detail by Tetsuro et al., 1992. The study shows that after 4 months at 40° C and 75% room humidity lansoprazole turns pale brown, and even in the absence of humidity under the same conditions, lansoprazole turns pale yellowish brown. The unusually high instability of lansoprazole even under weak acidic conditions is due to proton attack on the sulfoxide group. Lansoprazole seems to be especially sensitive to such attack compared to the other members of the 2- (2-pyridylmethyl) sulfinyl- benzimidazole family of drugs. Lansoprazole is unstable also under strongly basic conditions, but its degradation is minimized under weakly basic conditions. Therefore the degradation of lansoprazole in dosage forms can be minimized when formulated with stabilizing compounds suitable to produce such a weakly basic pH (Tetsuro et al., 1992).
Also it has been reported that too alkaline core can cause ionization of the enteric polymer and result in an increase of film permeability and film failure.

2.3. Solid-state chemical decomposition

Solid-state chemical decomposition of drugs has been the subject of many papers and the problem is further more complicated when the drug is formulated in a complex matrix such as a tablet or capsule (Table 2.2). Drug degradation occurs by four main processes: hydrolysis, oxidation, photolysis and trace metal catalysis. Hydrolysis and oxidation are the most common pathways, and in general light and metal ions catalyse a subsequent process.

Table 2.2. Factors affecting formulation stability

Drug & Excipient	Formulation	Environment
Chemical structure	Drug:excipient ratio	Temperature
Impurity profile	Processing method	Relative humidity
Physical form	Mixing/milling	Packing
Moisture content	Powder packing	Light
Particle size		Oxygen
Surface area		
Morphology		

Numerous studies have confirmed the importance of temperature and humidity as two of the most important variables that significantly affect the chemical stability of drugs in the solid state.

It is well known that products degrade faster when they are subjected to stress conditions, such as temperature, pH, humidity, etc. The most common acceleration factor is temperature and it's relationship is well characterized by the Arrhenius equation (Magari et al., 2004).

The length of time the product is stable at the recommended storage condition is referred to as the shelf-life. The source of the solvent for the solid-state decomposition reactions may be (Connors et al., 1986b):

- A melt from the drug itself or an ingredient in the formulation that has a low melting point
- Residual moisture of solvent from the production process
- Moisture adsorbed onto excipients
- Adsorbed atmospheric moisture
- A solvate or hydrate that loses its bound solvent with time or temperature fluctuations

Solid state degradation curves have a sigmoid shape with a lag phase followed by an acceleration phase. The acceleration phase can be apparent zero order, apparent first order, or higher orders with respect to drug, depending on the conditions of the experiment, such as humidity and temperature, and the mechanism of the degradation process.

Since only a fraction of the solid drug is in solution in the tablet or other solid dosage form, the overall loss of drug often follows apparent-zero-order kinetics. The word "apparent" is used here because more factors affect the magnitude of this activation energy than simply the effect of the temperature on the rate-controlling chemical reaction.

An apparent zero-order degradation curve, quite often seen for the decomposition of pharmaceutical products, can be rationalized as follows. The drug, drug-excipient blend, or tablet, adsorbs a thin layer of water on the surface of the solid product. The drug dissolves to the extend of its solubility in this water and only the dissolved drug decomposes. Temperature affects the solubility of the drug in a solvent layer, it increases the intrinsic rate of reaction, and it may alter the availability of solvent in which reaction occurs (Connors et al., 1986b).

When moisture is present in excess, decomposition is often accounted for by solution kinetics of a saturated solution, in which case, barring complications, the decomposition is zero order. If the decomposition is diffusion controlled, than it still appears zero order, but the solubility term is replaced by a smaller, constant concentration (Carstensen, 1974). Studies conducted on aspirin tablets in a microcrystalline cellulose base showed that aspirin degrades by a first-order reaction but other published data imply that the trend reverts to a zero-order pattern with higher moisture contents (Lee et al., 1965). It has already been mentioned that the excipient can have a positive or negative effect on stability of drug. Excipients that on partial dissolution in an adsorbed moisture layer may change the local pH to a level where it can be deleterious to drug stability will consequently accelerate the degradation of the drug. On the other hand, an excipient may stabilize the drug if it maintains a favorable local pH for optimal stability. Quite often

measurement of the pH of slurry of a possible tablet formulation can indicate whether stability could be a problem.

2.3.1. Arrhenius testing as a comparative technique in prediction of stability of solid dosage forms

In order to obtain rapid stability results for a product, it became a practice to store the product at elevated temperatures in order to force a degradation of active material in a short period of time. The loss of activity in a unit of time is defined as the degradation rate. This rate depends on the required activation energy for the chemical reaction, and is product specific (Magari et al., 2004).

The FDA guidelines state that the relationship can be adequately described by a linear, quadratic or cubic function on arithmetic or a logarithmic scale. Linear arithmetic is zero order and linear logarithmic is first order (FDA's guideline on submitting documentation for the stability of human drugs and biologicals).

Accelerated stability testing may be defined as the methods by which product stability may be predicted by storage of the product under conditions that accelerate change in a defined and predictable manner (Young, 1990).

In order to have an idea about the decomposition kinetic at determined temperature, the Arrhenius plotting is carried out. Natural logarithm of the absolute value of the decomposition constant *k* being plotted against reciprocal of the absolute temperatures *T*, see Equation 2.1 and Figure 2.4. Apparent activation energy *Ea* can be calculated by the slope of Equation 2.1.

$$k = A\exp^{\frac{-Ea}{RT}} \qquad \text{Equation 2.1}$$

In the Equation 2.1, *R* is the gas constant (1.99 cal/degree-mole or 8.314 J/mol K) and *A* represents frequency or steric factor which expresses the probability that the molecules contain a favorable orientation and will be able to proceed in a collision, *T* is the absolute temperature (t°C + 273.16°C). Ea is called activation energy of the chemical reaction. Activation energy is a measure of a barrier which prevents the reactants from immediately becoming products. Usual range of activation energies is about 12 to 24 kcal/mol for many reactions (50 – 96 kJ/mol).

It has to be kept in mind that this Ea does not have the same meaning as the activation energy for reactions in slutions Florence and Attwood, 2006. The Ea value in the solid state is affected not only by changes in the solubility of the drug in the moisture layer but also by the intrinsic rate of reaction. Linear regression can be extrapolated to the room temperature and thus shelf life of the product can be predicted.

Assumption must be made that the mechanism operative at the experimental temperatures is the same as at the extrapolated temperature (often room temperature).

In order for the reaction to proceed and overcome the activation energy, the temperature, orientation, and energy of the molecules must be substantial; this equation manages to sum up all of these things. This equation fits experimental data well over wide temperature range and it's a good approximation to the true temperature dependency of a reaction. It implies that a plot of the logarithm of the rate constant against the inverse of the absolute temperature is a straight line (Young, 1990). The easiest way to check the validity of Arrhenius law for the product is to plot the results within the available range. The most common causes for the invalidity of Arrhenius law are either that the degradation mechanism changes above a critical temperature or that there are two or more simultaneous decay mechanism with the different rate constants and heats of activation (Young, 1990).

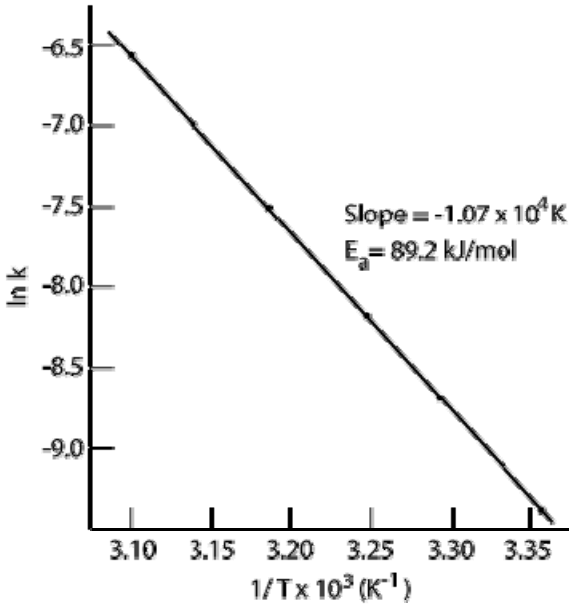

Figure 2.4. An example of Arrhenius plot

However, under certain conditions, Arrhenius equation is not applicable, particularly when higher-order reactions are involved and exponential relationship is not appropriate basis for approximation of degradation pattern of these reactions.

In the cases when storage temperature is very different from the range of temperatures used in the experiment, the prediction of shelf-life may be incorrect or the relationship between the temperature and the log of degradation rate is not linear (Magari et al., 2004).

Even though accelerated stability testing based on the use of Arrhenius equation shortens development time, it still involves time-consuming step of initial determination of the order of decomposition reaction. For a relatively small amount of degradation (around 10%) it is not possible to distinguish between zero, first order and simple second order kinetics using curve fitting techniques; consequently, the assumption of the first-order kinetics for any decomposition reaction should involve minimum error (Florence and Attwood, 2006). In most instances it is not

necessary to determine whether the component degrades according to a zero or a first order reaction, because over the area of interest both models can be approximated by a straight line (Bourdreau, 1984).

One purpose of a stability program should be to define stability of solid dosage form as a function of moisture content. The need for consideration of the effect of moisture on stability has been stressed by Carstensen, 1974, who stated that stability programs should always include samples that have been artificially stressed by addition of moisture, even though this may produce many experimental problems (Florence and Attwood, 2006).

In this study isothermal accelerated stability testing of a product as a function of moisture content has been employed in order to obtain degradation of the drug in the solid dosage form in a short period of time. The aim of the study was to apply Arrhenius equation for obtaining information on the most stable formulation as a comparative technique and to predict the stability in solid dosage form.

2.3.2. Order models

A number of empirical models have been used to represent pharmaceutical product decay with time. The usual model is based on reaction order where the rate of disappearance of the product is proportional to its concentration raised to an order (Young, 1990).

The order of a chemical reaction determines the shape of the concentration-time profile of a drug or drug product, whereas the rate constant determines its slope (Connors et al., 1986a).

Overall order is defined as the sum of the exponents of the concentration terms in the rate equation.

$$A + B \rightarrow E$$

Equation 2.2

$$-\frac{dC}{dt} = -k_{(n+m)}\left[A\right]^{n}\left[B\right]^{m}$$

Equation 2.3

The most important orders of interest in the pharmaceutical sciences are integral orders, those in which the sum of *n* and *m* is 0, 1, or 2, with the order being equal to the number of the molecules involved in the rate-determining step of the reaction. Since a true reaction of this type can only occur in solution, solid dosage decay is usually described as pseudo-order of apparent order (Young, 1990).

Knowledge of the order of a reaction is of a great importance in stability determination of drug substances, in particular in solution. The problem is frequently to judge whether the concentration-time profiles are linear (zero order) or curved (first or other order). The nature of any degradation relationship will determine the need for transformation of the data for any linear regression analysis. Usually relationship can be represented by a linear, quadratic or cubic

function on an arithmetic or logarithmic scale. Statistical methods should be employed to test the goodness of fit of the data on all batches to assume degradation line or curve.

A *zero-order reaction* has a rate which is independent of the concentration of the reactant. Increasing the concentration of the reacting species will not speed up the rate of the reaction.

$r = k$

r = reaction rate

k = reaction rate coefficient with units concentration/time

$$r = -\frac{d[C]}{dt}$$ Equation 2.4

$$-\frac{d[C]}{dt} = k_0$$ Equation 2.5

If this differential equation is integrated it gives an equation which is often called the *integrated* zero-order rate law:

$$[C] = -k_0 t + [C]_0$$ Equation 2.6

$[C]$ = concentration of the chemical of interest at a particular time

$[C]_0$ = initial concentration

Integrated rate equation links concentrations of reactants or products with time. Reaction is zero order if concentration data are plotted versus time and the result is a straight line. The slope of the resulting line is the zero order rate constant k.

For a zero-order reaction the half-life t_{50} is given by Equation 2.7.

$$t_{50} = \frac{[C]_0}{2k_0}$$ Equation 2.7

The shelf life, t_{90}, of a drug is usually taken to be the time for $[C]$ to reach 0.90 $[C_0]$, that is, 10% decomposition:

$$t_{90} = \frac{0.1 \cdot [C]_0}{k_0}$$ Equation 2.8

A first-order reaction depends on the concentration of only one reactant, other reactant can be present, but each will be zero-order. The rate law for first-order reaction is:

$$C \rightarrow \text{products}$$

$$r = k[C]$$ Equation 2.9

k = first order rate constant with units of 1/time

$$-\frac{d[C]}{dt} = k_1[C]$$ Equation 2.10

which *integrates* to:

$$\ln\frac{[C]}{[C_0]} = -k_1 t$$ Equation 2.11

$$\ln[C] = \ln[C]_0 - k_1 t$$ Equation 2.12

$$[C] = [C_0]e^{-k_1 t}$$ Equation 2.13

A plot of $\ln[C]$ versus time *t* gives a straight line with slope equal to the reaction rate constant. The half-life is the time for $[C]$ to become $[Co]/2$, that is one half of the original concentration. The half life of a first-order reaction, substituting $[C] = [C_0]/2$, can be determined using Equation 2.14:

$$t_{1/2} = \frac{\ln 2}{ak}$$ Equation 2.14

The shelf life, t_{90}, of a drug is usually taken to be the time for $[C]$ to reach $0.90[C_0]$, that is, 10% decomposition:

$$t_{90} = \frac{0.105}{k_1}$$ Equation 2.15

Accelerated aging by temperature, followed by shelf life prediction (Arrhenius equation) should be used with precaution when working with solid dosage forms. Because of the high degree of variability is usually associated with stability data, it is best if the order of degradation is determined a priori based on the known behavior of the product. If the order must be determined from the stability data alone the traditional approach would be to display graphically the determined amount of the drug decomposed after various time intervals according to the linear equations for the various orders of reactions until a straight line plot is obtained comparing the coefficient of determination, R^2. Since this method suffers form a number of deficiencies, particularly if the experimental data are noisy, quantitative measure of the

uncertainty of the parameters can be assessed using least squares linear regression. In the study data were plotted according to various orders and tested using linear regression for zero and first order kinetics.

2.4. Pellets as solid dosage form

Solid dosage formulation and design usually involves a serious of compromises, since producing the desired properties frequently involves competing objectives. The correct selection and balance of excipient materials and processes in a solid dosage formulation, to achieve the desired response is not in practice easy to achieve (Peck et al., 1989). Furthermore it is essential to develop tablet formulations and processing methods which may be validated.

Pellets are of a great interest to the pharmaceutical industry for a variety of reasons. Pelletized products not only offer flexibility in dosage form design and development, but are also utilized to improve the safety and efficiency of bioactive agents.

Pellets range in size, between 0.5 to 1.5 mm, though other sizes could be prepared, depending on the processing technique. Pharmaceutical pellets are agglomerates of fine powder particles, nearly spherical or cylindrical in shape with a narrow particle size distribution (Kleinebudde and Knop, 2007).

The technological advantages of spherical particles include the following:

1. Good flowability due to uniform size and spherical shape. This enables uniform and accurate filling of the capsules.
2. High physical integrity, meaning flow with minimal friction and dust generation
3. Superior properties for coating due to spherical shape, low area to surface volume, smooth surface and ability to withstand mechanical stress.

When pellets containing the active ingredient are administered in vivo in the form of suspension, capsules, or disintegrating tablets, they offer significant therapeutic advantages over a single unit dosage forms (Ghebre-Sellassie, 1989a). Because pellets disperse freely in the gastrointestinal tract, they invariably maximize drug absorption, reduce peak plasma fluctuations, and minimize potential side effects without appreciably lowering drug bioavailability. Pellets also reduce variations in gastric emptying rates and overall transit times. Thus, intra and inter-subject variability of plasma profiles, which are common with single unit regimens, are minimized.

When formulated as modified-release dosage forms, pellets are less susceptible to dose dumping than the reservoir-type, single unit formulations.

Pellets also allow the combined delivery of two or more bioactive agents that may or may not be chemically compatible, at the same site or at different sites within the gastrointestinal tract.

In addition pellets have a low surface area-to-volume ratio and provide an ideal shape for the application of film coatings. The specific surface area of pellets having a defined particle size is

smaller due to the spherical shape and smooth surface. This allows the use of less film forming polymer to achieve a required film thickness and reduces coating process (Kleinebudde and Knop, 2007).

Pellets are frequently used in gastric resistant or modified release forms. Recently, coated pellets are compressed to rapidly disintegrating tablets. For those purposes small pellets with the mean diameters below 0.5 mm are most suitable. Such pellets can be produces by the direct pelletization methods.

2.4.1. Pelletizing techniques

Pelletization is an agglomeration process that converts fine powders or granules of bulk drugs and excipients into small, free-flowing, spherical or semi-spherical units, referred to as pellets (Ghebre-Sellassie, 1989a). The type of coating technique strongly affects the film microstructure and, thus, the release mechanism and rate from pellets coated with polymer blends (Lecomte et al., 2004). There are several manufacturing techniques for production of spherical pellets. Broadly, they can be grouped in different ways according to production technique used, type of equipment or the intensity of mechanical forces involved. One of the more reacent methods for production of spherical pellets is rotary processing also called centrifugal granulation. In this type of equipment wet spheronisation, drying and coating can be performed in one closed system (single-pot). The most widely used pelletization processes in the pharmaceutical industry are extrusion/spheronization, solution/suspension layering, powder layering and direct pelletization. Pelletization processes are usually lengthy and expensive. Processing of a single batch may sometimes require hours or even days to be completed. Other pelletization processes that either have limited application or are still at the development stage include spherical agglomeration or balling, spray congealing/drying, and emerging technologies such as cryopelletization and melt spheronization.

The layering technique is the process in which drug in powder, solution or suspension form is layered onto seed materials (generally, a coarse material or nonpareil). This process leads to heterogeneous pellets, which consist of an inner core region and an outer shell region of a different composition (Kleinebudde and Knop, 2007).

2.4.1.1. Extrusion - spheronization

Extrusion-spheronization is a multistep process involving dry mixing of the active compound with excipients, granulation of wetted mass, extrusion of the mass, transfer of the mass to spheronizer to produce spherical shape, drying of the wetted mass in a dryer, and at the end screening to obtain required particle size.

2.4.1.2. Solution/suspension layering

Solution/suspension layering involves the deposition of successive layers of solution and/or suspension of drug substance and binders on starter seeds, which may be inert materials or crystals/granules of the same drug. Consequently, conventional coating pans, fluidized bed centrifugal granulators, and Wurster coaters have been used successfully to manufacture pellets. The efficiency of the process and the quality of pellets produced are in part related to the type of the equipment used (Ghebre-Sellassie and Knoch, 2002).

As a starter seeds usually sugar spheres consisting of a sugar-starch mixture are used or recently microcrystalline cellulose pellets and the pure drug crystals.

The most common configuration for bottom spray coating is known as the Wurster system. In this study solution/suspension layering of neutral pellets has been conducted applying novel fluidized bed technology from Hüettlin (see Figure 2.5).

This technology claims to improve the product movement in defined direction in all the equipment by the Diskjet gas distribution plate. Furthermore, a 3-component spray nozzle is used in order to improve the film formation on the pellets due to constant and reproducible drop size distribution. Accessibility of clogged nozzles without stopping and interrupting the process makes the equipment advantageous in respect to Wurster system. Hüettlin's three component nozzle is an air nozzle with an additional channel through which a second gas or component can be introduced to create a special microclimate around the nozzle which prevents excessive spray drying or clogging of the nozzle. Such microclimates near nozzle apertures are very useful when a film former with a relatively high minimum film-forming temperature (MFT) is used. The MFT of aqueous shellac suspensions, for example, lies between 35 and 55°C, depending on the plasticizer selected (Bauer et al., 1998).

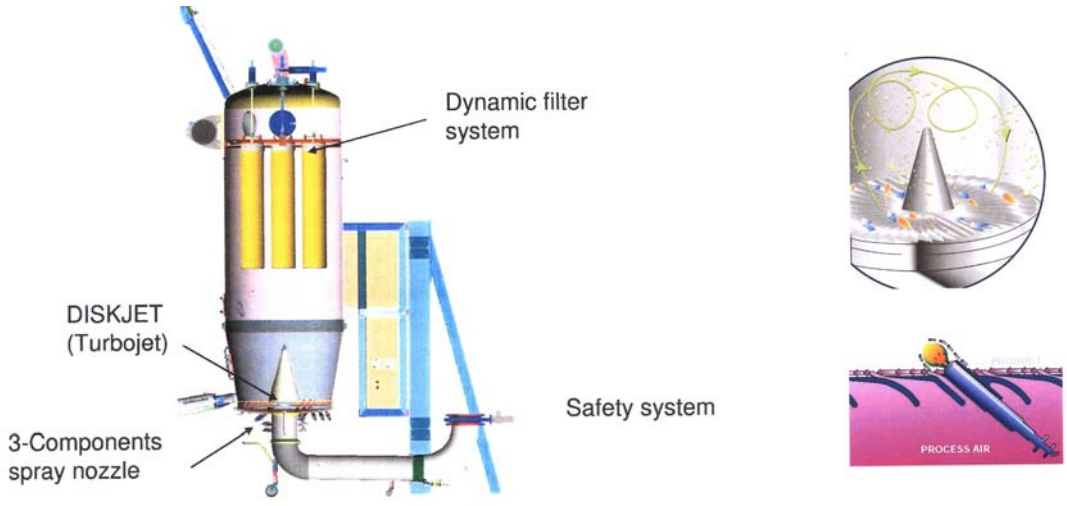

Figure 2.5. Bottom spraying fluidized bed from Hüettlin

Only a limited number of papers are available on the aqueous-based solution/suspension layering process in Hüettlin fluidized bed equipment. The most obvious difference between bottom spraying fluidized bed from other producers and the equipment used in this study is the presence/absence of Wurster insert.

The technology is applied to produce enteric coated lansoprazole pellets suggesting to improve lansoprazole stability in acidic media, due to the enhancement of the polymer film formation on the surface of the pellet. On the other hand enteric coating assures immediate release in alkalic media at the site of the action.

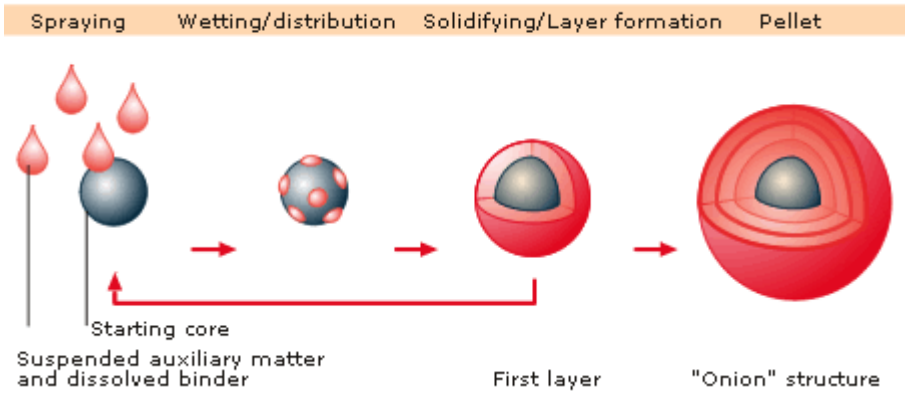

Figure 2.6. Principle of the solution and suspension layering process

2.4.1.3. Powder layering

Powder layering involves the deposition of successive layers of dry powder of drug or excipients or both, on preformed nuclei or cores with the help of a binding liquid (Ghebre-Sellassie and Knoch, 2002). Equipment which revolutionized powder-layering process as a pelletization technique is tangential spray of centrifugal fluidized bed granulator/rotary fluidized bed granulator.

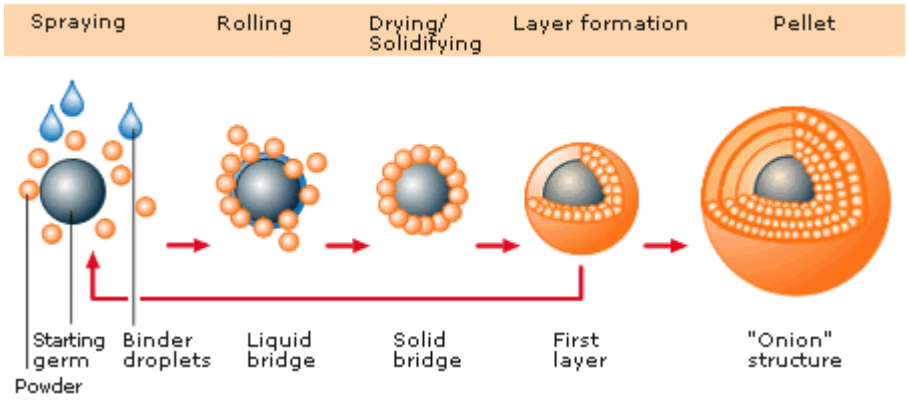

Figure 2.7. Principle of the powder layering process www.glatt.com,

Powder layering process can be chosen instead of the solution/suspension layering process in cases when the solution or suspension is too thick, or has a low potency , but the high pellets potency is required, when the process is too long, when the drug is not stable in the solution or comparatively low pellets density is desired (for rapid disintegration) (Jones, 2005a).
Owing to the simple process and equipment requirements layering processes are widely used for pelletization. Some of the disadvantages are:

- low amount of drug loading – not suitable for high-dose drugs
- final composition of pellets can vary if spray loss occurs

2.4.1.4. Direct pelletization

Direct pelletization process leads to formation of homogeneous pellets which have microscopically uniform structure and no core can be detected. The pelletization of powdered starting materials is facilitated by the addition of binder liquid and a suitable movement of wetted powders. The impact and acceleration forces that occur in this process result in the formation of agglomerates, which become rounded out into uniform and dense pellets. The speed of rotation has a direct influence on the density and size of the pellets. The solidification of the pellets is achieved by drying the liquid. Direct pelletization processes are mainly performed in high shear mixers and fluidized bed equipment (Kleinebudde and Knop, 2007).

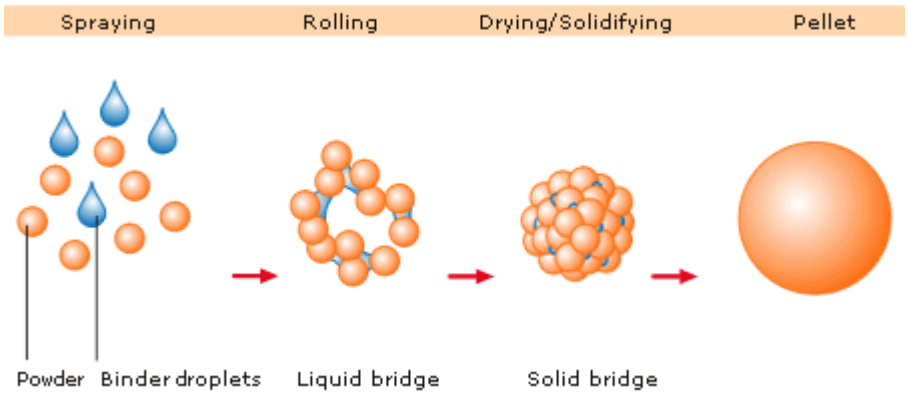

Figure 2.8. Principle of the direct pelletization process www.glatt.com,

Pelletization by wet granulation in fluidized bed rotary processor is a multivariable process in which several factors affect the final characteristics of produced pellets. The knowledge of these variables and their influence on the final pellet properties is essential in achieving a controlled process and desired pellet properties. Application of experimental design techniques, such as factorial design and optimization, present a useful tool for identification and correlation of significant factors that affect the process in order to develop a robust process (Korakianiti et al., 2000).

Properties of pellets obtained using direct pelletization in rotary tangential processor have been found to depend on process parameters like spray rate, rotor speed, type of rotor plate used (smooth and rough), gap space between the rotor disc and the unit wall, amount of liquid added, atomization pressure, inlet air temperature, etc.

Material consideration and variables

Pelletization material

Suitable formulation for spheronization should possess certain plasticity. Necessary plasticity can be achieved with addition of water or binder solution to suitable powder mixture. Because of its unique properties microcrystalline cellulose (MCC) represents a key excipient in the production of pellets by direct pelletization in rotary processor and extrusion/spheronization process.

Rotary processing was found to be effected by formulation variables such as type of MCC and content, type of filler and particle size of constituens (Kristensen et al., 2000). MCC is most widely used excipient in rotary processing due to its unique pelletization properties. One of the crucial factors for formation of pellets in rotary processor is the amount of MCC in a formulation. Generally, an amount of 15% to 30% (w/w) MCC has been reported necessary to produce spherical agglomerates with suitable properties (Kristensen and Schaefer, 2000). MCC not only gives the plasticity to wetted mass, but also emit binding properties that are essential to obtain pellet strength and integrity. More MCC results in larger agglomerates, wider size distribution, less friable and more spherical agglomerates (Kristensen and Schaefer, 2000). The actual amount needed to obtain pellets depends on the formulation used; type of other excipients as well as on the type of rotary processor. It has been found that MCC content is more critical when water soluble excipients, like lactose are used compared to the insoluble calcium hydrogen phosphate (Kristensen and Schaefer, 2000). However, a controllable spheronization process with consistent and acceptable characteristics required the content of MCC to be at least 20% (w/w) of the starting materials. A decrease in amount of MCC resulted in higher deposition and adhesion of moistened material, the formation of larger agglomerates to stick to the wall and the rotating plate of product container.

One disadvantage of MCC is that pellets containing MCC tend to swell in contact with liquid and do not disintegrate. As a conequence, especially drug substances having a low aqueous solubility, are released slowly from pellets containing MCC. In a rotary processor, the addition of of croscarmelose sodium as a disintegrant has been found to result in a faster release (Kristensen et al., 2002). It has been reported that MCC is not suitable for immediate release dosage forms of drugs with low aqueous solubility, wihout any additional excipient or disintegrant, but for sustained release form (Pisek et al., 2005).

Lactose was shown to be more suitable as a substituent for a portion of MCC than other fillers, like mannitol or calcium carbonate. Lactose 200 mesh was most commonly used in related

studies. The aqueous solubility of lactose enhanced the binding properties of the moistening liquid and plasticized moistened mass, thereby increasing the potential for granule growth with small moisture increment.

Balocel Sanaq

Balocel Sanaq is a unique excipient prepared by a special manufacturing process manly used for production of pellets and granulates. It is a powder premix which consists of 50% (w/w) microcrystalline cellulose, 35 % (w/w) lactose and 15 % (w/w) sodium carboxymethylcellulose.

Amount of granulation liquid

The other crucial formulation factor for direct pelletization is the amount of water or granulation liquid added. A linear relation between the amount of water (based on dry mass) and the fraction of MCC has been found (Figure 2.9) (Kristensen and Schaefer, 2000).

The moisture content of the mass at the end of the liquid addition is critical for the formation of pellets. The moisture content is influenced by the temperature, humidity, and flow rate of the fluidizing and atomizing air, and by the liquid addition rate. To control the process, the moisture content of the mass at the end of the liquid addition has to be exactly controlled using infrared moisture sensor or the alternative approach to the end point control might be to use indirect methods such as measuring the power consumption of the friction plate motor or torque on the shaft of the friction plate (Kristensen et al., 2000).

Disadvantages of MCC

Pellets based on MCC as a pelletization aid possess properties which are in some cases disadvantageous. Pellets containing MCC tend to swell but do not disintegrate during the application and as a consequence they release the drug according to a matrix release profile (Vecchio et al., 1994). Because of this reason, it has been hard to formulate MCC based pellets suitable for immediate release containing drug with low solubility, since the dissolution rate is too low. In this case MCC matrix pellets represent sustained release carrier system, where drug solubility plays a dominant role in dissolution behavior (Pisek et al., 2005). The retarding effect can further more be intensified by the size of the pellets. Smaller pellet fractions containing less MCC have better release profile than the pellets of bigger pellets fractions.

Combination of MCC with water soluble excipients and addition of disintegrants can be useful in order to achieve an immediate release profile.

Fraction of drug

Maximum fraction of the drug which can be incorporated in the pellets and the influence of the drug fraction on the process and product properties depend on the physico-chemical properties of the drug. With increase in the fraction of the drug, less pelletization aid is available, which can lead to process failure in production of pellets with wanted properties.

Particle size of drugs

Drugs with small particle size tend to adhere to the wall of product container and prevent rope-like movement of the wet mass required for the spheronization. Furthermore, this effect is more pronounced when a higher fraction of drug tends to be incorporated in the pellets.

Process variables

Spray rate

It has been determined that the lower spray rate applied in the process leads to the longer processing time resulting in a lower porosity of the pellets (Holm et al., 1996). Higher spray rates results in higher water content, shorter processing time and larger pellets, since there is shorter time for liquid to evaporate. A higher spray rate is also found to lead to a broader size distribution (Paterakis et al., 2002).

Rotor speed

The rotor speed in direct pelletization process affects mixing, liquid distribution, pellets growth and the shape of pellets. In some studies an increase in rotor speed led to larger pellets, with higher bulk density and improved roundness (Rashid et al., 1999) while in other studies it has been noted that increase in rotor speed led to production of smaller pellets (Korakianti, 2002). It has been suggested to use different rotor speeds during different stages of pellet production (Liew et al., 2000). Low – high – low pattern is proposed, suggesting the use of a low rotor speed during mixing and early wet-massing phase, until the mass is slightly wetted, which makes it more cohesive and less susceptible to be blown out of chamber, higher speed during the spheronization phase, and lowering the speed during drying phase. This pattern was also recommended by other authors since it leads to a more controllable agglomeration process (Kleinebudde and Knop, 2007). The effect of the rotation speed, however, depends on the actual formulations (Vertommen, 1996).

Moisture content

The moisture content at the end of the liquid addition stage is critical for the process. There is sensitive relation between moisture content and particle size, and moisture sensitivity depends strongly on the formulation, especially the fraction of pelletization aid. For formulation pelletization, process parameters have to be adjusted according to the physico-chemical properties of the drugs and excipients in the formulation and the desired characteristics of pellets, e.g. size. Correlation between the MCC content in the formulation and the water content at the end of liquid addition phase is presented in the Figure 2.9.

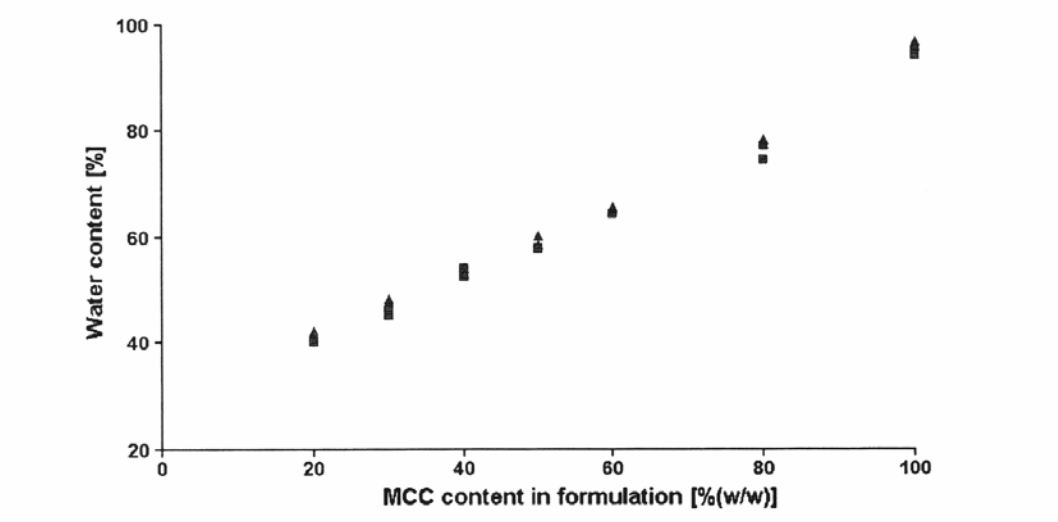

Figure 2.9. Correlation between the MCC content and the water content at the end of the liquid addition (Kristensen and Schaefer, 2000)

2.4.2. Pelletization equipment

Different manufacturing techniques are used for the production of spherical pellets. They can be broadly grouped according to the type of equipment used, the intensity of the forces which are involved in the production or by technique used. The production of spheroid pellets process does not differ a lot from the wet granulation process, in which moistening liquid is required. This also means that the equipment involved in the wet granulation can be used for production of pellets like fluidized bed granulator and high-shear mixer. Different types of spherical pellets require different type of equipment. Production of homogenous pellets is usually carried out in extruder spheronizator, high-shear mixer and rotary fluidized bed, while the production of heterogeneous pellets is carried out in top spraying or different types of bottom spraying fluidized bed equipment.

Fluidized bed equipment

The introduction of an expansion space between the product container and the filter chamber, and the inclusion of a liquid-spray nozzle in that space, gave rise to fluid bed agglomeration and in more resent years, unique processes for the coating of granulates, pellets and powders have expanded the use of granulation manufacturing equipment (Olsen, 1989).

In general, the main processing option can be characterized by spray nozzle position and orientation and by design principle of fluidization gas distribution into the processing chamber. Different processing options were created in order to manufacture different products in fluid bed equipment (Table 2.3) (Jacob, 2007).

There are three basic configurations in fluidized bed processing:

- top-spray processing (Figure 2.10, B)
- bottom-spray processing and Wurster processing (Figure 2.10, A; Figure 2.11)
- rotor processing (tangential spray) (Figure 2.10, C)

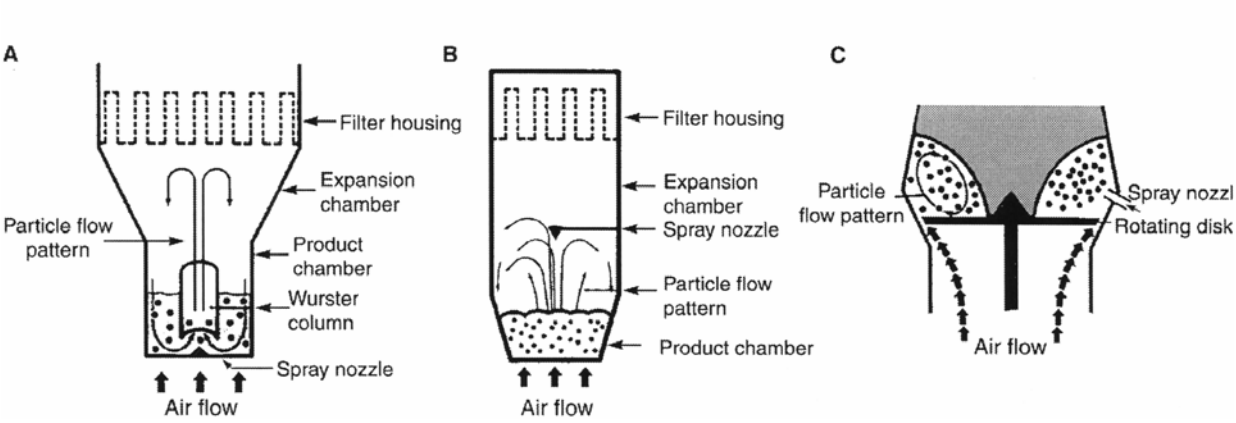

Figure 2.10. Schematic of a fluidized bed apparatus: (A) bottom spray with Wurster column insert; (B) top spray technique; (C) tangential spray technique (Felton, 2007)

The last two configurations are used for pelletizing and coating in laboratory, pilot and commercial scale equipment. The type of process used may significantly influence finished dosage form properties.

Table 2.3. Application of different types of fluidized-bed equipment

APPLICATION	*TOP SPRAY*	*BOTTOM/WURSTER*	*ROTOR*
Drying	+++		
Spray granulation/drying	+++	+	++
Pelletizing			
Solution/suspension layering	+	+++	+++
Dry powder layering			+++
Direct pelletization			++
Coating (fine particles/pellets)			
Organic solvent	+*	+++	++
Water based	++*	+++	+++
Hot melt	+++*	+	+

**substrate size and density are critical*

Conventional fluidized bed granulator

First powder, later granules or pellets are fluidized in the cylindrical product container by an air stream. The inlet air passes a screen or a perforated plate, fluidizes the particles and leaves the product container through a filter. This exhaust air filter prevents product losses and air pollution. The fluidizing air can be heated to the desired temperature to dry or melt the fluidized product. The molten binder or binder solution is sprayed onto the fluidized particles through a nozzle which has to be heated in case of molten binder. The spray nozzle is usually an air-atomizing nozzle which uses pressurized air to produce droplets from a liquid. The droplet size can easily be controlled by the atomizing air pressure. The position of the nozzle is above the fluidized product in most cases (Figure 2.10, B) (Kleinebudde and Knop, 2007).

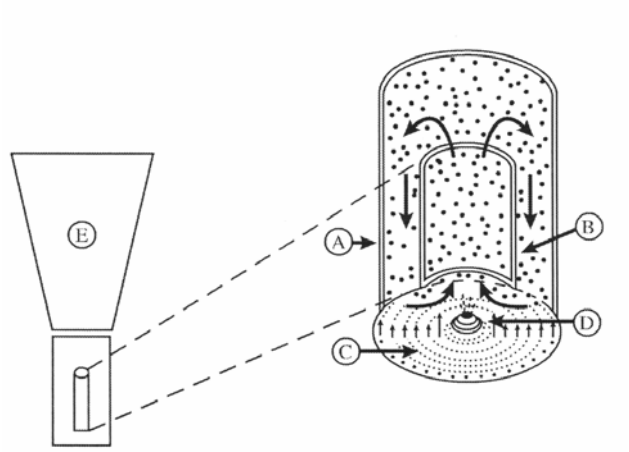

Figure 2.11. Schematic representation of the Wurster product chamber and process: (A) product chamber; (B) partition, (C) orifice plate; (D) nozzle and (E) expansion chamber (Felton, 2007)

Rotary fluidized bed processor

Since conventional fluidized bed granulator was not the method of choice for production of pellets, there was a need to modify the equipment in order to achieve highly spherical and high density pellets. Single-pot spheronizer system in which the whole cycle of liquid addition, agglomeration, spheronization, drying and coating can be performed is a rotary fluidized bed processor (Gu et al., 2004).

A rotary fluidized bed processor has a rotating friction plate instead of a screen at the bottom of the product container (see Figure 2.12). The inlet air passed through an air gap between the rotating plate and the wall of the product container. The movement of the particles in the equipment is helical and is the result of three forces:

- the centrifugal force from the rotating plate which tends to push the material towards the wall of the processing chamber
- the fluidizing force from the air stream through the gap
- the force of gravity allowing the product to fall towards the centre of the rotor plate.

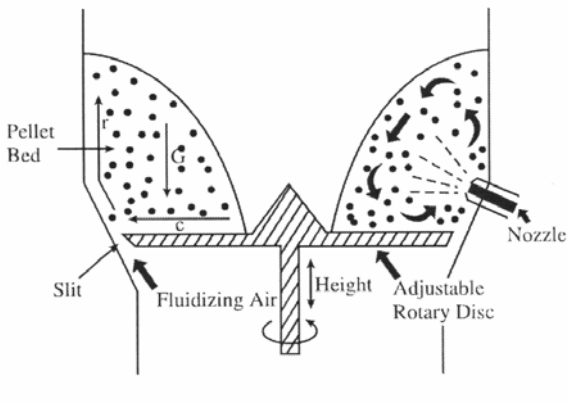

Figure 2.12. Schematic representation of centrifugal fluid-bed equipment, Glatt GPCG

This movement is described in different literature in different terms like of rope-like tumbling, twisted rope, spiral, spiral helix and others. The achievement of ideal product movement is of a high importance. In the beginning of the process this type of the movement is difficult to achieve because of the dry or moderate wet powder, but at the end of the process, in the spheronization phase, the movement is of a critical importance (Kleinebudde and Knop, 2007). If the movement does not occur in the spheronization phase, no spherical pellets will be obtained.
The nozzle or the nozzles in the rotary processor are often positioned tangentially in the wall of the container in the height of the fluidized product (Kleinebudde and Knop, 2007).

2.5. Mathematical description of fluidized bed

In typical fluidized-bed processor, the fluidizing gas enters the bed through an air distributor plate at the bottom of processor. The gas passes up through the bed of solids, setting them to the motion and causing it to fluidize. This gas/solid mixture behaves much like the liquid of a similar bulk density. In the upper part of fluidized bed, an expansion chamber slows down the particle velocity thrown up by bursting bubbles at the bed surface. During the fluidization of particles the filter bags are preventing elutriation of fines with the exit gas from the process vessel (Parikh, 2006). When the rate of air flow increases, the pressure drop across the bed also increases until, at a certain rate of flow, the frictional drag on the particles equals the effective weight of the bed. Mentioned conditions and the velocity of air corresponding to it, are termed incipient fluidization and incipient velocity, respectively. This relationship between the air velocity and pressure drop is shown in Figure 2.13.

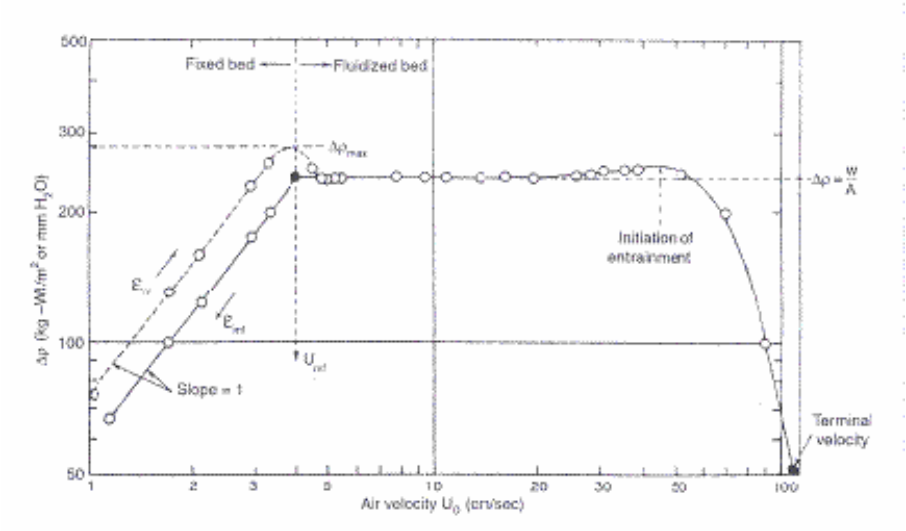

Figure 2.13. Typical pressure drop curve as a function of gas velocity

With the increase in the air velocity, at one point bed behavior changes from packed bed to suspended particles, and this superficial velocity required to first suspend the bed particles is known as minimum fluidization velocity and presents a lower limit of operating velocities. Corresponding approximate pressure drop can then be used to approximate pumping energy requirements. Usually, in the agglomeration process operating air velocity is five to six times higher than the minimum fluidization velocity. At the point of incipient fluidization, the pressure drop of the bed will be very close to the weight of the particles divided by a cross-sectional area of the bed (W/A). For the fluidized bed normally used in pharmaceutical industry density of gas is much less than the density of the solids, so the balance of the forces can be presented with the following equation:

$$\Delta p_{mf} = \frac{W}{A} \qquad \text{Equation 2.16}$$

Which can also be expressed as:

$$\Delta p = \frac{HA\,(1-\varepsilon_{mf})(\rho_p - \rho_f)g}{A} = H\,(1-\varepsilon_{mf})(\rho_p - \rho_f)g \qquad \text{Equation 2.17}$$

Where

Δp	=	pressure drop
H	=	fluidized bed height
A	=	bed cross sectional area
ε	=	void space of the bed at minimum fluidization
ε_{mf}	=	porosity of the bed at minimum fluidization
ρ_p	=	particle density
ρ_f	=	fluid density
g	=	gravitational acceleration

At the same time, the estimated pressure drop in packet beds at minimum fluidization is best described by the Equation 2.19:

$$\frac{\Delta p}{H} = 150\frac{(1-\varepsilon_{mf})^2}{\varepsilon_{mf}^3}\cdot\frac{\mu U_{mf}}{d^2} - 1.75\frac{(1-\varepsilon_{mf})}{\varepsilon_{mf}^3}\cdot\frac{\rho U_{mf}^2}{d} \qquad \text{Equation 2.18}$$

where: μ = fluid viscosity

U_{mf} = minimum fluidization velocity

D = the particle diameter

The first summand in Equation 2.19 represents the laminar flow component, whereas the second one stands for the turbulent flow component. The minimum fluidization flow is reached when the upward drag force exerted by the fluid on the particles is equal to the apparent weight of particles in the bed.

As the velocity of the gas is further increased, the bed continues to expand and its height increases with only a slight increase in the pressure drop. At a certain velocity of fluidizing air, known as entrainment velocity, particles are carried over by the gas. Particular fluidization is called particular in the case when the volumetric concentration of solid particles is uniform throughout the bed at all times.

Aggregative fluidization is when the concentration of particles is not uniform throughout the bed and changes with time. Different types of fluidized bed can be distinguished, depending upon the movement of bubbles through the bed. Figure 2.14 shows various types of fluidized beds.

A *slugging bed* is a fluid-bed in which the gas bubbles occupy entire sections of the product container and divide the bed in two layers.

A *boiling bed* is a bed in which the gas bubbles are approximately the same size as the solid particles.

A *channeling bed* is a fluidized-bed in which the gas forms channels through which most air passes.

A *spouted bed* is a bed in which the gas forms a single opening through which some particles flow and fall on the outside.

The pattern of movement of the gas in and out of bubbles depends upon several factors, including minimum fluidization velocity and particle size. These movements affect heat transfer between the air bubbles and particles. Air distributor at the bottom of the product container has a controlling influence on the uniform distribution of the gas, minimization of dead areas and maximization of particle movement.

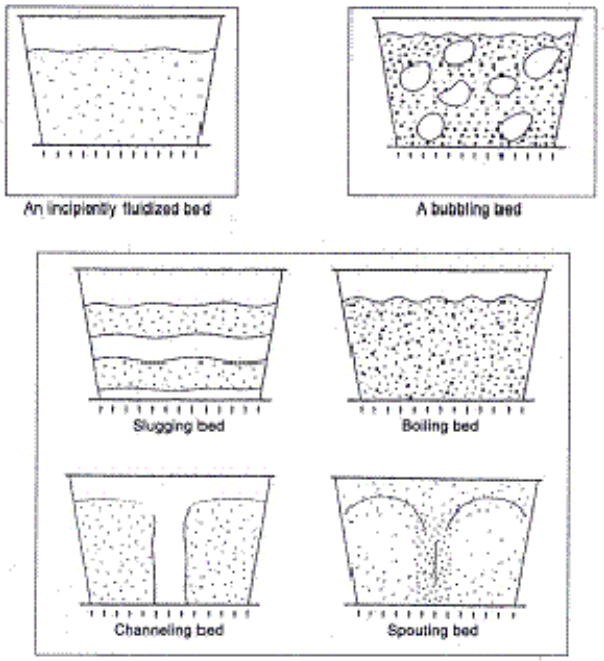

Figure 2.14. Various types of fluidized beds

The extent of segregation can be controlled in part by maintaining high fluidizing air velocities and high-bowl-height-to-bowl-diameter ratio. The standard velocities for are based upon the cross-sectional area at the bottom of the product container. The air velocity can be calculated according to the following equations:

$$\text{Velocity (m/sec)= Air flow (cubic meter per hour – CMH)} \div \text{Area (m}^2\text{)} \times 3600 \qquad \text{Equation 2.19}$$

$$\text{Air flow (cubic meter per hour – CMH)} = \text{Air flow (CFM)} \times 1.696 \qquad \text{Equation 2.20}$$

Low air velocities of 0.8 – 1.4 m/s are required for drying, while in the early stages of drying high air velocities are required in order for product to lose its moisture. Air flow velocities are normally between 1.0 – 2.0 m/s.

The energy required to heat the granules is small compared to energy necessary to evaporate the solvent. Due to the intense mixing of the particles the exit gas and the particles have the same temperature. The mass energy balance limitations are quite evident:

- The exit gas humidity cannot exceed the saturation humidity in the gas at the exit temperature. Once the air is saturated, no more liquid can be removed from the system.
- The energy required to evaporate the liquid cannot exceed that available from the incoming gas.

Collapse of the fluidized bed will happen if any of these limits is exceeded.

2.6. Theory of pellet formation and growth

2.6.1. Bonding forces

Attraction between solid particles

The mechanism of pellet formation can be explained through the mechanism of granule formations since the same forces cause solid particles to adhere to each other when they are brought close enough together.

Attractive forces are short-range forces that cause solid particles to adhere to each other only if they are brought close enough together. Attractive forces may be molecular (valence and Van der Waals), electrostatic or magnetic.

Valence forces are effective only up to distance of 10°A, and since the surface roughness can lead to a separation greater than 10°A they can be excluded from the pellet-forming bond. Van de Waals forces are thought to be the ones which make the most significant contribution to all intermolecular attractive effects.

Electrostatic forces are found with fine powders and are produced during size reduction or due to interparticle friction, or sometimes only the contact between the particles can produce these forces. The electrical double layer that develops whenever particles touch each other is much more significant and generates adhesional forces that are permanent.

Magnetic forces are rarely found to be pellet-forming bonds. However, should they exist, they are expected to produce very strong bondings between particles (Ghebre-Sellassie, 1989b).

Interfacial forces and capillary pressure in movable liquid surfaces

Formation and growth of pellets can be divided in four stages (see Figure 2.15):

1. Pendular state
2. Funicular state
3. Capillary state
4. Droplet state

The initial step in wet agglomeration process is the step of bringing liquid binder into contact with powder particles and attempt to distribute this liquid evenly throughout the fluidized particles. This leads to formation of initial agglomerates and it is referred to as nucleation (Dybdahl, 2005).

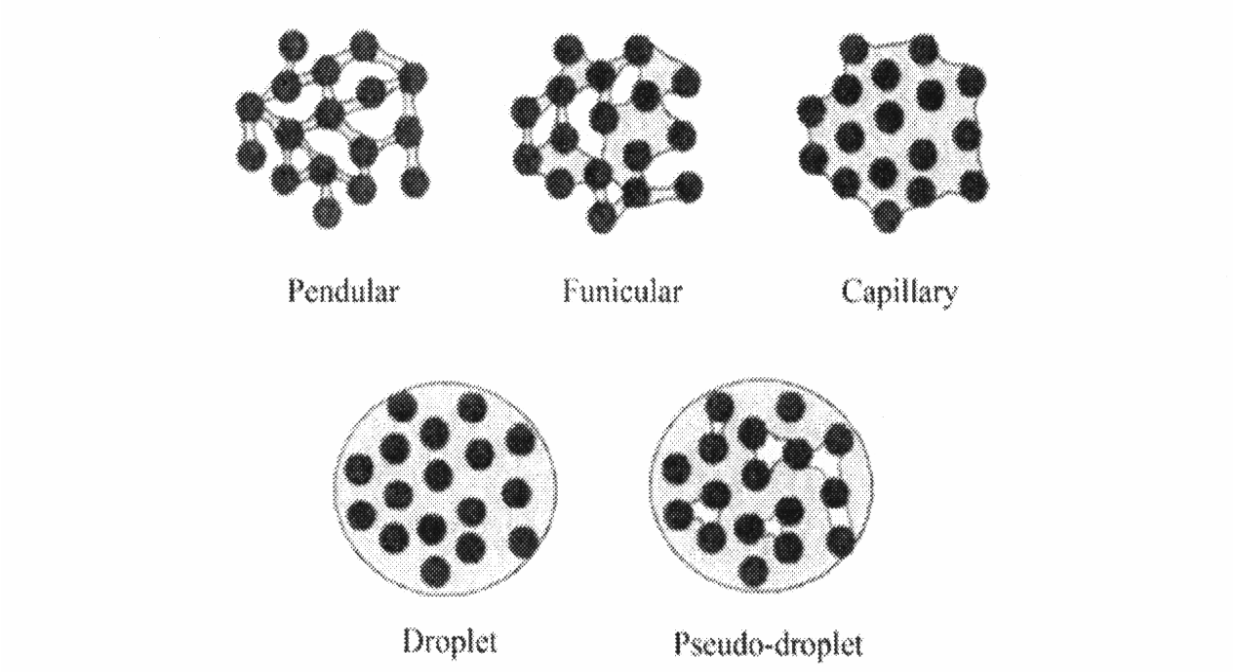

Figure 2.15. Different formal spatial structures of liquid-bound agglomerates depending of liquid saturation

When liquid is added in the powder mixture, part of the void space in a randomly packed material is filled with a liquid forming lens-like rings (liquid bridges) at the contact points between the particles forming agglomerates. The number of contact points of any particle is a function of the distribution and surface geometry of the adjacent particles.

This state where the ratio of the liquid to the void volume is low is called pendular state (Figure 2.15). Mutual attraction of particles is brought about the surface tension of the liquid and the negative suction pressure generated at the liquid bridges.

In the funicular state, as in the pendular state, liquid bridges containing gas and pores filled with liquid are present, but here the liquid forms a continuous phase and pockets of air are dispersed throughout the agglomerate (Figure 2.15).

The capillary state is reached when all the void space in agglomerate is fully occupied by liquid but the quantity of liquid is not sufficient enough to surround the agglomerate (Figure 2.15). Capillary pressure and interfacial forces create strong bonds between the particles, which disappear once the liquid evaporated.

In the droplet state the liquid completely envelopes the agglomerate. The primary particles are held together only by the surface tension of the droplet. There is no interparticle capillary bonding and this situation almost never happens in fluid beds.

Adhesional and cohesional forces in bonding bridges that are not freely movable

Viscous binders and thin adsorption layers provide bonds that are based on immobile liquid bridges. Highly viscous binders adhere to the surface of solid particles and generate strong bonds that are similar in characteristic to those that exist with solid bridges.

Thin-adsorption layers are also immobile and can form strong bonds between neighboring particles by either smoothing surface roughness and increasing particle contact area or by decreasing the effective interparticle distance and allowing the intermolecular attractive forces to participate in the bonding mechanism.

Solid bridges

Liquid bridges are only temporary structures and more permanent bonding is achieved with evaporation of the solvent during further fluidization and formation of solid bridges. Solid bridges are formed by different mechanisms:

1. Crystallization of dissolved substances. As the dissolved medium evaporated, the dissolved solids crystallize out and form bonds at the contact points
2. Hardening binders. Upon drying or curing, binders which are used during pelletization, harden and form solid bridges.
3. Melting. Substances that melt on the input of energy tend to solidify when cooled forming solid bridges, and their strength depends on the chemical composition of the melted material.

2.6.2. Elementary growth mechanism

The most classified pelletization process which involves a rotating drum has been divided into three consecutive regions: nucleation, coalescence, layering and abrasion transfer.

Nucleation (Figure 2.16, A) occurs whenever a powder is wetted with liquid and presents first stage of the pellets growth. The primary particles are drawn together and attached together by liquid bridges which are pendular in nature. The size of primary particles, the viscosity of the bonding particles, the moisture content, wettability of the substrate and the processing conditions influence the size, the rate and the extend of nuclear formation.

Nucleation is followed by a transition phase with two major mechanism, coalescence and layering. Coalescence phase is characterized with formation of large-sized particles by random collision of nuclei containing slight excess of moisture. Although the number of nuclei is reduced, the total mass of the system remains unchanged during this step. Layering (Figure 2.16, C) involves successive addition of fines and fragments on surface of nuclei. The number of nuclei remains the same, but the total mass of in the system increases due to increasing particle size as a function of time. The fragments and fine particles are formed during the process in the stage of particle size reduction due to attrition, breakage and shatter.

The final phase is called ball growth and the main mechanism in this phase is the abrasion transfer (Figure 2.16, D) which involves the transfer of materials from one granule formed to another without any preference in either direction. Particles will experience a change in size as long as the conditions that lead to the transfer of material exist.

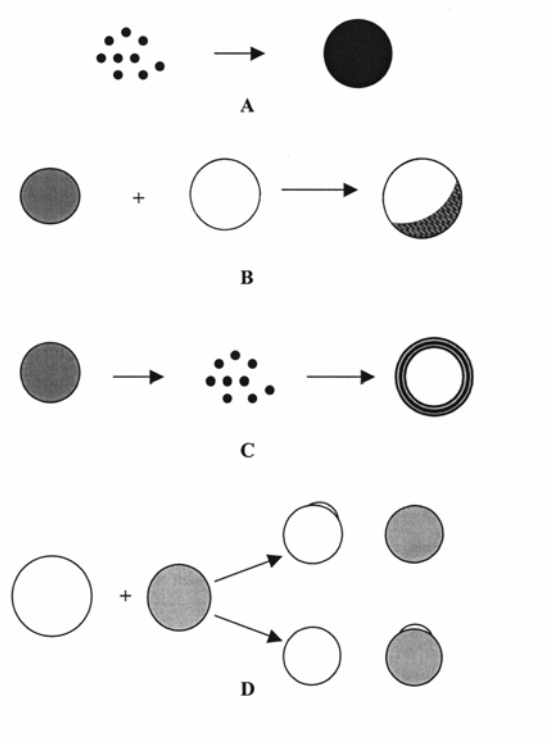

Figure 2.16. Pellet growth mechanisms. (A) Nucleation, (B) Coalescence, (C) layering and (D) abrasion transfer (modified from Ghebre-Sellasie 1989) (Kristensen, 1987)

In the case of solution/suspension layering growth of pellets involves deposition of successive layers on existing nuclei, which may be inert seed, crystal or granule.

In solution suspension layering, the drug particles are dissolved or suspended in the binding liquid, with or without the binder. Droplets of the binding liquid spread on the surface of the nuclei. During drying, liquid evaporates and the dissolved substances crystallize out and capillary forces which are formed draw the particles towards each other and towards the inert seed, forming solid bridges. In suspension layering, particles have low solubility and are bonded by solid bridges formed from the hardening binder, meaning that higher concentration of binder might be necessary.

In this process fines are produced as a result of attrition or spray drying, especially when the process is not optimized.

2.7. Coating of pellets

Film coating consists mainly of polymers, which are applied to the cores in the form of solutions or dispersion in which other excipients are dissolved or suspended. After drying of solvent or

dispersing agents, the polymer and other excipients remain on the cores as coherent, uniform film. Film formation from solutions or dispersions occurs by different mechanism (Bauer et al., 1998). First functional polymers have been used to coat pharmaceutical solid dosage forms for protective, decorative and functional purposes.

2.7.1. Mechanism of film formation

A film forming polymer in polymeric dispersion is atomized and deposited from the nozzle onto the surface of pellets intended for coating. Polymeric dispersion contains submicron-size spheres and each sphere consists of hundred of polymer chains. Deposited on the surface, they coalesce into a continuous film as the aqueous phase evaporates, since interfacial tension between water and polymer pushes particles into contact point in a close-packed arrangement (Figure 2.17). Energy required for the coalescence of spheres results from the surface tension of the polymer generated by the negative curvature of the particle surface may be described by Frankel's equation:

$$\theta^2 = \frac{3\gamma t}{2\pi r} N \qquad \text{Equation 2.21}$$

Where: θ = one-half the angle of coalescence (contact angle) at time t

γ = the surface or interfacial tension

r = the radius of a sphere

N = the viscosity of the spheres

This equation illustrates the inverse relationship between internal viscosity *(N)* of the spheres and the driving forces (*γ)* necessary to fuse or coalesce discrete particles. It is evident that smaller radius polymer spheres require less driving force (capillarity) to completely fuse or coalesce.

2.7.2. Glass transition temperature

Film formation of polymer dispersions is correlated to the glass transition temperature of the polymer itself, because the flexibility of the polymer chains changes as the temperature decreases or increases. The glass transition temperature (Tg) is the temperature at which the viscosity of a melted polymer increases considerably on cooling, or decreases noticeably on heating. In molecular terms, it is the temperature at which the flexibility of polymer chains changes by several orders of magnitude.

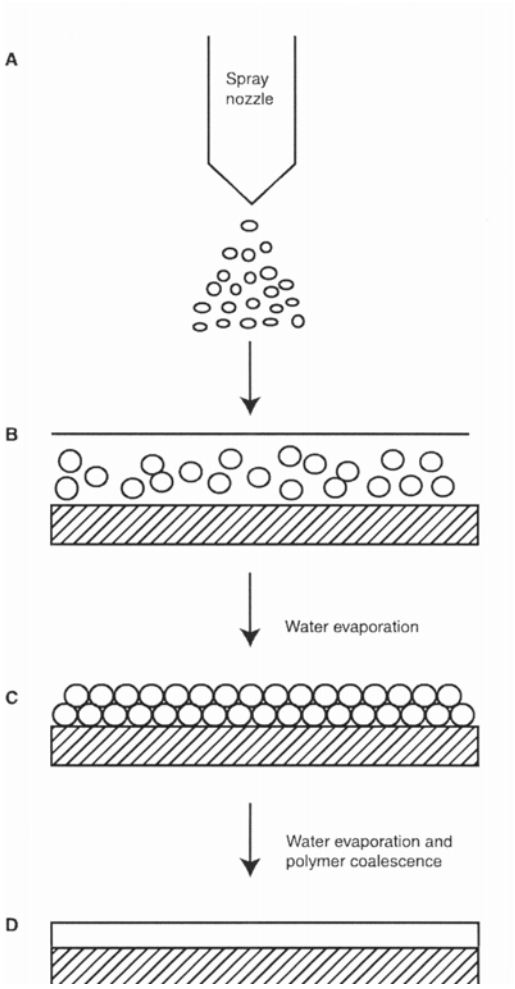

Figure 2.17. Schematic representation of the film formation process for and aqueous polymeric dispersion: (A) atomization of polymeric dispersion; (B) deposition of the polymeric dispersion on the substrate surface; (C) packing of the polymer spheres with water filling the void spaces; (D) formation of continuous polymeric film (Felton, 2007).

2.7.3. Minimum film - forming temperature

The term minimum film formation temperature (MFT) is the temperature in degrees Celsius above which a continuous film is formed under distinct drying conditions (Lehman, 1997). It is an important parameter to determine appropriate bed temperature of the fluidized bed apparatus (Frohoff-Huelsmann et al., 1999). The coated dosage forms are than cured, namely treated at high temperatures for short time. This procedure completes the film formation, especially in the case of dispersions with high MFT (over 40°C) (Dashevsky et al., 2005).

Many polymers which are used for film coating of pharmaceutical dosage forms have brittle properties at ambient temperature. In the literature it is known that MFT values decrease with increasing level of plasticizer, with the increasing standing time of the aqueous polymer dispersion and in some cases with increasing amount of pore former. The dispersion medium water also has a plasticizing effect.

2.7.4. Film-formers for enteric resistance coating

Polymers are macromolecules having a molecular weight range between 10,000 and several million Daltons and consist of a number of repeating units in the structure. They can cause a prolonged drug release in order to extend the intake intervals or enteric resistance in order to protect the drug against the acidic media in the stomach.

Shellac

The oldest group of polymers for enteric resistance coating, consists only on shellac, which is of natural origin and has been used for hundred years for enteric coatings and taste masking as well as prolonged release (Hogan, 1995). Shellac is obtained from the resinous secretion of the insect Kerria lacca (Pearnchob et al., 2003).

Due to higher coating levels shellac is able to retard the drug release, but these formulations lack of drug release in the gastric environment. Shellac consists mainly of a mixture of polyesters, basically composed of shelloic and alleuritic acid, which are responsible for its gastric resistance properties. However, as a product of natural origin it is subjected to batch-to-batch variation of the quality in dependence of the purification process and the resulting content of wax, coloring material and other impurities. According to the literature coating materials such as shellac and resin do not fulfill modern requirements because they are not sufficiently soluble in the digestive tract (Lehman, 1997).

Incorporation of hydrophilic polymers into the shellac formulation, according to the study conducted by Qussi and Suess, 2005, could prevent dissolution of drug in simulated gastric fluid for 2 hours and increase the drug release from pellets.

Aqueous dispersion

Aqueous dispersions are dispersed substances in the dispersing agent, water like gas in water (foam), fluid in water (emulsion), or solid in water (suspension). When the dispersed phase is a polymer, it is called polymer dispersion and the dispersed phase can be solid, fluid or any intermediate condition. The term latex is used for colloidal polymer dispersions.

The aqueous systems have an advantage from an environmental standpoint and are less toxic and cheaper than organic systems.

The particle size is the most important specification of latex and is between 10 and 1000 nm. Latexes are characterized by low viscosity even when they have high solid content like 30%.

Methacrylic acid copolymers

Methacrylic acid copolymers belong to a group of polymers which are insoluble in acid media and dissolve by salt formation above pH 5-6. They are full synthetic copolymers exhibiting an acidic carboxyl group which is responsible for the enteric resistance. The backbone is based on a continuous carbon chain stabilized by methyl groups resulting in poly(methyl methacrylate) (PMMA) which was also used crystal clear, unbreakable organic glass (Lehman, 1998).The enteric resistant polymer is available as Eudragit® L and S. Hence they are used for enteric film

coatings, since they are insoluble in dilute acids, gastric fluid and pure water, they dissolve in buffer solutions above pH 5.5 (L 100-55), pH 6 (L 100) and pH 7 (S 100).
Methacrylic acid copolymers are produced by emulsion polymerization and subsequent spray drying. They are soluble in isopropyl alcohol, acetone, ethanol and methanol, also in mixtures with up to 40% water.
These products are commercially available in the form of spray-dried powders, and in the form of aqueous dispersion with 30% solids (Eudragit L 30 D-55).
Eudragit L 30 D-55 is an anionic copolymer based on methacrylic acid and ethyl acrylate, with free carboxyl groups in a ratio of 1:1 with the ester groups. The carboxylic groups begin to ionize in an aqueous media at pH 5.5 and above, rendering the polymer resistant to the acidic environment of the stomach, but soluble in intestinal fluid.

2.7.5. Film coating equipment

With aqueous dispersions, the process conditions such as spraying rate, drying temperature, amount of drying air and spraying pressure must be carefully chosen because if, as a result of processing conditions, the product bed temperatures are too low, they will be insufficient to achieve the desired filming above minimum film-forming temperature. The product temperature during coating should be approximately 20°C above the minimum film formation temperature in order for good film formation to occur (Dashevsky et al., 2005). On the other hand, excessively high product bed temperatures allow the dispersion agent to evaporate so rapidly that the film-former is spray dried (Thoma and Bechtold, 1999).
Different types of fluidized bed equipment used for coating process are presented in the Figure 2.10 and Figure 2.11.

2.8. Characterization of pellets

In order to meet the requirements of size distribution, surface area, shape, surface roughness, density and friability, including the reproducibility of morphologic properties of the pellets, pellets have to be tested.

2.8.1. Size distribution

The size distribution of pellets should be as narrow as possible because it will ensure a minimum variation in coating thickness and coating performance within the batch. If the pellets are intended for compression, wide size distribution may lead to segregation and variations in content uniformity.
The most common and widely used method for determination of size distribution is *sieve analysis*. The reasons for its extensive use are simplicity, low costs, low time consumption. Some of the disadvantages of this simple method are the inability of the sieve to detect variation

in the shapes of particles. The procedure involves the mechanical shaking of a sample through a series of sieve sizes and weighing these sieves before and after the analysis. Critical parameters of the method are sieve loading, type of motion (vibration or tap), intensity and duration of intensity.

Another method for measurement of pellet size distribution is light scattering, and it is a method most suitable for spherical particles. In laser diffraction method particles pass through a beam of light, they scatter the light, which is directed onto a diode array detector directly opposite the incident light. Sizing of the particles is based on the angle of diffracted light, with small particles diffracting at wider angles than larger particles.

Assuming a log-normal distribution, a plot of particle size versus the cumulative percentage of undersize particles can be used to determine the geometric mean weight diameter d_g, the size corresponding to the 50% value, which is also equal to the mean diameter Randall, 1995. When making a log-probability plot it is common to find that experimental data are scattered, specially the one with very small and very large particles. For this reason when determining the best straight line, it is recommended by some authors that only experimental points within the 20 to 80% range are used (Fonner et al., 1981).

2.8.2. Shape and surface roughness

In order to obtain good performance of coated pellets it is necessary to have spherical and smooth particles suitable for subsequent coating, usually for achieving modified-release. The commonly used method is the analysis of microscopic or non-microscopic pictures of interest.

Scanning electron microscopy (SEM) is a technique of choice for measuring the shape and surface smoothness of the pellets to support visually the other qualitative and quantitative results (Costa et al., 2004).

2.8.3. Porosity

The morphology of pellets and total structure can change in any variation in formulation or material properties, affecting porosity, which is considered to have a great influence on coating, flow and packing during tablet or capsule filling Rashid, 2001. It also influences the rate of release of drug from pellets by affecting the capillary action of dissolved drug (Rashid, 2001). The pores can be analyzed, qualitatively, by scanning electron microscopy and, quantitatively by mercury porosimetry (Mehta, 1989).

The “PoreSizer” measures the volume distribution of pores in material by mercury intrusion or extrusion. It is a 30,000 psia (207MpA) mercury porosimeter covering the pore diameter range from approximately 360 to 0.006 μm / 3 nm to 200 μm. The unit has two built-in low pressure ports (range of low pressure measurement 0 to 30 psia which corresponds to pore diameter 360

to 6 μm) and one high pressure chamber (with the high pressure measurement range of 0 – 30 000 psia which corresponds to pore diameter 6 – 0.006 μm).

Mercury has a high surface tension and is non-wetting to all materials with exception of a few noble materials. These properties cause a mercury surface in contact with a solid to assume the minimum surface area and the largest radius of curvature possible at a given pressure. An increase in pressure on the mercury shifts the balance between surface tension and surface area causing the radius of the curvature of the mercury contacting the solid to become smaller. When the radius is equal to that of a pore entrance, mercury fills the volume within the pore.

The method is based on the capillary rise phenomenon in which excess pressure is required to force a non-wetting liquid into a narrow volume. The mercury is forced into the pores of the sample using an externally applied pressure, with the smallest pores requiring the highest pressure to effect the filling (Brittain et al., 1991).

Mercury porosimetry is based on the capillary law governing liquid penetration into small pores. This law, in the case of a non-wetting liquid like mercury and cylindrical pores and open at both ends, is expressed by the following Washburn equation:

$$D = -(1/P)4\gamma\cos\varphi \qquad \text{Equation 2.22}$$

Where: D = *pore diameter*
P = *applied pressure*
γ = *surface tension*
φ = *contact angle*

The surface tension (γ) and the contact angle (φ) of mercury were 485 dynes/cm and 130 – 140 degrees, respectively.

The total pore surface area (assuming that all the pores are cylindrical) can be calculated using the Equation 2.23 (Juppo et al., 1997):

$$S = \frac{1}{\gamma\left|\cos\theta\right|}\int_{0}^{Vtot} PdV \qquad \text{Equation 2.23}$$

Where: P = applied pressure
V = volume of intruded mercury

Mean pore diameter (D_{mean}) is calculated by the following Equation 2.24:

$$D_{mean} = \frac{4V_{tot}}{S} \qquad \text{Equation 2.24}$$

The bulb volume of the cell which is usually used for pellets is 5 cm^3 for powders.

2.8.4. Density of pellets

Variation of density of pellets from batch to batch affects the potency of finished capsules, produces segregation during mixing and causes problems in batch size determination during coating.

Bulk and tap density of pellets is measured using automated tapper, by measuring the volume of a known mass into a graduated cylinder, and is influenced by the diameter and size distribution of pellets. They are indicative of the packing properties of particles.

True density indicates the extent of densification or compactness of substance. The pycnometric density is determined by measuring the volume occupied by a known mass of particles which is equivalent to the volume of gas displaced by the particles. In this case only open pores are included in the measured volume since the sealed pores are inaccessible to the gas (5.0, 2005).

2.8.5. In-vitro dissolution testing

Dissolution is defined as the process by which a solid substance enters in the solvent to yield a solution. A dissolution test measures the rate of release of the drug. Before the drug is absorbed from the gastrointestinal tract (GIT), it has to be released and dissolved first. For a development compound, dissolution testing is used primarily to help and evaluate new formulations by evaluating the drug release from dosage forms, evaluating the stability of these formulations, but for the commercial products dissolution testing is used primarily to confirm manufacturing and product consistency and to assess post-approval changes and the need for bioequivalence studies (Brown et al., 2004).

The dissolution characteristics of drugs can be influenced by different factors such as the physical characteristics of the dosage form, the wettability of dosage unit, the penetration ability of the dissolution medium, the disintegration, deaggregation and swelling process of the dosage form. The basic steps in dissolution process can be divided in three steps. First step is described as a step in which dissolution media and/or components of dissolution media move towards the solid-liquid interface with certain velocity. It is assumed that the solid is surrounded by a layer of liquid with a certain thickness. At the solid's surface adsorption takes place and this represents second step in dissolution mechanism. Third step is described with the molecular diffusion of the dissolved drug molecules from the surface towards the bulk solution.

According to this step classification, dissolution process can be either reaction limited or diffusion limited, depending which step in the process is slower. Both of these two steps depend on the agitation conditions, composition of dissolution medium and as previously mentioned drug and dosage form properties.

Two main models for interpretation of dissolution mechanisms are diffusion layer and the interfacial barrier model. In both models assumption is made that there is a stagnant layer of liquid around the solid particles. Interfacial barrier model assumes that the rate limiting step of the dissolution model is the reaction at the solid-liquid interface (Macheras et al., 2006), meaning that the slower step is the transfer of drug from the solid phase to the solution. For the diffusion layer model, the step that limits the rate is the rate of diffusion of the dissolved drug molecules through the stagnant liquid layer to the bulk solution.

Diffusion layer model is the most commonly used, but also some alterations have been proposed.

Mathematical description of the dissolution process

Different mathematical formulas that express the dissolution results as a function of some of the dosage characteristics are used in practice.

Some basic principles of the dissolution process of a solid dosage form are given by the film theory (Nernst, 1904). Be a solid immersed in an agitated liquid, surrounded by a stagnant liquid layer with a thickness h. At the solid's surface, the concentration of dissolved solid is equal to its saturation concentration S. Be c the concentration of the dissolved solid in the agitated dissolution medium. At the steady state, Fick's first law can be employed (see Equation 2.25).

$$J = -D\frac{\partial c}{\partial x} \qquad \text{Equation 2.25}$$

where *J* is the diffusion current, defined as the amount of substance passing vertically through an unit surface area per time. *D* stands for the diffusion coefficient, whereas *∂c/∂x* represents the constant concentration gradient corresponding to the slope (*C-S)/h* (see Figure 2.18).

Considering the dissolved mass m and the surface area of the dissolving solid O, the Fick's law can be expressed according to Noyes Whitney (Noyes and Whitney, 1897) (see Equation 2.26).

$$\frac{dm}{dt} = \frac{OD}{h}(S - c) \qquad \text{Equation 2.26}$$

Dividing both member of Equation 2.26 through the volume of the dissolution media *V*, Equation 2.27 is obtained.

$$\frac{dc}{dt} = \frac{OD}{hV}(S - c) \qquad \text{Equation 2.27}$$

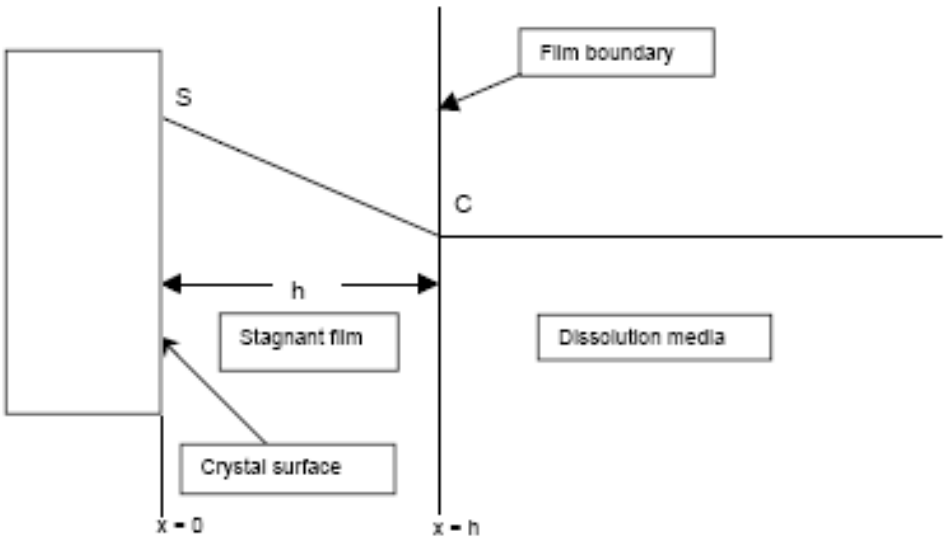

Figure 2.18. Fick's low graphic illustration

If the middle distance between the discussed molecules is negligible compared to the diameter of the molecules, Einstein relation can be applied (see Equation 2.28).

$$D = \frac{RT}{N_a\ 6\pi\eta r} = \frac{kT}{6\pi\eta r} \qquad \text{Equation 2.28}$$

where N_A indicates the Avogadro number, R the universal gas constant, k the Boltzmann constant, T the temperature, η stands for the viscosity of the dissolution medium and r for the radius of the molecule. It is redundant to say that the molecular mass of a certain compound in a molecular-disperse solution does not have a big influence on the diffusion coefficient D, since the radius of a spherical particle corresponds approximately to the third root of its molecular mass. Another theory, called the surface renewal or penetration theory (Danckwerts, 1951), proposes the existence of a dynamic (and not stagnant) laminar layer h, meaning that the surface would be continually replaced by fresh liquid.

Mechanisms of release from coated pellets

In the case of release from pellet dosage forms coated with polymers insoluble in GIT may occur in three different mechanisms (Dressman and Bernhard, 1994):

a. solution/diffusion through the continuous plasticized polymer phase;
b. solution/diffusion through plasticizer channels;
c. diffusion though aqueous pores.

Solution/diffusion through the continuous plasticized polymer phase assumes that the polymer forms a phase in which the plasticizer and other additives are homogeneously dispersed. The

diffusion of a solute molecule within an amorphous polymer phase is an activated process involving the cooperative movements of the penetrant (drug) and the polymer chain segments around it. It is by this stepwise process that hindered molecular diffusion occurs. Release by diffusion/solution through the plasticized polymer phase is presented in Figure 2.19.
Based on the Fick's law (Equation 2.25), the release rate in presence of the above mentioned mechanism can be described by the Equation 2.29.

$$J = \frac{P_m}{\delta}(C_s - C_b) \qquad \text{Equation 2.29}$$

where J ist the flux (release rate per unit surface area of coating) C_s and C_b are the concentration of drug at the coating interface and the bulk, respectively, and δ is the coating thickness. The permeability coefficient of the coating polymer P_m can be written as:

$$P_m = \frac{D\varepsilon}{\tau\beta}K = D'K \qquad \text{Equation 2.30}$$

where D ist the molecular diffusivity of the drug, K the distribution coefficient of the drug between the polymer membrane and fluid in the core (imbibed water), ε the volume fraction of the chain opening, β a chain immobilization factor and τ the tortuosity factor. The frequency with which a diffusion step occurs depends on the size and shape of the drug, tightness and bonds between adjacent polymer chains and the stiffness of the polymer chain. Further below its glass transition temperature (T_g), the less permeable the polymer. Plasticizers lower the T_g, increase free volume and increase diffusivity. Accordingly, this mechanism is dominant in continuous film, flexible polymers which lacks pores.
Overall permeability of the polymer to the drug will depend on the ability of the drug to partition into the polymer as well as its ability to diffuse through the polymer.

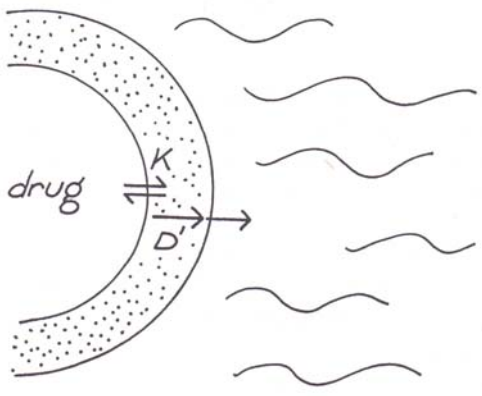

Figure 2.19. Drug release from coated pellets via solution/diffusion through the polymer film

The second mechanism occurs when the plasticizer is not uniformly distributed in the coating polymer and its content is high where plasticizer takes the form of a continuous phase in the form of patched channels. This mechanism is shown in Figure 2.20 and the release rate for this model can be described by the Equation 2.31, which derives from the Equation 2.30 replacing P_m, the permeability of the coating polymer, with P_{pl}, the permeability of the plasticizer.

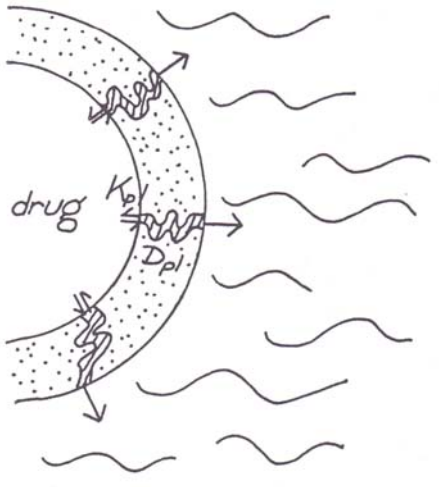

Figure 2.20. Drug release from coated pellets via solution/diffusion through plasticizer channels

$$P_{pl} = \frac{D_{pl}\varepsilon_{pl}}{\tau_{pl}} K_{pl} \qquad \text{Equation 2.31}$$

In this case, K_{pl} is the distribution coefficient of the drug between plasticizer and the core fluid (imbibed water), τ_{pl} the tortuosity of the plasticizer channels, and ε_{pl} the volume fraction of plasticized channels. For this mechanism to be dominant, the following condition must be satisfied:

$$P_{pl} \approx 10^{-8} cm^2/s = \frac{D_{pl}\varepsilon_{pl}}{\tau_{pl}} K_{pl} \qquad \text{Equation 2.32}$$

Diffusivity in the plasticizer will generally be lower than in water since plasticizers tend to be relatively viscous. Assuming a $D_{pl} \approx 10^{-6}$ cm^2/s, a plasticizer load of 40% with half forming channels ($\varepsilon = 0.2$) and a low tortuosity ($\tau = 2$), the ability of the drug partition should be at least 0.1. In the reality, this mechanism was demonstrated to be too slow to explain the release rates observed.

Diffusion through aqueous pores intervenes when a continuous, but inhomogeneous coating layer is punctuated with pores. This mechanism is more likely to be operative for the coatings

formed from aqueous dispersions and when the pellets come in contact with an aqueous medium, these pores fill with solution thus facilitating the diffusion of the drug. During the coating and curing processes, the pseudolatex particles often do not fuse completely, thereby creating a porous coating. The pores may be of 1μm size and the release mechanism is illustrated in Figure 2.21.

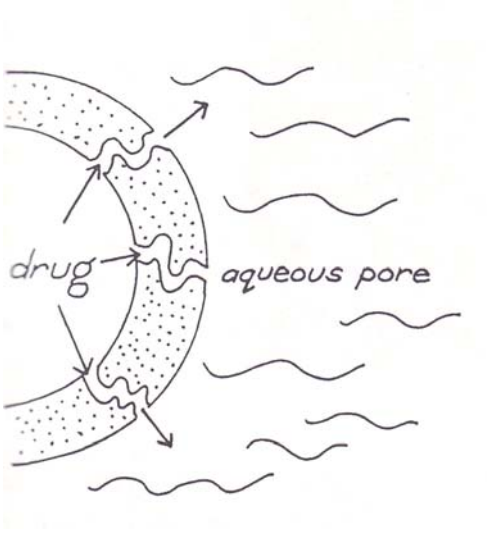

Figure 2.21. Drug release from coated pellets via diffusion through aqueous channels

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For diffusion through aqueous pores, the permeability coefficient P_p is given by:

$$P_{pl} = \frac{D_p \varepsilon_p}{\tau_p}$$ Equation 2.33

Where D_p is the aqueous diffusivity of the drug, ε_p the volume fraction of the aqueous channels, and τ_p their tortuosity. The partition coefficient K is the unity in this case.

For diffusion through aqueous pores to be the mechanism driving the release rate, P should be in the order of 10^{-8} cm^2/s. If SEM consistently indicates the presence of pores in the coating, it is likely that diffusion through the pores will contribute significantly to the overall release rate

3. Objective of the study

In order to shorten the development time which presents today's focus of pharmaceutical industry and obtain early assessment of stability, the main purpose of the study was to investigate and gain understanding about the factors affecting the stability of lansoprazole delayed release pellets.

Since there was a need to investigate the thermal behavior of lansoprazole in order to clarify the differences reported in the literature and to determine a reliable method for determination of the melting point of lansoprazole followed by decomposition of the substance, which makes a determination of the melting point very difficult.

Study will try to clarify the influence of type of neutral pellet (Suglets® and Etispheres®), type of stabilizing agent and presence of protective coating on surface morphology, porosity and stability of enteric coated pellets containing acid-liable drug (lansoprazole). The influence of type of neutral core on the stability of pellets has not yet been investigated.

Possibility of stabilizing lansoprazol using Na-CMC and rotary processor (direct pelletization) will be investigated. Since the process has been characterized as a multivariable process the study will include an optimization of factor settings of two process variables, spray rate and rotor speed and one formulation variable, drug loading on geometric mean diameter of pellets and moisture content at the end of liquid addition phase using statistical optimization techniques in order to obtain spherical lansoprazole pellets of acceptable size. Experiments will be planned using statistical design of experiment (DoE) and the data obtained analyzed to yield valid and objective conclusions.

Furthermore, the objective of the study is to determine the temperature effects in the presence of moisture on the degradation rate constant of lansoprazole applying accelerated degradation with Arrhenius testing as a comparative technique in stability prediction of pellets prepared using different pelletization techniques.

4. Materials and Method

4.1. Materials

4.1.1. Drug substance

Lansoprazole

Batch no. F51217 and F41403, Cipla, Mumbai, India

(See Figure 4.1)

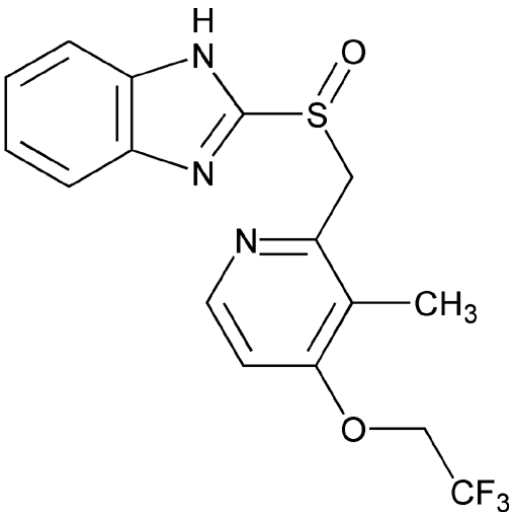


Figure 4.1. Chemical structure of lansoprazole

Empirical formula: $C_{16}H_{14}F_3N_3O_2S$

*1*H-*Benzimidazole, 2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridinyl]methyl]sulfinyl]-2-[[[3-Methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]-methyl]sulfinyl]benzimidazoles*

Appearance: White to brownish white odorless crystalline powder

Molecular weight: 369.36

Melting point: 166°C with decomposition (producer reference)

BCS: Class II

pKa: 8.78 and 3.82

logP: 2.761 ± 0.779 (25°C)*

Stability: unstable at pH below 7

* SciFinder Scholar, Calculated properties for lansoprazole, Advanced Chemisrty Development (ACD/Labs) Software, V8.14 for Solaris 1994 – 2008.

4.1.2. Excipients

Neutral sugar pellets, *Suglets*®

Batch no. 503 P, NP Pharm, Bazainville, France

Appearance: White spherical granules with sweet taste, soluble in water

Particle size: 850 – 1000 µm

Components: saccharose (up to 92%) and maize starch

Manufacturing date: 03/2005

Neutral microcrystalline cellulose pellets, *Ethispheres® 850*

Batch no. 609 ZZ, NP Pharm, Bazainville, France

Appearance: White spherical granules, insoluble in water, high physico-chemical inertness (improves stability of finished products)

Particle size: 710 - 1000 µm

Manufacturing date: 04/2006

α-Lactose monohydrate, *Lactosum D-80*®

Molkerei Meggle Wasserburg GmbH, Wasserburg, Germany

Particle size: 200 mesh (54%< 32 µm, 86%< 63 µm, 96%< 100 µm)

Hydroxypropilcellulose (HPC), *Klucel LF*®

Hercules Inc., Wilmington, Delaware, USA

Viscosity: 5% aqueous solution 75 – 150 cps

Hydroxypropilmethylcellulose (HPMC), *Methocel E5*®

Colorcon, Budapest, Hungary

Viscosity: 2% aqueous solution 5-g cps

Magnesium carbonate, heavy

Calmags GmbH, Germany

Sodium dihydrogenphosphate dodecahydrate

Merck KGaA, Darmstadt, Germany

Ethyl acrilate methyl methacrylate polymer, Eudragit L 30 D-55®

Röhm Pharma Polymers, Darmstadt, Germany

30% aqueous dispersion of anionic polymethacrylate for enteric coating

Film solubility: above pH 5.5

Shellac, *Marcoat 125*®

Aqueous Shellac solution, Emerson Resources, INC-Syntapharm Group, Product no. 0802200701

Appearance: Clear amber solution

Odor: Characteristic sweet odor

Solids: Dewaxed Orange Shellac NF

Solid content: 25% ± 1% by weight

pH: 7.2 ± 0.3

Specific gravity: 1.04 ± 0.01

***Balocel*®**

Pharmatrans Sanaq AG, Basel, Swizerland

Powder premix for pelletization

Constituents: 50% microcrystalline cellulose, 35% lactose monohydrate, 15% sodium carboxymethylcellulose

Polysorbate 80, *Tween 80*®

Seppic GmbH, Cologne, Germany

Dispersing agent

Tryethylcitrate, *Eudraflex*®

Merck KGaA, Darmstadt, Germany

Plasticizer

Glycerol monostearate 40-50, *Imwitor® 900K*

Sasol, Hamburg, Germany

Glyceryl monostearate 40 – 55%

Glidant

Lansoprazole USP standard

Lot. GOD307, USP Rockville, MD

USP reference

4.2. Characterization of drug substance and neutral pellets

4.2.1. Solubility of lansoprazole

Solubility experiments were performed with a large excess of the substance. It was anticipated that decomposed lansoprazole could be replaced with the intact substance during experiment (Kristl et al., 2000).

Excess of the substance was tested for solubility in water using agitation method, in 100 ml flasks at temperature of 25°C ± 0.1°C in a water shaking bath for 48 h, with shaking amplitude of 66. Samples were taken after regular time intervals and quantified by UV/VIS spectrometry described in chapter 4.5.6 at 284 nm.

Standard calibration curve was prepared from stock solution of lansoprazole which was prepared weighing 10 mg of lansoprazole in 100 ml volumetric flask, adding 1 ml of methanol and filling up to the volume with distilled water. Different concentrations were prepared diluting the standard stock solution with water and quantified.

In order to assure working in the sink conditions solubility of lansoprazole was also determined at 37°C in phosphate buffer pH 6.8 using the same procedure. Sink conditions were defined as follows: the total concentration of the drug dissolved should not be significantly higher than 10% of their saturated concentration (Gibaldi and Feldman, 1967).

4.2.2. Fürrier-transform infrared spectroscopy (FTIR) of lansoprazole

FTIR spectra of lansoprazole was obtained using PerkinElmer FTIR spectrophotometer (Waltham, Massachusetts, USA) using diffuse reflectance technique (KBr disc technique) as a part of qualitative analysis by comparing it with the spectra of lansoprazole USP standard. Samples of lansoprazole powder and lansoprazole USP standard were previously ground and mixed with KBr, an infrared transparent matrix. The KBr discs were prepared by compressing the powder and the scans were obtained in the mid-infrared regions of the spectrum form 4000 – 400 cm^{-1} at resolution of 1 cm^{-1}.

4.2.3. Thermal properties of active substance

4.2.3.1. Differential-scanning calorimetry (DSC)

The samples (2.5 – 5.0 mg) were placed in aluminium sample pans with holes, 50 µm, sealed and scanned from 60°C up to 220°C/300°C (40°C above the determined signal) with different heating rates (2.5°C/min, 5°C/min, 10°C/min, 20°C/min, 30°C/min and 40°C/min) in a Perkin Elmer Pyris 1 DSC/Diamond DSC, in order to see the effect of scanning rate on the thermal behavior of the studied substance.

4.2.3.2. Thermogravimetric analysis (TGA)

Since thermogravimetrical analysis measures changes in sample weight as a function of time or temperature. Desolvation and decomposition processes are frequently monitored by TGA (Fiese and Hagen, 1986). Loss of mass that occurred due to heating was determined under conditions that gave maximum noticeable degradation during the DSC studies. Comparing TGA and DSC data recorded under identical conditions can greatly help in the interpretation of thermal processes.

The samples (14 - 15 mg) were placed in sample pan holder and scanned from 60°C up to 230°C (40°C above the determined signal) with different heating rates (5°C/min, 10°C/min, 20°C/min, 30°C/min and 40°C/min) in a Perkin Elmer Pyris 6 TGA under the dynamic flow of nitrogen (100 ml/min) and the loss of mass recorded.

Also, samples were scanned from 60°C up to 350°C (120°C higher temperature range than the previous scanning) with four different heating rates (1°C/min, 2.5°C/min, 10°C/min and 20°C/min) in order to examine if there is another thermal event which can be visible on the TGA curve.

4.2.3.3. Hot-stage microscopy (HSM)

Degradation during thermal analysis may provide misleading results and for that reason HSM has been used in order to clarify the thermal behavior. Behavior of lansoprazole upon heating was visualized by Hot-stage microscope using a hot stage unit (Lynkam THMS 600) optical microscope (Olympus BX 51) with 10x magnification. A small amount of lansoprazole was placed on a glass slide, covered with the cover glass and heated from 60°C up to 220°C with the heating rates of 1°C/min, 2.5°C/min, 10°C/min and 20°C/min.

4.2.4. Powder X-ray diffractometry of lansoprazole

Powder X-ray diffractometry of lansoprazole samples was performed with an Siemens, Model D 5005, X-ray diffractometer over 5-60°2θ range at a scan rate of 1°/min where the tube anode was Cu with Kα= 0.154 nm monochromatized with a graphite crystal. The pattern was collected with 40kV of tube voltage and 40 mA of tube current in step scan mode (step size 0.05, counting time 1 s/step) (Zhang et al., 2007).

4.2.5. Particle size measurement

In order to determine appropriate method for determination of particle size distribution by laser diffraction, two methods were used. Dry powder laser diffraction method was proposed by producer of the substance (Batch no. F51217, Cipla, Banglore, India), and wet laser diffraction method which is in some cases more appropriate for determination of particle size distribution of pharmaceutical substances. Particle size distribution measurement has been performed on

Mastersizer 2000 (Malvern Instruments) using dry powder feeder unit Scirocco 2000 and wet unit Hydro 2000.

Dry powder laser diffraction has been conducted with optical characteristics of particle refraction index 1.500, absorption 0.001. Vibration feed rate was set to 50% and dispersion medium pressure (air pressure) was set to 2 bars.

In wet method as dispersant medium, water was used. Since lansoprazole has low wettability, 10% polysorbatum 80 solution is used as surface active substance to obtain good dispersion of substance Wet powder laser diffraction measurement has been conducted with optical characteristics of particle refraction index 1.500, absorption 0.001. Weight residual was 0.499%, and pump for dispersion medium was set on 1750 rpm.

For determination of particle size distribution of neutral pellets, dry powder feeder unit was used with different measurement settings. Optical characteristics were set to particle refraction index 2.500 with the absorption 0.1. An obscuration value in the range of 1-10% in all measurements as obtained (2.9%). Vibration feed rate was set to 50% with air as dispersion medium, with pressure of 2 bars. With the software (Malvern) the particle size distribution, including mean and median particle size was calculated from raw data.

4.2.6. Bulk and tapped density

Bulk (poured) density and tapped density was measured using automated tapper (Stav, J. Engelsmann, Ludwigshafen, Germany). 100 g mass of sample, giving the volume between 50 and 250 ml, was poured into the graduated cylinder. The volume noted, without any tapping of the cylinder, is the bulk volume. After fitting, cylinder was tapped 500 times and the volume was noted. Sample was further tapped until 1250 times and again the volume was checked. If the difference between the volume after 500 and 1250 tapps was higher than 2 ml sample was tapped 1250 times more, giving the volume after 2500 times of tapping. The relative bulk density (ρ_{bulk}) and relative tapped density (ρ_{tapped}) were calculated as ratio of volume and mass used for determination, respectively.

Hausner ratio (R) and Carr's index (CI) USP, 2006a were calculated using the Equation 4.1 and Equation 4.2:

$$H = \frac{\rho tapped}{\rho bulk} \qquad \text{Equation 4.1}$$

$$CI = \frac{(\rho tapped - \rho bulk)}{\rho tapped} \cdot 100 \qquad \text{Equation 4.2}$$

where:

H	=	Hausner ratio
ρ_{bulk}	=	bulk density (g/cm^3)
ρ_{tapped}	=	bulk density (g/cm^3)
CI	=	Carr index (%)

4.2.7. Scanning electron microscopy

SEM pictures of lansoprazole powder were sputtered with gold palladium and than observed with a scanning electron microscope (SEM) Philips ESEM XL 30 FEG at a voltage of 10 KV, using magnification of 300, 3000 and 10000.

SEM pictures of neutral pellets were obtained after a neutral pellet and cross section of pellet was sputtered with gold palladium and than observed with a scanning electron microscope (SEM) Philips ESEM XL 30 FEG at a voltage of 5 and 10 KV, using magnification of 100, 300, 1000 and 3000.

4.2.8. Specific surface area measurement

Brunauer, Emmett and Teller (BET) surface measurements were performed using NOVA 2000, Version 8.00, Quantachrome. The samples of lansoprazole powder (≈ 900 - 1000 mg, n=3) were placed in glass sample cell (outer diameter 9 mm with bulb) with glass rod and degassed under vacuum for 24 hours, on degassing temperature of 25°C. Additional sample of non-degassed sample was analyzed to get an idea about the way the surface area is affected if the sample has not been pre-treated.

Surface area measurement of neutral pellets (sugar pellets and MCC pellets) was conducted in triplicate. Samples of sugar pellets in weight of 1.5520 – 1.5550 g were degassed under vacuum for 24 hours on 25°C, in sample cell without bulb (outer diameter 9 mm). Mass of sample of MCC neutral pellets was 0.5210 – 0.5340 g and they were degassed using the same, previously described procedure for sugar pellets.

Multipoint measurement was carried out using N_2 as adsorbate gas, on temperature of liquid nitrogen (-196°C) in the limited range of the adsorption isotherm, usually in the P/Po range of 0.05 to 0.35. First step in the calculation of specific surface area was calculation of weight of monolayer W_m using the BET plot Equation 4.3:

$$\frac{1}{W((Po/P)-1)} = \frac{1}{WmC} + \frac{C-1}{WmC}\left(\frac{P}{Po}\right) \qquad \text{Equation 4.3}$$

In which W is the weight of gas adsorbed at a relative pressure P/P_0 and W_m is the weight of adsorbate constituting a monolayer of surface coverage. The term C, the BET C constant, is related to the energy of adsorption in the first adsorbed layer and consequently its value is an indication of the magnitude of the adsorbent/adsorbate interactions (Quantacrome).

From the slope *s* and intercept *i* of the BET plot, W_m can be calculated:

$$S = \frac{C-1}{WmC} \qquad \text{Equation 4.4}$$

$$i = \frac{1}{WmC}$$ Equation 4.5

$$W_m = \frac{1}{s+i}$$ Equation 4.6

Further step in the application of the BET method is the calculation of the surface area. The total surface area of the sample can be expressed as:

$$St = \frac{W_m N A_{cs}}{M}$$ Equation 4.7

Where N is Avogardo's number (6.023 x 10^{23} molecules/mol) and M is the molecular weight of the adsorbate.

Specific surface area of the solid is calculated using the following equation:

$$S = \frac{S_t}{W}$$ Equation 4.8

4.2.9. True density

True density of lansoprazole powder and neutral pellets (n=3) was determined using helium pycnometer (Mycromeritics Accupyc 1330, Norcross, GA, USA). Mass of sample was approximately 1.6 g for lansoprazole powder, which presented 2/3 of the sample cell volume, while mass of neutral pellets for the analysis was approximately 5 g. Purging procedure was set to number of purges 5 and purge fill pressure was set to 1.5 bar (19.5 psia). For the measurement number of runs was 5, with run fills pressure of 1.5 bar (19.5 psia) and equilibration rate: 0.01 - 0.001 psia/min.

4.3. Preparation of pellets using solution/suspension layering

4.3.1. Active and protective layering

Following eight different formulations have been prepared (see Table 4.1) using solution/suspension layering technique. Lansoprazole loaded pellets were prepared by layering a drug-binder (HPC, 4 w/w%) suspension onto sugar spheres (Suglets®, saccharose 92% and maize starch) or MCC pellets (Etispheres®) in bottom spraying fluidized-bed Unilab-5 (Hüettlin, Schopfheim, Germany) with the 1.0 mm nozzle, until the desired drug loading (8.8% w/w) was achieved.

Table 4.1. Formulations under study

Run	*Pellet type*	*Protective coat (HPMC)*	*Alkaline agent*
1	*Sugar*	*Yes*	*Mg carbonate*
2	*MCC*	*Yes*	*Mg carbonate*
3	*Sugar*	*No*	*Mg carbonate*
4	*MCC*	*No*	*Mg carbonate*
5	*Sugar*	*Yes*	*Sodum phosphate*
6	*MCC*	*Yes*	*Sodum phosphate*
7	*Sugar*	*No*	*Sodum phosphate*
8	*MCC*	*No*	*Sodum phosphate*

First phase of the process was active layering onto the neutral spheres. Formulation of active suspension is presented in the Table 4.2.

In order to maximize the interactions between drug and neutral pellets, the size ratio of micronized lansoprazole powder and neutral pellets was 1:100. Since lansoprazole has a low wettability (Kristl and Vrecer, 2000), and it is known that successful interaction between the drug and the binder solution is greatly influenced by the wettability of the drug, sodium lauryl sulfate was included in the formulation as a wetting agent. Also, adsorbents prepared by depositing lansoprazole and surfactants on porous adsorbants (as solid dispersions) have been employed to improve the dissolution and oral bioavailability.

Neutral pellets (sugar of MCC, see Table 4.1) were loaded into the fluidized bed in quantity of 3.5 kg and heated on 37°C for 15 minutes. Active layering solution was prepared in a way that the first part consisted of HPC which was dissolved in distilled water, in quantity necessary to obtain 4.1 w/v% solution and left over night, prior to coating (see Table 4.2).

Table 4.2. Active layering formulation part I

Substance	*Proportion solids (w/w %)*
HPC	2.64
*Water**	-

**evaporated during the coating*

Second part of the active solution was prepared according to the formulation presented in Table 4.3.

Active layering formulation I and II were mixed for 15 minutes using a propeller mixer type (Eurostar digital, IKA®-WERKE) and sprayed with spray rate of 10 – 23 g/min until the desired drug loading was achieved (8.8% drug load).

Table 4.3. Active layering formulation part II

Substance	*Proportion solids (w/w %)*
*Sugar spheres***	61.59
*MCC spheres***	61.59
Lansoprazole	8.80
Lactose monohydrate	3.52
Sodium laurylsulphate	0.26
*Magnesium carbonate heavy***	0.3
*Dinatriiphosphas dodecahydrate***	0.70
*Water**	-

**evaporated during the coating*

*** not used in the same trials (see Table 4.1)*

Prepared lansoprazole-loaded pellets were dried in the fluidized bed for 20 minutes on temperature of 40°C, and further on coated with the protective coating containing hydroxypropyl methylcellulose 7 w/v% (seal-coating) with spray rate of 15 – 21 g/min. The amount of HPMC used for each layering was always dissolved in the distilled water a day prior to coating.

Table 4.4. Protective layering formulation

Substance	*Proportion solids (w/w %)*
*HPMC***	61.59
*Water**	-

** evaporated during coating, **not used for all trials (see Table 4.1)*

Pellets were dried for 20 minutes on 40°C and intended for further coating with enteric polymer (Chapter 4.3.2).

Process parameters for all three phases of pellet preparation (active layering, protective layering and enteric coating) are presented in Table 4.5.

Table 4.5. Process parameters used for solution/suspension layering

Process parameters	*Process phase*		
	Active layering	*Protective coating*	*Enteric coating*
Inlet air volume (m^3/h)	230	230	230
Inlet air temperature (°C)	45	45 – 53	40
Atomizing air pressure (bar)	1.0 – 1.17	1.16	0.5 -1.27
Microclimate pressure (bar)	0.1 – 0.25	0.3	0.3 – 0.5
Product temperature (°C)	37 - 40	37 – 40	28 - 30
Spray rate (g/min)	10 -23	15 - 21	15 - 32

4.3.2. Enteric coating of drug loaded pellets

4.3.2.1. Enteric coating with Shellac

In order to determine the coating level needed for obtaining gastric resistance with Shellac aqueous solution, 10%, 15% and 20% of shellac solid substance has been applied on lansoprazole pellets from run 8 (see Table 4.1).

Enteric coating fomulation consisted of 34% (w/w) aqueous Shellac solution (25% solid Shellac substance), 33% w/w HPMC (5 cPoas) and 33% w/w of polyethylene glycol 6000 (Macrogolum 6000) as a plasticizer (see Table 4.6). First Shellac aqueous solution was mixed with plasticizer for 15 minutes and than HPMC was added to formulation in the form of 5% w/w aqueous solution which was prepared a day before. Mixing of the final solution was performed for 2 hours at 51 rpm's (Eurostar digital, IKA®-WERKE). Enteric coating with Shellac was performed in bottom spraying fluidized bed Unilab-5 (Hüettlin, Schopfheim, Germany) with the 1.0 mm nozzle diameter. It was necessary to add 1% of talc into the product container because of the electrostatic charge and retention of pellets on the walls of product container. 500 g of pellets was loaded in fluidized bed with reduction insert for the product container and coated with shellac solution up to the weight of 10%, 15 % and 20 % of solid shellac. After achieving a desired weigh gain, pellets were dried in between stages and samples of pellets were removed using a sampling tube, and coating was continued.

Table 4.6. Shellac coating formulation

Substance	*Proportion solids (w/w %)*
Shellac solution (34% w/w aqueous solution)	25
HPMC (5% w/w aqueous solution, 5 cPoas) (on dry polymer)	33
Polyethylene glycol 6000 (plasticizer)(on dry polymer)	33
Solid content of the spray solution	15.6

Since shellac is completely insoluble in water, a film formed from shellac may not be permeable enough to provide the targeted release profile. Therefore, water-soluble additives may be added to provide a channel in membrane for permeation in aqueous environment Qussi and Suess, 2005. The process parameters for the coating are stated in Table 4.7.

Table 4.7. Process parameters for coating with shellac (Unilab-5)

Process parameters	
Inlet air volume (m^3/h)	130
Inlet air temperature (°C)	34 – 36
Atomizing air pressure (bar)	0.37 – 0.41
Microclimate pressure (bar)	0.14 – 0.18
Product temperature (°C)	21 – 23
Spray rate (g/min)	10 – 20
Process time (min)	approx. 210 min

Draying of pellets was perfomed on inlet air temperature of 33 °C, for 20 minutes at the air flow of 135 m^3/h. Samples of pellets coated with shellac were tested on gastric resistance in 0.1N HCL (pH 1.2) and on dissolution in phosphate buffer pH 6.8.

4.3.2.2. Investigation of coating level of methacrylic acid copolymer

In order to determine the coating level needed for obtaining gastric resistance, pellets from the run 5 (see Table 4.1) were coated in different weight gains (20%-26% w/w) of solid enteric polymer and samples of pellets were removed at 2% weight gain increments. Enteric coating suspension (20% solids w/w) contained Eudragit L 30 D-55 as enteric coating polymer (20% of dry polymer), triethyl citrate as plasticizer, glycerol monostearate as a glidant, and polysorbate as dispersing agent (formulation presented in Table 4.8).

Enteric coating was performed in bottom spraying fluidized bed Unilab-5 (Hüettlin, Schopfheim, Germany) with the 1.0 mm nozzle diameter. Preparation of the enteric coating suspension was performed according to the product sheet guidelines for Eudragit L 30 D-55 Degussa, using a Ultra-Turrax® T50 basic and propeller type mixer Eurostar digital (IKA®-WERKE). Enteric coating dispersion was gentle stirred during the coating process. Process parameters for enteric coating are presented in the Table 4.5.

Table 4.8. *Enteric coating formulation*

Substance	*Proportion solids (w/w %)*
Eudragit L 30 D-55 (30% aqueous dispersion)	20
Triethyl citrate (on dry polymer substance)	10
Glicerol monostearate (on dry polymer substance)	5
Polysorbate 80 (on GMS substance)(80% dispersion)	40
Solid content of the spray suspension	20

Samples of prepared pellets were tested on gastric resistance in 0.1N HCL (pH 1.2) and modified acid stage media acetate buffer (pH 4.5), and on dissolution in phosphate buffer pH 6.8 in order to determine necessary quantity of enteric polymer to achieve gastric resistance. Further more, the influence of quantity of enteric coating polymer on stability of lansoprazole pellets was investigated using Arrhenius testing.

4.3.2.3. Coating of pellets with Eudragit L 30 D-55

Eudragit L 30 D-55 (20% of solid polymer) was applied on pellets prepared according to the Table 4.1, using the same procedure described previously in Chapter 4.3.2.1. Process parameters are presented in the Table 4.5.

4.4. Preparation of pellets using direct pelletization

4.4.1. Optimization of pellet size using experimental design

The objective of the study was to obtain spherical lansoprazole pellets of acceptable size, for pellets are intended for further coating.

In order to determine the optimum levels of spray rate, rotor speed and drug load for the production of desired size and shape of the pellets, and since the moisture content is influenced by previously mentioned factors (Chapter 2.4.1.4), all other process parameters, as well as the quantity of MCC in the formulation and the amount of binder solution, were determined in pre-

experiments and were kept constant through out all the experiment. Drug load was interesting because lansoprazole is a low wettable substance and the obtained content of drug in pellets could provide valuable information on the process. Furthermore, since there was no possibility of power consumption measurement, moisture content measurement was conducted at the end of the liquid addition phase, supposing that it could reveal us the level of moisture necessary for successful pelletization. Even though, the inlet air humidity is an important parameter and influences powder bed moisture content, this parameter was noncontrollable during the experiments since the equipment could not condition the inlet air.

Drug containing pellets were prepared in fluidized bed rotary processor (GPCG-1, Glatt GmbH, Binzen, Germany) using direct pelletization technique. Since the quality of statistically designed experiment depends on the good parameter settings, preliminary experiments were performed to establish suitable settings for the process and formulation variables. Using the settings found on the basis of the pre-experiments, a Vertex-Centroid design for the optimization of pellets size was performed.

Pelletization procedure

The starting materials were sieved through 0.5 mm sieve, mixed for 6 minutes in Turbula mixer, type T2C (Willy, Bachofen AG, Basel, Switzerland) and 600 g of powder mixture was loaded into the equipment. Formulations for the two drug loading levels are presented in the Table 4.9. From the Table 4.9 it can be seen that lactose monohydrate has been used as filler in order to keep the mass of loaded powder constant. It was assumed that difference of only 8% in the quantity of lactose monohydrate will have no influence on the pellets characteristics. Temperature and flow rate of fluidizing air were set to 28°C and 80 m^3/h in all experiments. After the fluidizing air was initiated, air gap has been set to approximately 2.8 kPa by elevating the rotor plate and rotation of rotor plate has been started.

Table 4.9. Excipient proportions used in the study

Excipients	*Proportion (w/w %) 8% drug load*	*Proportion (w/w %) 16% drug load*
MCC	35	35
Lactose monohydrate	38.5	30.5
NaCMC	10.5	10.5
Magnesium carbonate heavy	7	7
Sodium lauryl sulphate	1	1

Balocel quantity was kept constant for all runs (70 w/w %)

Powders were mixed for 5 minutes in preheated equipment and than HPC binder solution (4% w/v) was sprayed tangentially onto the moving powder mixture presented in the Table 4.9,

through 0.8-mm nozzle diameter. The spray rate and the drug load were varied according to the design (see Table 4.12). The amount of binder solution which was necessary to obtain pellets was 470 g. It was determined in pre-experiments and kept constant for all experiments along with the other process variables.

Table 4.10.Process parameters during process phases (GPCG-1)

Process parameters	*Process phase*		
	Binder addition	*Spheronization*	*Drying*
Air flap (%)	40	40	43
Air flow (m^3/h)	80	80	80
Inlet air temperature (°C)	28	28	35
Atomizing air pressure (bar)	2.5	-	-
Pressure difference product (kPa)	2.8 - 3.1	2.6 - 2.8	2.5 – 2.8
Rotor speed (%)	Design	80 – 90	50
Time (min)	21 - 35	7	10 - 15

Immediately after stopping the liquid addition, samples of about 2 g were drawn with the sampling tube of the equipment for the determination of the moisture content and the nozzle was removed. Moisture content was determined using infra-red balance (Mettler LP16 drying unit, Mettler PE360, Mettler Toledo). One gram of the sample was tested on moisture content at temperature of 105°C for 15 minutes.

After addition of determined quantity of binder solution, process was transferred to spheronization phase, followed by drying phase. Process parameters of all three phases are presented in the Table 4.10.

A Vertex-Centroid Design, quadratic (D-optimal), was used to find the optimal levels for spray rate, rotor speed and drug load on chosen dependent variables, geometric mean diameter by weight and moisture content. The independent variables and their levels are shown in the Table 4.11. The geometric mass mean diameter was calculated according to the procedure and equation given by (Fonner et al., 1981). Generated design and evaluation of the results was conducted using Stavex.

Table 4.11. Independent variables and their levels

Factors		*Level -*	*Level +*
Spray rate (rpm)	A	6	10
Rotor speed (%)	B	50	80
Drug load (%)	C	8	16

Design consisted of all possible combinations off all factors, at all levels. Table 4.12 presents a matrix design with 14 generated runs which were carried out in randomized order.

Table 4.12. Composition of experimental formulations (runs) Vertex-Centroid Design, quadr.; (D-opt.)

Run	*Spray rate*	*Rotor speed*	*Drug load*
	rpm	*%*	*%*
1	6	50	8
2	6	50	16
3	6	80	8
4	6	80	16
5	10	50	8
6	10	50	16
7	10	80	8
8	10	80	16
9	10	65	12
10	8	80	12
11	8	65	16
12	6	50	12
13	6	65	8
14	8	50	8

Influence of MCC and lactose ratio on production and properties of pellets

As a part of preliminary investigation influence of quantity of lactose monohydrate, which was used as water soluble filler necessary to obtain the same mixture loading, on size and properties of pellets was investigated. Even though the difference of MCC and lactose monohydrate loading in the design was 8% (w/w) (see Table 4.9) wider range has been tested. Influence of 15% of difference of lactose monohydrate in the formulation on pellet size, porosity, true density and dissolution was tested (see Table 4.13).

Table 4.13. Tested formulations on influence of quantity of lactose monohydrate

Excipients	*Proportion (w/w %)* *Trial 1*	*Proportion (w/w %)* *Trial 2*
MCC	35	35
Lactose monohydrate	34.5	49.5
NaCMC	10.5	10.5
Magnesium carbonate heavy	7	7
Sodium lauryl sulphate	1	1
Lansoprazole	12	12

Production of pellets was performed using process parameters described in binder addition phase in Table 4.10 with the spray rate of 10 rpm's and rotor speed of 65%. Quantity of binder solution was kept constant at 470 g, as described previously in the pelletization procedure.

4.4.2. Protective coating of pellets

Protective coating of optimized pellets obtained with direct pelletization was performed only on the pellets from run 9 (Table 4.12), since these pellets had desired pellets size (range of 500 microns) and sphericity (Table 5.12).

Protective coating was performed on a small scale fluidized bed Mini-Glatt (Glatt GmbH, Binzen, Germany) using a bottom spraying technique, without Wurster insert, with the nozzle size 0.5 mm. Batch size of 80 g of pellets was coated with 2.0 % (w/w) hydroxypropylmethyl cellulose solution. Coating of pellets with the solutions containing higher amount of binder was not applicable with Mini – Glatt. Before coating it was necessary to add 1.0 % (w/w) of talc to pellets to decrease the electrostatic charging and prevent retention of pellets on the walls of fluidized bed. Pellets were coated up to the weight gain of 4% using process parameters described in Table 4.14. Binder solution was applied using pump (Flocon 1003 Periflo), with the hose size 4.8 mm in diameter and the quantity of applied binder was measured using balance (PG5002-S Delta-Range, Mettler – Toledo). Temperature inside the fluidized bed was monitored with temperature sensor Testo 925 (Testo GmbH, Lenzkirch, Germany).

Table 4.14. Protective coating process parameters with the Mini - Glatt

Process parameters	*Process phase*	
	Coating	*Drying*
Inlet air pressure (bar) (m^3/h)*	0.45 – 0.55 28.5 – 32.7*	1.4 68.7*
Inlet temperature (°C)	33 – 34	35
Atomizing air pressure (bar)	1 – 1.2	-
Spray rate (g/min)	1.05 – 1.4	-
Fluidized bed temperature (°C)	24 – 26	33
Duration of process (min)	170	25

*Values expressed in m^3/h according to the equation: y=42.284x+9.479 R2=0.9077, y: m3/h, x: bar

4.4.3. Enteric coating of pellets

Enteric coating suspension was applied on selected pellets according to the scheme presented in Table 4.17. Enteric coating dispersion in the quantity of 20% solids was not applicable in the case of Mini-Glatt, because of the continuous blockage of the nozzle and for this reason enteric dispersion containing 10% solids has been applied (same composition as described in Table 4.8 with addition of distilled water to adjust 10% solids). Coating was conducted on bottom spraying fluidized bed Mini-Glatt (Glatt GmbH, Binzen, Germany), without Wurster insert, using a 0.8 mm nozzle. Batch size of 60 g of pellets was coated until 20% of solid enteric polymer has been applied using the same pump type, temperature sensor and balance as described for protective coating (Chapter 4.4.2). Process parameters for enteric coating on Mini-Glatt are presented in the Table 4.15.

Table 4.15. Enteric coating process parameters (Mini-Glatt)

Process parameters	*Process phase*	
	Coating	*Drying*
Inlet air pressure(bar) (m^3/h)*	0.3 – 0.9 22.2 – 47.5	1.2 60.2
Inlet temperature (°C)	30 – 32	35
Atomizing air pressure (bar)	1.5 – 1.7	-
Spray rate (g/min)	0.38	-
Fluid bed temperature (°C)	25 – 27	33
Duration of process (min)	210	20

*Values expressed in m^3/h according to the equation: y=42.284x+9.479 R2=0.9077, y: m3/h, x: bar

The process had to be interrupted for several times because of the nozzle blockage and agglomeration of the pellets.

4.5. Characterization of drug-loaded pellets

4.5.1. True density

The true density of pellets was determined using an AccuPyc^{TM} 1330 Helium Pycnometer (Micromeritics, Norcross, USA) with a sample cell of a known volume 12.0530 cm^3. The mass of the sample was calculated as a difference between mass of filled pycnometer sample cell and a mass of empty pycnometer sample cell. The volume was determined by purging the sample 10 times with helium. First 5 runs were considered as an equilibrating procedure. Average value for density was taken from the next 5 runs. The procedure was carried out three times for each sample and mass of sample was approximately 5 grams.

4.5.2. Size distribution of pellets

Laser diffaction method

Particle size distribution measurement of pellets prepared with solution/suspension layering has been performed on Mastersizer 2000 (Malvern Instruments, Worcestershire, UK) using dry unit Scirocco 2000. The measurement was carried out 3 times for each sample.

Dry powder laser diffraction has been conducted with optical characteristics of particle refraction index 2.500, absorption 0.1. An obscuration value in the range of 1-10% in all measurements as obtained (2.8 – 3.0%). Vibration feed rate was set to 50% with air as dispersion medium with pressure of 2 bars. With the software (Malvern) the particle size distribution, including mean and median particle size was calculated from raw data.

Sieve analysis

The size distribution of pellets produced by direct pelletization, for the particle size optimization, was determined by sieve analysis of 100 g of sample using a vibrating sieve (Retsch, S&H AG, Arlesheim, Germany). The sieves of 0.9, 0.125, 0.180, 0,250, 0.355, 0.500, 0.710, 1.0 mm were shaked for 10 minutes on vibration amplitude of 55.

4.5.3. Shape and surface morphology of pellets

Coated and uncoated pellets and the cross-section of the pellets were sputtered with gold palladium and than observed with a scanning electron microscope (SEM) Philips ESEM XL 30 FEG at a voltage of 5 and 10 KV using magnifications of x60, x120, x300, x1000.

4.5.4. Porosity of pellets

Porosity measurement of pellets, with and without enteric coating, was conducted on a PoreSizer 9320 System (Micromeritics) with software version 2.05. The PoreSizer measures the volume distribution of pores in material by mercury intrusion or extrusion. Empty penetrometer for powders (penetrometer no. 920-61708-01) was calibrated (n=3) and determined bulb volume was 5.2927cm^3. Approximately 2 g of sample (2/3 of the volume of the penetrometer) were filled in penetrometer. Sample was evacuated until the pressure reached 50μm Hg and than mercury was introduced. Low pressure run was conducted in a left low pressure port until the pressure reached 25 psia. Mass of penetrometer with the sample and mercury was checked on a balance and noted, and than penetrometer was transferred to the high pressure chamber, where the mercury was introduced in the pores with the pressure up to the 30 000 psia.

4.5.5. Measurement of pellet pH

Measurement of pH slurry of pellets was conducted using pH meter (Metrohm, pH Meter 744) after calibration with standard calibration solutions of pH 4.05 and pH 7.09. The uncoated pellets were ground into fine particles using mortar and pestle and quantity of 2 g of pulverized

material was mixed for 5 minutes in 20 ml of distilled water, and pH of prepared slurry of pellets was measured (Bruce et al., 2003).

4.5.6. Assay

HPLC

Lansoprazole content in pellets (n=3) prepared using solution/suspension layering was determined using high pressure liquid chromatography (HPLC) under following conditions: mobile phase water:acetonitrile:triethylamine (60:40:1) with pH of 7.0; diluent water:acetonitrile:triethylamine (60:40:1) pH of 10.0; flow rate about 1 mL/min; detection wavelength 285 nm. Internal standard solution 4'-ethoxyacetophenone was prepared dissolving 3 g of 4'-ethoxyacetophenone (Sigma Aldrich) in 400 ml of acetonitrile, HPLC grade (Merck, I285991 612). This method was official method described in USP 28 (USP, 2006b).

Resolution solution was prepared in concentration of 0.1 mg/ml of USP lansoprazole and USP lansoprazole related compound A RS **[**2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl]sulfonyl]bezimidazole**]** ($C_{16}H_{14}F_3N_3O_3S$, M_w 385.36, USP standard, lot no. ROB 311, USP) in diluent. Obtained resolution between two major peaks was 7.

Lansoprazole standard solution was prepared in concentration of 3.0 mg/ml in a mixture of 0.1M sodium hydroxide and acetonitrile (3:2). 25 ml of the solution and 5 ml of internal standard solution was diluted up to 50 ml with a diluent, and furthermore diluted with diluent to obtain concentration 0.1 mg/ml of lansoprazole.

Volume of 10 µL of resolution solution, standard solution and final solution were injected in HPLC (Shimadzu LC-2010A) using Hypersil BDS column (C18, 4.6-mm × 25-cm column, 5-µm, Thermo Scientific, Waltham, US).

Mass of pellets equivalent to 300 mg of lansoprazole were weighed to a 300 ml conical flask. 60 ml of 0.1N NaOH was added and sonicated until complete disintegration. After disintegration 20 ml of ACN and 20 ml of internal standard solution was added, shaked well and centrifuged for 15 minutes on 4000 rpm's. Supernatant was diluted with diluent to obtain concentration of lansoprazole about 0.1 mg/ml.

Quantity of lansoprazole (mg) was calculated using Equation 4.9:

$$\frac{LC}{D} \cdot \frac{Ru}{Rs} \qquad \text{Equation 4.9}$$

where L is the labeled quantity of lansoprazole in mg, C is concentration in mg/ml of lansoprazole standard in the standard solution, D is the concentration in mg/ml of lansoprazole in the sample preparation, based on the labeled quantity of lansoprazole in the pellets taken

and the extend of dilution; and Ru and Rs are the peak responses ratios obtained from the sample and standard preparation, respectively.

UV/VIS spectrophotometry

Second method which was employed for determination of lansoprazole content in pellets prepared by direct pelletization is UV/VIS spectrophotometry proposed by (ölzaltin, 1999). Lansoprazole stock solution was prepared by dissolving 100 mg of lansoprazole in 60 ml of 0.1 N NaOH and adjusting to 100 ml with phosphate buffer pH 6.6. Further dilutions were performed with 0.01 N NaoH solution.

The mixture of 0.1 N NaOH and phosphate buffer pH 6.6 (3:2) was used as a reference solution. Absorbance was measured UV/VIS Spectrophotometer Beckman DU 530 on wavelength of 292 nm.

A sample of pellets equivalent to the mass of 15 mg of lansoprazole (n=3) was weighted and transferred to a 100 ml volumetric flask. 0.1 N NaOH was added in the quantity of 60 ml and the mixture was sonicated for 15 minutes, and than filled up to the volume of 100 ml with phosphate buffer pH 6.6. Further dilutions, for the linear range were made with 0.01 N NaOH.

Resulting pellets from the direct pelletization which were intended for accelerated stability testing were tested on content using both techniques in order to confirm published UV/VIS spectrophotometry method.

4.5.7. Gastric resistance and dissolution of coated pellets

Samples of pellets in determined size (n=6) were tested for dissolution according to the USP monograph for “Delayed Release Lansoprazole Capsules” in SOTAX AT7 (SOTAX AT/, Allschwil/Basel, Switzerland) dissolution paddle apparatus. Rotating speed of paddles was 75 rpm and temperature of medium was kept at 37°C ± 0.5°C.

Non-encapsulated samples were tested on gastric resistance in 500 ml of 0.1 N HCl with pH 1.2 for 60 minutes. Buffer stage medium consisted of 900 ml which was a mixture of acid stage medium or modified acid stage medium and phosphate buffer concentrate with adjusted pH to 6.8.

Samples were removed from the dissolution media in different time intervals during 60 minutes of acid stage and further 60 minutes of buffer stage testing. An equal volume of dissolution medium, warmed to 37°C, was added after each sampling in order to keep a constant volume.

Quantity of drug release was determined using UV/VIS Spectrophotometer Beckman DU 530 on wavelength of 306 nm for the acid stage, and 286 and 650 nm for the buffer stage.

In the USP 28 monograph for “Delayed Release Lansoprazole Capsules” it is stated that no more than 10% release of lansoprazole in one hour in 0.1 N HCl and no less than 80% release of lansoprazole after one hour in phosphate buffer pH 6.8 is allowed.

4.5.8. Gastric resistance and dissolution of pellets in modified acid stage medium pH 4.5

Enteric performance and dissolution of lansoprazole pellets is routinely evaluated using USP delayed release method in acid media at pH 1.2 in order to determine the effective quantity of enteric polymer and buffer media at pH 6.8. While this method may be appropriate for general enteric performance, the in-vivo stomach pH for patients may be significantly different. As a result of drug action, the gastric acid secretion will be reduced and it has been reported that in-vivo stomach pH for PPI patients on a multiple dose regimen is higher (>pH 4) and modified bio relevant media, which better simulates the gastric environment, has been suggested (Fegely et al., 2006; Rohss et al., 2004). Non-encapsulated samples were tested in 500 ml modified acid stage medium, consisting of acetate buffer with pH 4.5.

Buffer stage medium consisted of 900 ml which was a mixture of modified acid stage medium (acetate buffer pH 4.5) and phosphate buffer concentrate with adjusted pH to 6.8.

Samples of pellets in determined size (n=6) were tested for dissolution according to the USP monograph for “Delayed Release Lansoprazole Capsules” in SOTAX AT7 (SOTAX AT, Alschwill, Basel, Switzerland) dissolution paddle apparatus. Rotating speed of paddles was 75 rpm and temperature of medium was held at 37°C ± 0.5°C.

Samples were removed from the dissolution media in different time intervals during 60 minutes of acid stage and further 60 minutes of buffer stage testing. An equal volume of dissolution medium, warmed to 37°C, was added after each sampling in order to keep a constant volume.

Quantity of drug release was determined using UV/VIS Spectrophotometer Beckman DU 530 on wavelength of 286 nm.

In the USP monograph for “Delayed Release Lansoprazole Capsules” it is stated that no more than 10% release of lansoprazole in one hour in 0.1 N HCl (in this test modified acid stage medium) and no less than 80% release of lansoprazole after one hour in phosphate buffer pH 6.8 is allowed.

4.6. Effect of temperature on degradation rate constant in solid state and prediction of shelf-life

In this study isothermal stability testing of a product, with addition of moisture effect on stability, and the prediction of shelf-life using an Arrhenius equation has been employed. First it was necessary to determine the order of decomposition reaction and generally accepted method in stability is least squares linear regression.

4.6.1. Solution/suspension layered pellets

Accelerated stability testing was performed on lansoprazole delayed release pellets containing 20% enteric coating (calculated on solid polymer) prepared using solution/suspension layering (see Table 4.1) in order to evaluate the effect of presence of different alkaline compounds (pH adjusters), presence and absence of protective coating, and influence of type of neutral core on chemical stability of lansoprazole at different storage conditions. Accelerated stability testing of pellets in open glass bottles was performed at thermostatically-controlled ovens at temperatures of 30°C, 40°C, 55°C and 60°C at 79% RH.

Accelerated stability testing was also conducted on pellets from run 5 with enteric coating polymer in quantity of 20, 22, 24 and 26%, at previously described temperature and humidity conditions, to investigate the effect of percentage of Eudragit L 30 D-55 on stability of lansoprazole in solid dosage form.

Non-encapsulated samples were collected at different time intervals and examined on lansoprazole content using HPLC conditions described in chapter 4.5.6.

4.6.2. Pellets prepared with direct pelletization

For the comparison of the stability of lansoprazole in pellets prepared using different techniques, pellets prepared in the run 9 (see Table 4.12) using direct pelletization procedure were investigated, since they had desired pellet size and acceptable sphericity. Pellets were prepared using rotary processor (GPCG-1, Glatt, Germany) with the drug load of 12%, spray rate of 10 rpm's, and rotor speed of 65 %. Other process parameters were as described previously in Table 4.10. Same accelerated stability conditions described previously (Chapter 4.6.1) have been applied. Formulation variables which have been taken into consideration are presence and absence of magnesium carbonate heavy, presence and absence of protective coating (HPMC formulation presented in Table 4.4). Pellets core formulations are presented in the Table 4.16.

Table 4.16. Direct pelletization core formulations for stability studies

Ingredients	*Proportion (w/w % Formulation I*	*Proportion (w/w %) Formulation II*
Lansoprazole	12	12
MCC	35	35
Lactose monohydrate	34.5	41.5
NaCMC	10.5	10.5
Magnesium carbonate heavy	7	-
Sodium lauryl sulfate	1	1

Further more, pellet cores with formulation I and II were coated according to the scheme presented in the Table 4.17 using the procedure described in Chapter 4.4.2 and 4.4.3.

Table 4.17. Scheme of pellet formulations from direct pelletization under stability study

	Protective coating	*Enteric coating*	*Trial no.*
Formulation I	yes	Yes	1
	no	Yes	2
Formulation II	no	Yes	3
	yes	Yes	4

5. Results and Discussion

5.1. Solubility of lansoprazole

Solubility study of lansoprazole was conducted at times beyond the equilibration to verify that a state of true equilibrium is reached. Estimation of solubility in water and phosphate buffer pH 6.8 was performed in different time intervals in order to determine the real thermodynamic equilibrium.

The solubility of lansoprazole in water at 25°C and phosphate buffer pH 6.8 at 37°C is presented in Table 5.1.

Table 5.1. The solubilities of lansoprazole in water at 25°C and phosphate buffer pH 6.8 at 37°C

Medium	*Solubility (µg/ml) ± RSD (%) (time)*	
Water (n=3)	34.478 ± 0.413 (18 h)	33.571 ± 5.46 (24 h)
pH 6.8 (n=3)	44.162 ± 8.259 (24 h)	43.892 ± 1.46 (32 h)

The solubility of lansoprazole in water at 25°C was very low. It was not surprising since it was known that lansoprazole has low water solubility and that solubility of lansoprazole increases with the increase in pH. The system equilibrium concentration was achieved in 24 hours of measurement and the average sample concentration from 18 to 24 hours differed by less than 2%. Averaged solubility was 34.0245 µg/ml in water at 25°C. No reference in the literature on the experimentally determined solubility of LSP in unbuffered water at 25°C was found. Kristl and Vrecer, 2000, found that the solubility of LSP at 25°C in pH 7 is 29.55 µg/ml.

The concentration of lansoprazole in buffer solution at a temperature of 37°C and pH of 6.8, averaged 44.162 µg/ml and was achieved in 24 hours. Obtained results were higher than the results obtained previously by Zhang et al., 2008. They found that solubility of LSP at 37°C in phosphate buffer pH 7.4 is 32.101 µg/ml. The results show that LSP becomes increasingly ionized with the increase in pH.

5.2. FTIR and X-Ray diffractometry

In order to confirm the identity of lansoprazole, FTIR spectroscopy and PXRD was conducted.

The infra-red spectrum of lansoprazole powder was in agreement with the infra-red spectrum of lansoprazole USP standard. Both powder samples showed the same characteristic absorption peaks at 3240, 2984 and 2930, 1476 and 1458, 1282 and 1268, respectively, denoting stretching vibrations of –NH, aromatic –CH-, –NH bending and aromatic C-O stretching (Cipla,

2005a; Zhang et al., 2007). Corresponding FTIR spectrum is presented in the Appendix in Figure 8.1.

Lansoprazole shows characteristic peaks at 12.557°, 14.499°, 16.952°, 17.884°, 18.892°, 22.264° and 27.854° (Zhang et al., 2007). Lansoprazole X-ray diffractogram is presented in Appendix in the Figure 8.2.

5.3. Thermal properties of lansoprazole

Although the drug is commercially available on the market, literature reports on thermal characterization and melting behavior of the drug substance are contradictory. US Pharmacopoeia 25 states that lansoprazole melts at about 166°C, with decomposition USP, 2006b. For lansoprazole a range of melting point was reported (T_m=178 – 182°C) (O'Neill, 1996) however the producer of the lansoprazole used in the present work reported a melting point of 168 - 169°C (Cipla, 2005b) and no measurement conditions were provided. In the study of Zhang et al., 2008, DSC curve of lansoprazole obtained on heating rate of 5°C/min exhibits a sharp endothermic peak at 178.6°C.

The reason for these contradictory reports could be that the melting behavior of sulfoxides known as proton pump inhibitors is uncommon showing significant heating rate dependence and melting of the substance is followed by decomposition of the substance, which makes a determination of the melting point very difficult. The other reason for inconsistency of reported data could be that different methods for determination have been applied. As a result, there is confusion in the melting point values quoted in the literature.

In a recent study, Rosenblatt et al., 2005, reported on the thermal behavior of one proton pump inhibitor – omeprazole. Using thermal and chromatographic analyses, the authors reported that the melting point depression at low heating rates is due to eutectic behavior of the drug with its decomposition products formed at low heating rates. At heating rates above 20°C/min, the melting point of omeprazole becomes independent of the heating rate due to absence of decomposition products. Furthermore, no differential scanning calorimetric data on the influence of scanning rate on the thermal behavior of lansoprazole were available. Kotar et al., 1996, have declared that lansoprazole exists in two polymorphic forms designated as form A and form B. Form B is unstable and is completely converted to the stable form A under physical stress (milling) or even after some time at ambient temperature. DSC curve of the form A showed only an endothermic peak at 180°C, while the form B showed one exothermic peak at 102°C and one endothermic peak at 180°C. In the literature no DSC curve has been found or provided by Kotar et al., 1996.

Obtained DSC curve of lansoprazole (see Figure 5.1) on all heating rates exhibited two different events. Endothermic peak corresponds to the melting of the drug and it is immediately followed by sharp symmetric exothermic peak. High heating rates (20 - 40°C/min) led to endothermic peaks at higher temperatures, while lower heating rates (2.5 - 10°C/min) led to a shift of the peak to lower temperatures, what can be seen in the Figure 5.2. Difference between the onset temperature obtained with the lowest heating rate and with the highest applied heating rate was around 15°C. The most pronounced shift of the melting peak between two consequent measurements was found to be 6.5°C and it was observed between heating rates of 2.5°C/min and 5°C/min (see Figure 5.2), while with the application of higher heating rates this difference decreased to 1°C. From this values can be seen that higher heating rates led to a smaller difference in the melting point between consecutive measurements (see Figure 5.2).

However, even the highest applied heating rate of 40°C/min did not show independence on the heating rate and the shift of the melting point (T_{onset} = 187°C) was still observed in comparison with the melting point obtained with 30°C/min (T_{onset} = 188°C).

Same dependence of the heating rate was observed with the USP standard of lansoprazole (Figure 5.2).

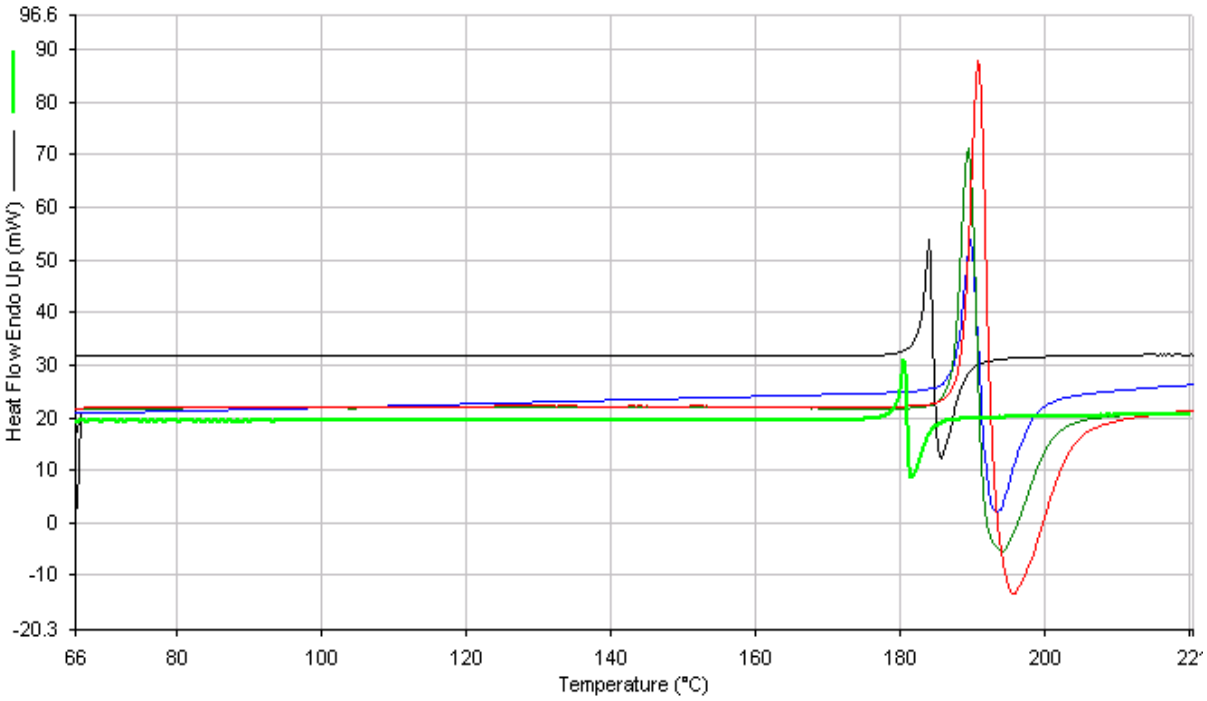


Figure 5.1. Shift of the DSC curve of lansoprazole using different heating rates

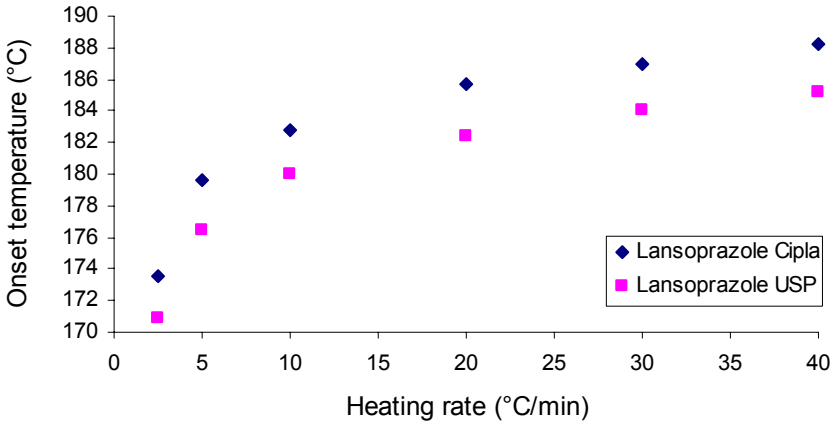


Figure 5.2. Onset temperature of the endothermic event observed by DSC

From the Table 5.2 can be seen that the onset temperatures obtained from the USP standard of lansoprazole differ from the ones obtained with the sample of lansoprazole, but also show high heating rate dependence. Difference can be explained with the purity and the smaller particle size of lansoprazole USP standard.

Table 5.2. Comparison of onset temperatures obtained by DSC and Hot-stage microscope

Heating rate (°C/min)	*Onset temperature endothermic DSC (°C) Cipla*	*Onset temperature DSC (°C) USP*	*Melting temperature Hot Stage (°C) Cipla*
2.5	173.04	170.94	174.30
5.0	179.56	176.46	179.00
10	182.76	179.98	183.50
20	185.74	182.35	184.30
30	187.01	184.08	185.70
40	188.22	185.25	186.50

Contrary to the findings of Rosenblatt et al., 2005, for omeprazole, lansoprazole exhibited variation in the melting point values even at the high heating rates. This indicated that in the case of lansoprazole, the heating rate dependence was larger than in the case of omeprazole. For the endothermic peak it was found that the height of the peak increased with the increase in heating rate, and even the lowest applied heating rate (2.5°C/min) did not lead to its disappearance. This finding was contrary to the findings of Rosenblatt et al., 2005, who noticed that heating rate of 2.5°C/min and lower, lead to disappearance of the endothermic peak in lansoprazole, meaning that decomposition in the sample starts very early and no melting point can be detected, but no DSC curve was provided. Also, they reported that exothermic peak was clearly visible at 30°C/min, while in our case exothermic peak was observed on all heating rates in the form of sharp symmetric peak.

At heating rate of 2.5°C/min, the observed melting peak temperature was 173.04°C ± 0.17 (T_{onset} = 173.04°C ± 0.06) with an apparent heat of fusion of 89.08 J/g ± 1.84. The exothermic effect was peaked at 175.33 ± 0.14 (T_{onset} = 174.75°C ± 0.12) with enthalpy value of 138.46 J/g ± 0.69. With an increase in the heating rate, increase in the peak height, as well as an increase in the enthalpy, was observed for both endothermic and exothermic event. At heating rate of 40°C/min, melting peak temperature was 190.85°C ± 0.03 (T_{onset} = 188.22°C ± 0.09) with an apparent heat of fusion of 166.56 J/g ± 11.51. Peak temperature of exothermic event at 40°C/min was 195.41 ± 0.11 (T_{onset} = 191.88°C ±0.01) with enthalpy value of 248.38 J/g ± 5.08. More detailed results of thermal events are presented in the Appendix (Table 8.1 and Table 8.2).

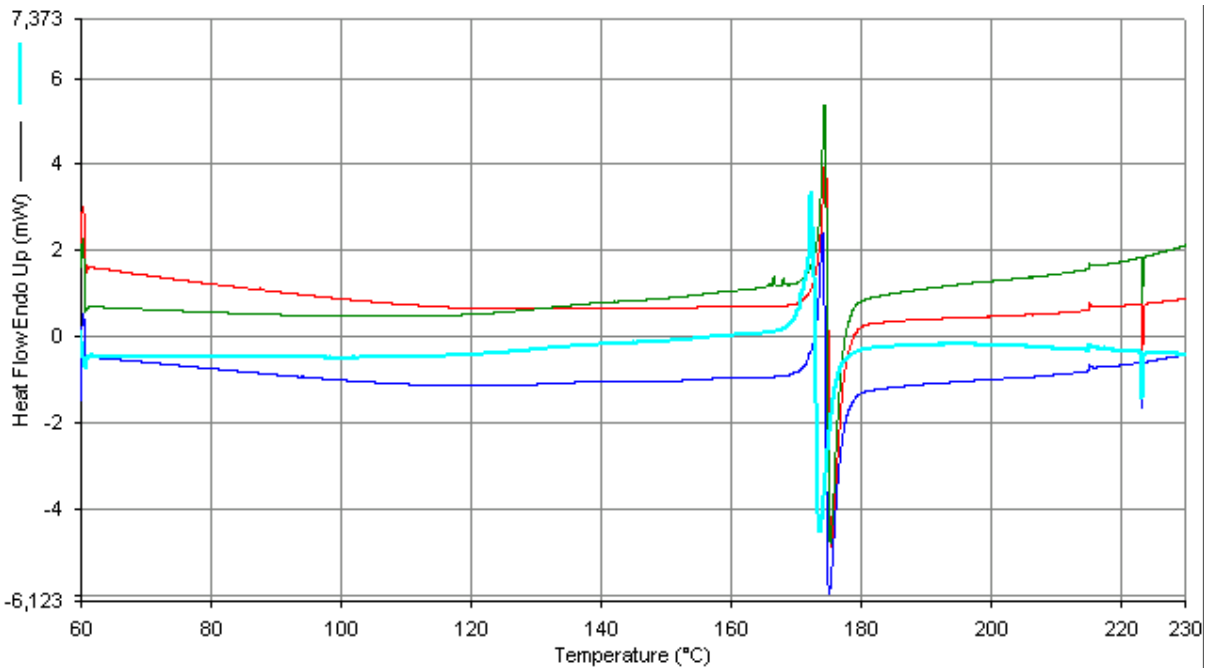


Figure 5.3. DSC thermogram with heating rate of 2.5°C/min of lansoprazole Cipla and lansoprazole USP standard (light blue)

Lansoprazole was further investigated by thermogravimetric analysis, which reveals a weight loss upon heating of the sample. The total weight loss is the difference between the initial weight and the final constant weight of the dry solid. The temperature, at which weight loss began, depended on the heating rate applied (see Figure 5.4). Two regions were observed in the obtained TGA profiles. The first region, assuming that the only volatile component is water, should present a weight loss connected with the water content and its starting point depended on the heating rate applied. At the lowest applied heating rate of 1°C/min the weight loss started at around 70°C and reached a weight loss of 6% until the inflection point. For the highest heating rate applied (10°C/min) presented in the Figure 5.4, water loss region was not

observed. According to the results of Karl Fisher titration, sample of lansoprazole contained 0.06% of water, so the weight loss found at the 1°C/min, may not be related to water loss, but to the weight loss of substance caused by early decomposition.

The second region of TGA curve presented a decomposition of the product. From the Figure 5.4 can be seen that heating the sample up to the temperature of 300°C a considerable weight loss of substance from 27% up to the 53% of weight occurred, which confirmed a decomposition of the substance. For lower heating rates, weight loss began at lower temperature (around 177°C for 1°C/min) and reached higher values of weight loss (53% for 1°C/min) comparing to the values obtained with the higher heating rates (190°C for 20°C/min heating rate with weight loss of 27%).

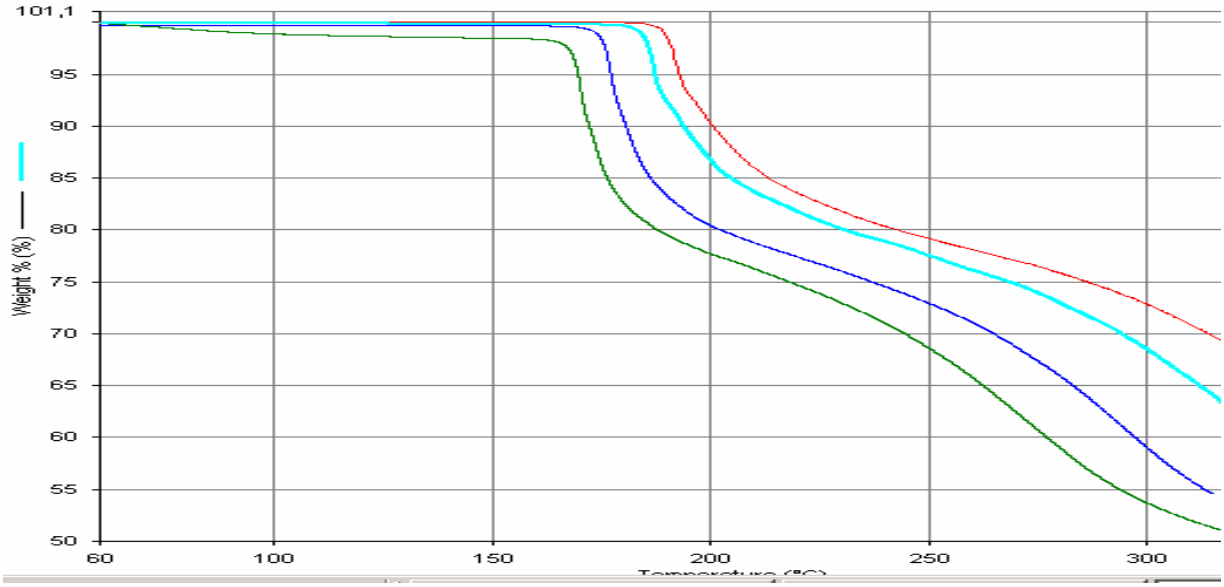


Figure 5.4. TGA curve of lansoprazole: heating range from 60°C to 350°C, with 1°C/min-green line, 2.5°C/min-dark blue, 10°C/min-light blue line and 20°C/min red-line

For comparison of thermogravimetric results with the values obtained with DSC (see Figure 5.5) temperature of 1% weight loss was determined.

For the lower heating rates (2.5°C/min, 5°C/min, 10°C/min and 20°C/min) the temperature of 1% weight loss is almost identical to the onset temperature of exothermic event obtained with DSC. From the Figure 5.5 can be seen that for the higher heating rates of 30°C/min and 40°C/min, a small difference in temperature values of 1% weight loss from the onset values of DSC exotherm is observed.

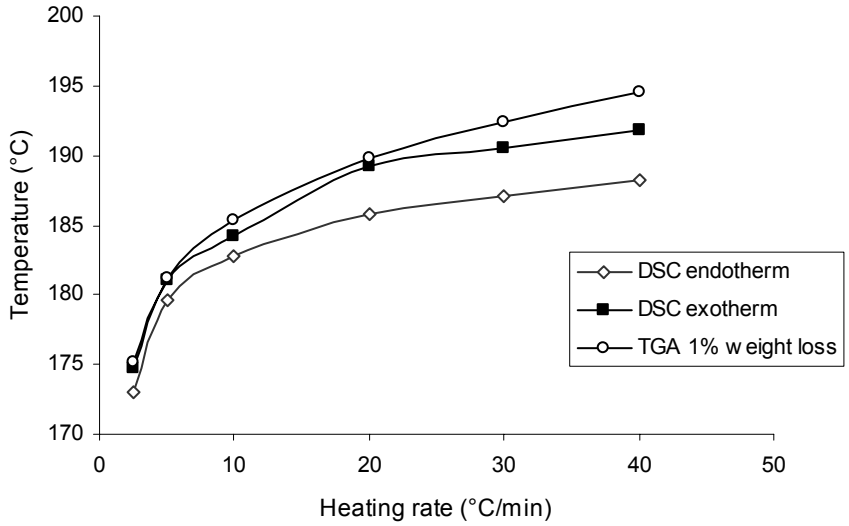


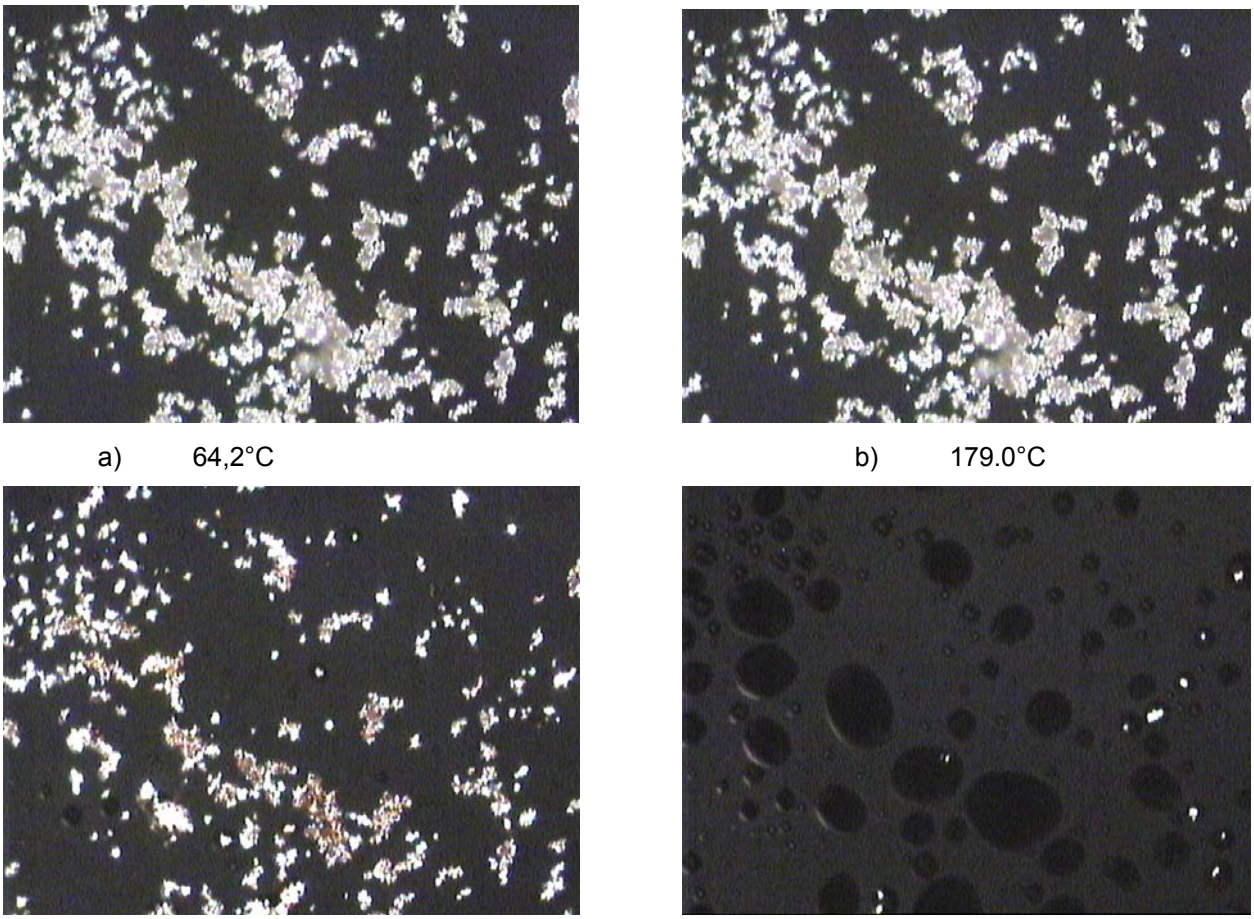
Figure 5.5. Comparison of the DSC onset temperatures with the values of 1% weight loss measured with TGA

In the case of the onset values of endothermic peak, the temperature of 1% weight loss showed smaller difference on lower heating rates (2.5°C/min, 5°C/min and 10°C/min), but the difference become bigger with the increase in the heating rate.

The 1% weight loss, as a part of decomposition process**,** starts at the higher temperature than the melting of the substance (presented in Figure 5.5) and corresponded to the onset values of exothermic event obtained with DSC. Since the temperature of 1% weight loss is higher than the onset temperature of endothermic peak, even at the low heating rates, presence of a sharp symmetric peak at all heating rates can be explained.

Possible decomposition of the substance prior to reaching the onset temperature of melting, and formation of eutectic of lansoprazole and its degradation products on low heating rates, could be an explanation of the melting point depression, as it was observed for omeprazole by Rosenblatt et al., 2005. In addition to this assumption, goes the fact that the smallest difference between the onset temperature of melting and temperature of 1% weight loss was observed at low heating rates of 2.5°C/min, 5°C/min and 10°C/min (see Figure 5.5).

Melting behavior, which was visually observed with the hot-stage microscope, found the melting temperature of LSP to be smaller than the one obtained with the DSC. The result was not surprising since the hot-stage microscopy presents only a visual tool for investigation of thermal properties. Therefore, it is subjected to variability prone to inter-individual observation.



a) 64,2°C b) 179.0°C

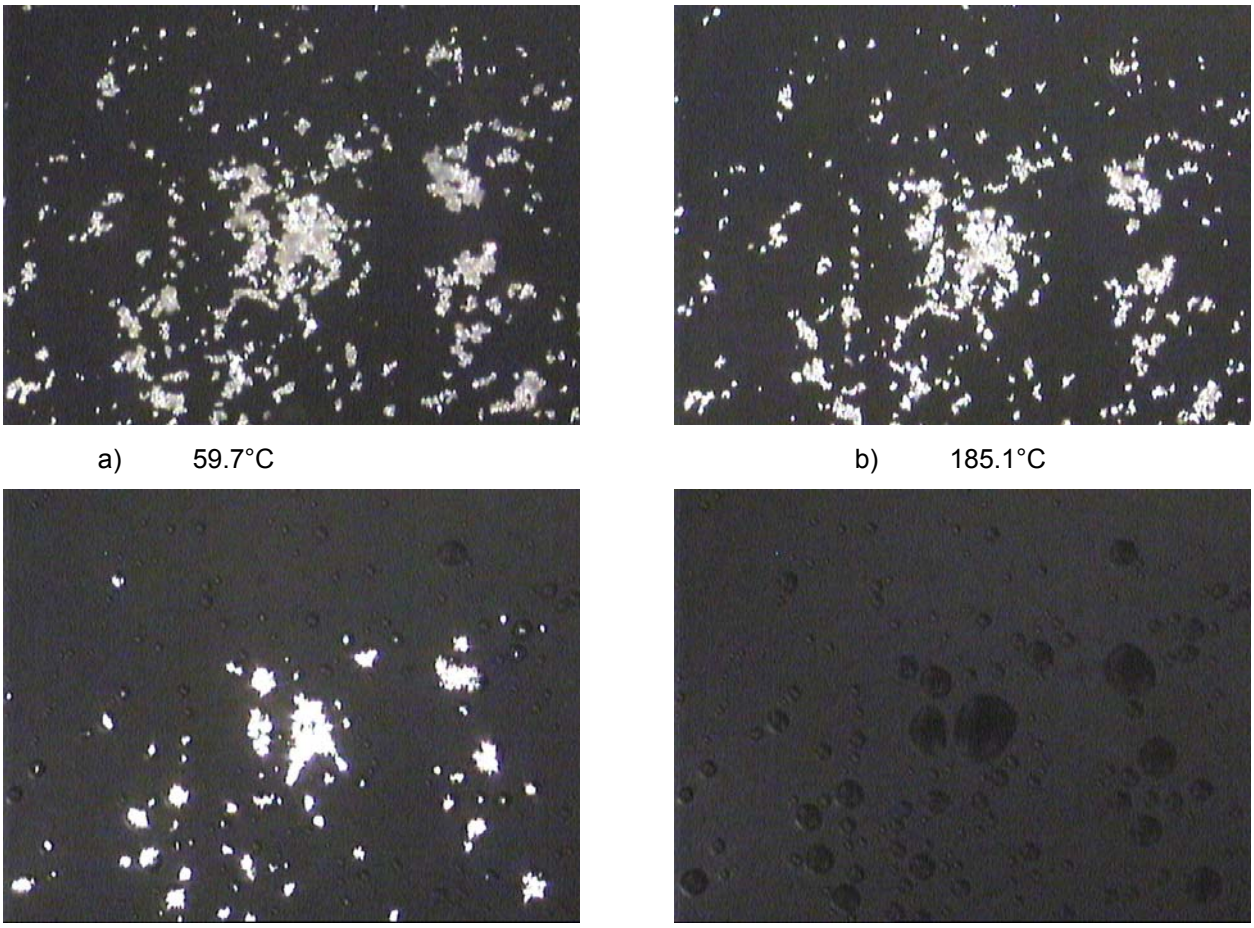
c) 182.4°C d) 183.7°C

Figure 5.6. Melting and decomposition of lansoprazole observed by Hot-stage microscopy with applied heating rate of 5°C/min: a) unheated sample, b) beginning of melting, c) melting of the substance with decomposition and d) end of melting

HSM confirmed that the endothermic event observed on DSC corresponded to the melting of the substance. In the Figure 5.6 a process of melting and decomposition of lansoprazole at the heating rate of 5°C/min is presented. It can be seen that at the same time with the melting, decomposition of the substance occurred, what can possibly be seen as liquefaction and darkening of the crystals (Figure 5.6, C). The process of decomposition prior to melting was even more pronounced when the lower heating rates of 1°C/min and 2.5°C/min were applied.

In the case when the higher heating rate was applied (40°C/min) process of melting presented in the Figure 5.7 did not reveal presence of simultaneous process, since no darkening of the crystals was evident.

Melting point of lansoprazole was also determined using a simple capillary method described in European Pharmacopoeia with Kofler bench (Mettler Toledo DR45). Repeated measurement of three samples gave the value of 166°C. Apparatus automatically registers the melting point and for this reason influence of the operator is excluded.



a) 59.7°C b) 185.1°C

c) 187.0°C d) 188.4°C

Figure 5.7. Melting and decomposition of lansoprazole observed by Hot-stage microscopy with applied heating rate of 40°C/min: a) unheated sample, b) beginning of melting, c) melting of the substance and d) end of melting

Using the combination of DSC, TGA and Hot-stage microscopy it could be confirmed that the endothermic event obtained by DCS measurement corresponded to reported literature melting point range by O`Neill et al., 1996, and exothermic event can be assigned to decomposition process. Applied heating rates beyond 20°C/min leave no space for formation of eutectic and shift of the melting peak became less pronounced.

In conclusion, a very dynamic method and exactly standardized measurement conditions, particularly with regards to heating rate (e.g., in DSC), have to be employed to enable reliable determination of a melting point of these decomposable substances. Even though lansoprazole melting point was highly influenced by heating rate, higher heating rates should be employed since they give lower variability of results (Table 8.1 and Table 8.2 in Appendix). Presence of exothermic peak at 102°C, characteristic for polymorphic form B reported in the work of Kotar et al., 1999, was not observed on all applied heating rates, and for this reason it can be concluded that LSP had polymorphic form A.

5.4. Powder characterization of drug substance

The powder characteristics, including values for particle size, different densities (bulk, tapp and true density), and specific surface area are presented in Table 5.3.

Table 5.3. Lansoprazole powder characteristics

Characteristic of powder	*Obtained values ± RSD (%)*
Particle size (μm) (n=3)	
d (0.1)	1,298 ± 0.36
d (0.5)	3,752 ± 0.55
d (0.9)	9,129 ± 0.32
True density (g/cm^3) (n=3)	1.509 ± 0.08
Bulk density (g/cm^3) (n=3)	0.385 ± 0.75
Tapp density (g/cm^3) (n=3)	0.505 ± 0.66
Hausner ratio	1.312
Carrs index (%)	23.76
Specific surface area (m^2/g) (n=3)	1.458 ± 12.27

Even though the sizing of some powders can be fraught with problems (like nonreproducible results caused by cohesive nature of powders, poor control of ambient humidity, variable rate of introduction of particles, etc.) reported by Heng and Chan, 1997, and the measurement using the wet method is more preferred (Randall, 1995) dry powder feeder was used. Dry powder laser diffraction method for particle size measurement, which was proposed by producer of the substance, was determined to be more appropriate than the wet laser diffraction method, since the wet laser diffraction method showed a presence of agglomerates (see Figure 5.8). The air flow stream, which was used as a carrier in dry laser diffraction method, seemed to be more efficient in breaking the agglomerates present in the sample.

From Table 5.3 it can be seen that 90% of drug substance had particle size under 9 μm, while 50% of drug substance had particle size under 4 μm. In order to have a successful drug layering, the suspended material must be small in relation to the size of the substrate onto which it is being applied or the potency will be compromised, the overall yield will be low, a higher binder content may be needed and the resulting surface will be rough and porous. Particle size of the drug substance in relation to the substrate has to be 1:50 in order to have a good potency and yield (Jones, 2005b). Li et al., 1989, emphasized the importance of particle size and overall particle size distribution of drug on the yield and surface of indomethacin pellets

prepared with solution layering. They found that the finer powder under 10 μm gives the higher yield and smoother surface of pellets.

Considering the SEM photographs (see Figure 5.9) low particle size and presence of agglomerates were confirmed. Lansoprazole particles appeared to be aggregates composed of plate-like crystals of equivalent size.

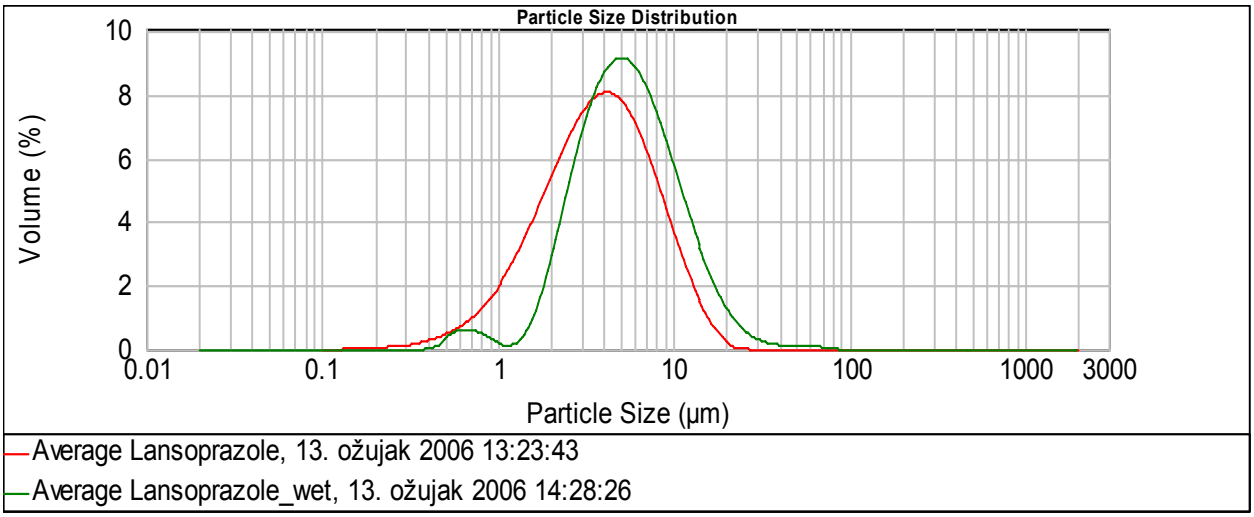
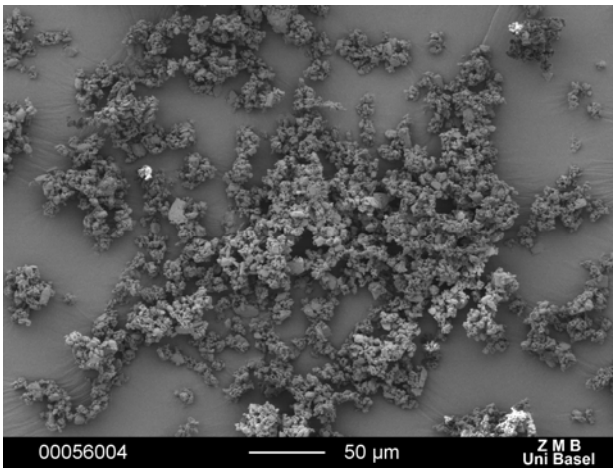


Figure 5.8. Particle size distribution of lansoprazole powder measured with dry and wet laser diffraction method

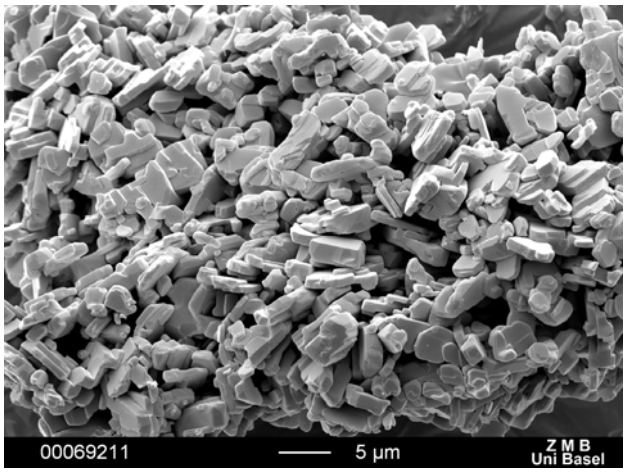
Measurement of bulk density and specific surface area is very sensitive to sample preparation, especially if the powder used has a small particle size like lansoprazole. The flowability of the powder is related to the particle size and shape. Hausner ratio and Carrs index are measures of the relative importance of interparticulate interactions. In a free-flowing powder, such interactions are generally less significant, and the bulk and tapped densities will be closer in value. For poorer flowing materials, there are frequently greater interparticle interactions, and a greater difference between the bulk and tapped densities will be observed (USP, 2006a).

From the SEM pictures (see Figure 5.9) and the values obtained for densities, according to the classification by Wells, 1988, for Hausner ratio and Carr classification for Carr's index powder can be characterized as a poorly flowable. A Hausner ratio of less than 1.20 indicates good flowability of the material, whereas a value of 1.5 or higher suggests a poor flow (Wells, 1988). The Carr index values of 5-10, 12-16, 18-21, and 23-28 indicated, excellent, good, fair and poor flow properties of the material, respectively.

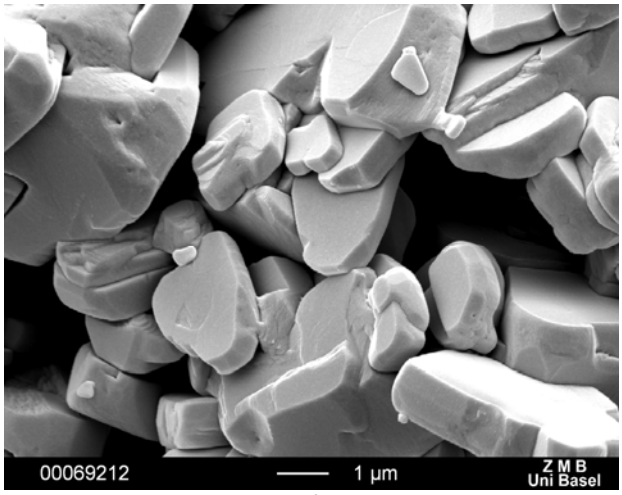
From characteristics of the powder it can be concluded that the drug layering onto the neutral core could be conducted using solution/suspension technique, while powder layering technique could be compromised with low flowability of the drug, if the process of drug addition is not optimized.



a)



b)



c)

Figure 5.9. SEM photographs of surface of lansoprazole powder a) magnification x300 b) magnification x3000 c) magnification x10000

5.5. Properties of sugar and MCC neutral pellets

Substrate morphology and porosity plays and important role in the process of coating of multiparticulate systems. Higher substrate surface requires a higher quantity of deposited material to achieve the same action as a substrate with lower surface value (for example: dissolution).

The properties of neutral pellets (sugar and MCC) including values for particle size, true density, specific surface area and porosity are presented and summarized in Table 5.4.

From the summarized properties of neutral pellets (Table 5.4) and SEM pictures (see Figure 5.11 and Figure 5.10) it can be seen that the neutral pellets differed in size, shape and surface

properties. Sugar pellets were bigger in size and more spherical in shape. More than 50% of sugar pellets had a size around 981 µm, while 50% of MCC pellets had 834 µm.

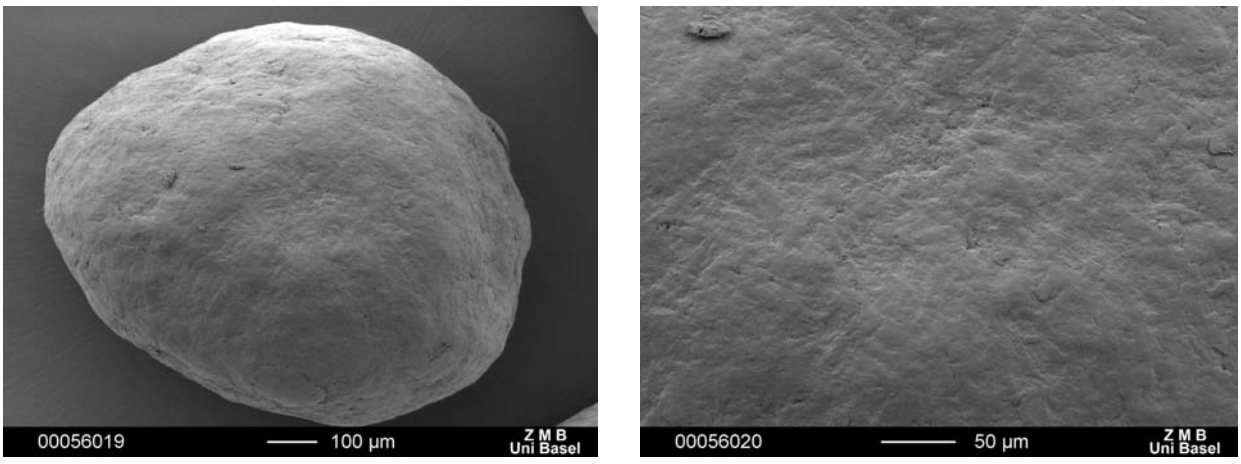
Table 5.4. Properties of neutral pellets (n=3)

Property of pellets (n=3)	*Obtained values ± RSD (%)*	
	Suglets®	*Ethispheres®*
Particle size (µm)		
d (0.1)	721.21 ± 0.21	618.23 ± 1.22
d (0.5)	981.04 ± 0.24	876.30 ± 1.28
d (0.9)	1347.63 ± 0.23	1241.59 ± 1.09
Specific surface area (m^2/g)	2.09 ± 1.44	0.50 ± 16.97
True density (g/cm^3)	1.52 ± 0.023	1.45 ± 5.26
Porosity (%)	12.74 ± 0.39	15.33 ± 12.31

According to the porosity values obtained for sugar and MCC pellets, neutral sugar pellets had lower porosity of 12.74% compared to MCC pellets which had porosity of 15.33%. The porosity of core particles is more critical in aqueous coating processes with latexes, while with the organic coating solution there is a good chance of filling the pores and stabilizing the surface area in the first phase of coating process (Lehman, 1994).

Specific surface area of MCC pellets appeared to be significantly lower than the specific surface area of sugar pellets. This value correlated with the values obtained for particle size and porosity, since both of these properties contribute to the surface area.

From the SEM pictures MCC pellets (Figure 5.10) also seemed to be spherical and smoother than the sugar pellets, from a visual point of view (see Figure 5.10, A).



a) b)

Figure 5.10. SEM photographs of MCC spheres: a) surface, magnification x100, b) surface, magnification x300

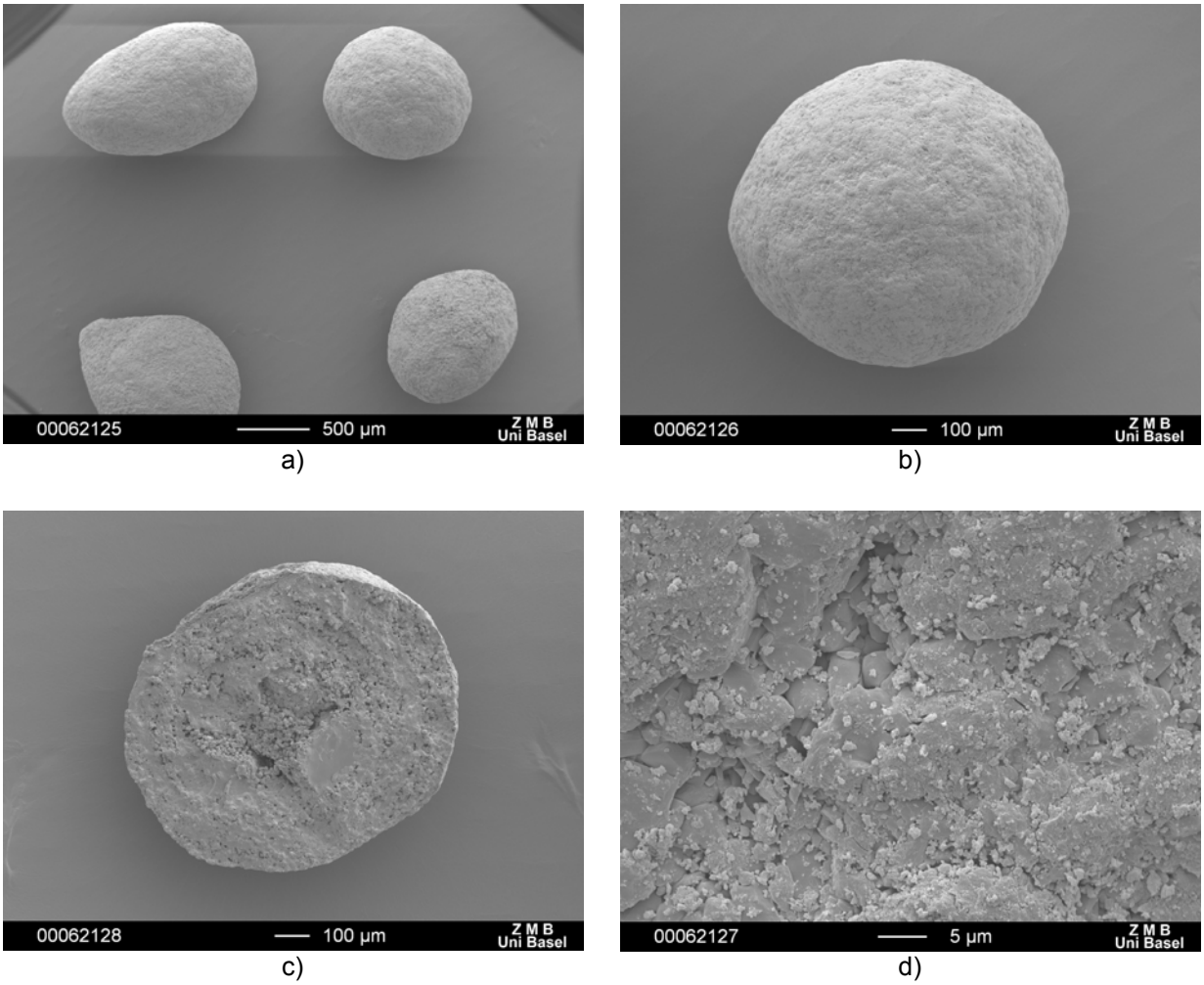


Figure 5.11. SEM photographs of sugar spheres: a) surface, magnification x30, b) surface, magnification x70, c) cross section, magnification x70, d) surface, magnification x2000

During the active layering phase of neutral pellets in solution suspension technique of pellet preparation, sugar pellets were more sensitive to the spray rate than the MCC pellets. Lower spray rates had to be applied until the first layer was formed since pellets became sticky. The reason for this behavior could be found in sucrose being soluble in water, since the aqueous solution of binder was used. At the beginning of the coating process of MCC pellets in the fluidized bed, electrostatic charge was formed until the core was sufficiently moistened.

5.6. Dissolution of pellets with shellac as enteric coating polymer

In order to decide on the type and the coating level of enteric polymer necessary to obtain gastric resistance, pellets were coated with aqueous shellac dispersion containing 10%, 15% and 20% of solid shellac polymer. Gastric resistance and dissolution of lansoprazole from shellac coated pellets in 0.1N HCl and phosphate buffer pH 6.8 is presented in Figure 5.12.

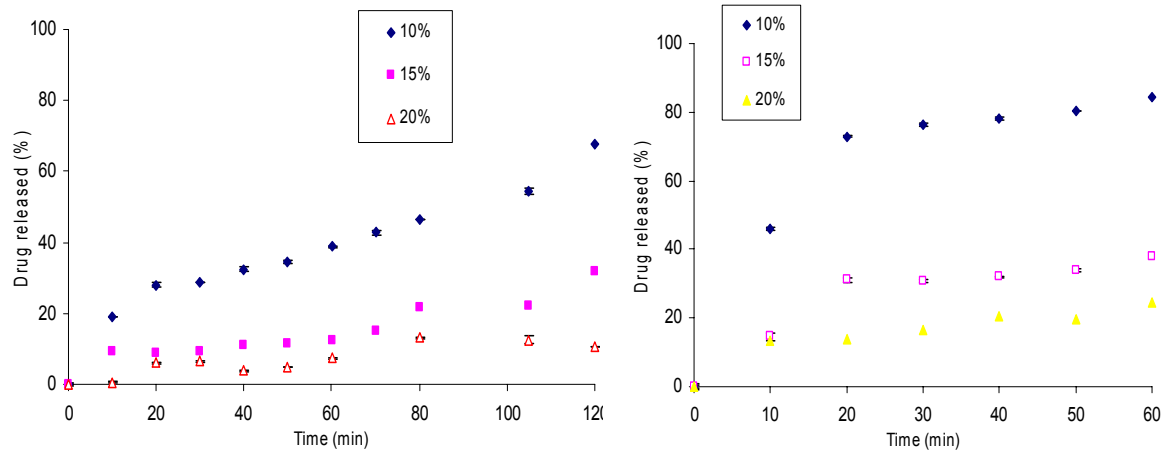


Figure 5.12. Dissolution profiles of Shellac coated pellets (Run 1): a) untreated pellets in pH 1.2 and pH 6.8, b) cured pellets (40°C, 8 hours) in pH 1.2. The bars represent standard error of the mean (n=6)

Higher coating levels of solid shellac resulted in better gastric protection of the drug, but release of the drug in the buffer stage could not be achieved. Lower coating levels could not prevent the drug release in acid media, but the drug release in buffer media was higher. As it is shown in the Figure 5.12 a), coated pellets containing 10% and 15% of solid shellac could not prevent the release of lansoprazole in simulated gastric media with pH 1.2. Pellets containing 10% of enteric coating polymer released 38.7%, while pellets with 15% coating level released 12.2% of lansoprazole. Only pellets containing 20% w/w of shellac were able to meet the requirements of US Pharmacopoeia for lansoprazole delayed release ($t_{60min} \leq 10\%$) with the 7.4% of drug release in the 0.1N HCl. The reason for this high release in pH 1.2 could be explained by the presence of HPMC, a water soluble excipient which acts as a pore former (described in Chapter 4.3.2.1). Qussi and Suess, 2005, demonstrated that incorporation of 25% w/w of HPMC in the shellac/plasticizer coating solution increases porosity tenfold and the coating system could not prevent release of the drug in the acid media, but the drug release in pH 7.4 was complete. On the other hand, none of the coated pellets showed instant and complete drug release in the phosphate buffer media with the pH of 6.8 ($t_{60min} \geq 80\%$). Our result was contrary to the literature data. In the literature, Pearnchob and Siepmann, 2003, obtained a gastric protection with water soluble salt of shellac at a coating level of 10%, also achieving a rapid drug release in pH 6.8.

Influence of thermal treatment on dissolution of shellac coated pellets was further investigated, even though a high concentration of plasticizer has been used. The curing procedure is often used to achieve complete film formation and to avoid changes in drug release during storage due to a further coalescence of particles, decrease in polymer mobility and reduction of free volume. While the majority of published studies (Pearnchob and Bodmeier, 2003; Siepmann et al., 2008) refer to curing as a procedure which slows drug release, in the case of lansoprazole pellets coated with shellac curing had an opposite effect. Curing time for 8 hours on 40°C on a

tray doubled the amount of drug released compared to untreated pellets (Figure 5.12, a). Pellets with 10%, 15% and 20% of solid shellac released (t_{60min}) 84, 38 and 25% of drug in acid media, respectively. Possible explanation of the curing result could be that curing caused a leakage of the enteric coating and migration of lansoprazole through the pores to the surface of pellets, resulting in an increase of drug release (Figure 5.12, b), since it is reported by Bruce et al., 2003, that depending on the drug solubility in the polymer drug can migrate into the coating.

The results showed that the coating level is an important parameter and that low coating level can result in release of drug in acidic media, while high drug loading can lengthen the dissolution time in the intestinal area. Slow release of lansoprazole from shellac coated pellets in pH 6.8 could be attributed to the fact that the pKa value of shellac lies in the range of 6.9 – 7.5 (Qussi and Suess, 2005). Possibly, highest coating level applied in this study (20% of solid polymer) with the 33% w/w of hydrophilic polymer could lead to a complete dissolution of drug in the buffer pH 7.4.

Therefore no further investigation has been conducted in order to optimize the enteric coating formulation of shellac for dissolution in pH 6.8. Even though, the study of Riedel and Leopold, 2005, showed that shellac, applied as enteric coating polymer had the lowest influence on benzimidazoles, omeprazole, another type of enteric polymer had to be chosen to achieve gastric resistance of pellets.

5.7. Influence coating level of Eudragit L 30 D-55 on properties of pellets

Obtained SEM photographs of pellets coated with different levels of anionic copolymer based on methacrylic acid and ethyl acrilate (1:1) (Eudragit L30 D-55) are shown in Figure 5,13.

The morphology and surface of the pellets is clearly dependable on the type and the amount of applied layer. With the application of the consecutive layers on the surface of sugar pellets, surface became smoother and sphericity was maintained (see Figure 5,13).

Increase in particle size of pellets coated with different amount of enteric coating polymer is presented in Table 5.5.

Table 5.5. Particle size of lansoprazole pellets with different coating level of Eudragit L30 D-55 (n = 3)

% (w/w) of solid enteric polymer	*d (0.1) ± RSD (%)*	*d (0.5) ± RSD (%)*	*d (0.9) ± RSD (%)*
20	832.66 ± 0.72	1118.42 ± 0.65	1485.76 ± 0.53
22	850.78 ± 1.57	1140.63 ± 1.49	1510.28 ± 1.33
24	854.72 ± 0.24	1145.87 ± 0.19	1516.4 ± 0.13
26	852.64 ± 1.25	1143.82 ± 1.36	1513.14 ± 1.23

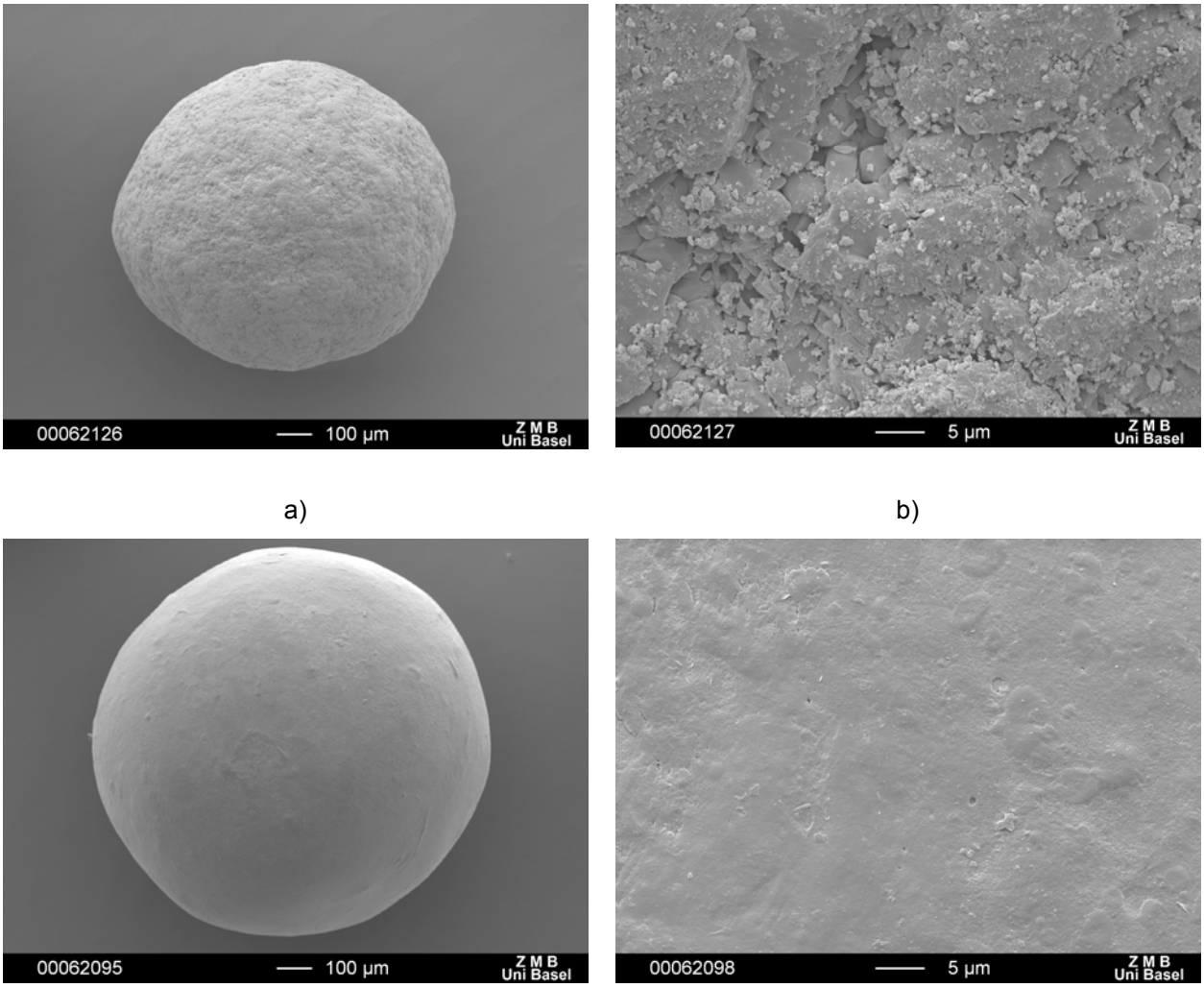
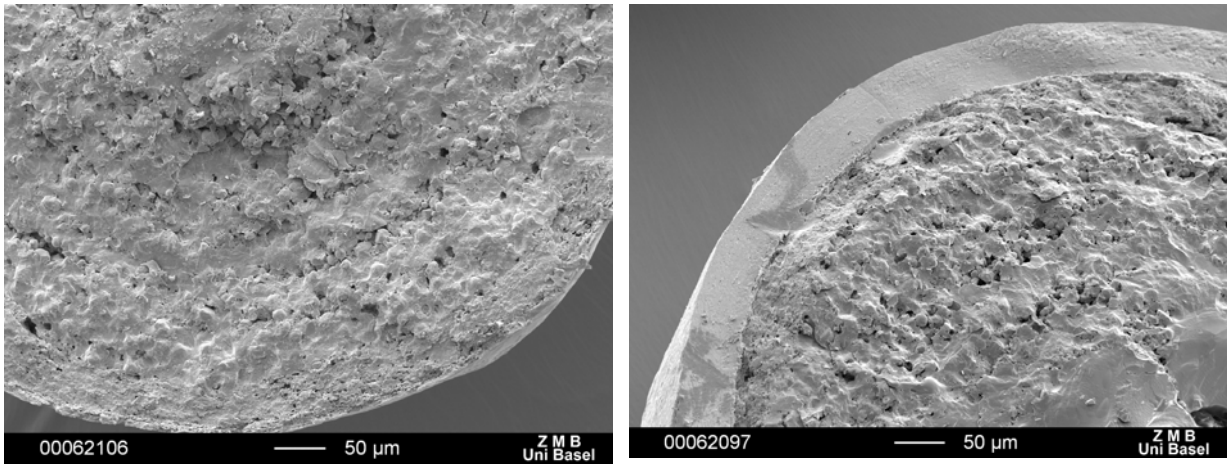


Figure 5.13. SEM photographs of surface of neutral sugar pellet magnification a) x70 and b) x2000, surface of lansoprazole pellet with pellet with 26% of enteric coating magnification c) x70 and d) x2000

Increase in size from the neutral sugar pellet to the enteric coated pellets containing 20% of enteric polymer was approximately 130 µm (12.3% increase in size) and further addition of successive layers up to 26% of solid enteric coating polymer led to increase of 163 µm (14.3% increase).

According to the literature data, it is necessary to apply films of approximately 20 to 30 µm thickness to achieve a mechanical stability and enteric resistance (Lehman, 1994).



a) b)

Figure 5.14. SEM picture of cross section of a) sugar neutral pellet and c) lansoprazole pellet with 26% of solid enteric polymer with magnification of x200

Values for the porosity, apparent density and average pore diameter obtained by mercury porosimeter are presented in the Table 5.6. Results showed that all tested pellets, regardless of the percentage of applied enteric coating polymer, had pores within the same range of size and the only significant difference in the cumulative volume of intruded mercury has been observed between pellets coated with 26% (solid enteric polymer) and pellets with lower quantity of methacrylic acid copolymer. Pellets containing 26% of solid enteric polymer had the lowest average pore diameter of 16.1 nm.

Table 5.6. Experimental data obtained using mercury porosimeter

Enteric coating (% of solid polymer)	*Average pore diameter (nm) ± RSD (%)*	*Bulk density (g/ml) ± RSD (%)*	*Apparent (skeletal) density (g/ml) ± RSD (%)*	*Porosity (%) ± RSD (%)*
20	17.9 ± 0.64	1.1487 ± 2.27	1.284 ± 2.34	10.55 ± 4.71
22	18.1 ± 3.36	1.1164 ± 1.03	1.252 ± 1.36	10.81 ± 3.78
24	17.9 ± 4.74	1.1237 ± 0.70	1.249 ± 0.65	10.05 ± 0.94
26	16.1 ± 1.29	1.1210 ± 1.76	1.226 ± 1.27	8.61 ± 5.4

Incremental intrusion volumes were plotted against pore diameters that represented pore size distribution. Figure 5.15 shows the pore size distribution of pellets with increased enteric polymer coating level. The figure demonstrates that no change in pore distribution was observed with the increase in coating level from 20% to 24%, while the mean pore diameter decreased with the application of 26% of enteric polymer.

In Figure 5.16, the total intrusion volume was plotted against pore diameters showing the intrusion profile of pellets with different coating levels. The intrusion volume of mercury was significantly lower for the pellets coated with the 26% than the 20, 22 and 24% (w/w calculated

on solid polymer) of enteric polymer. Porosity was significantly affected with addition of enteric polymer at coating level higher than 24% (w/w calculated on solid polymer).

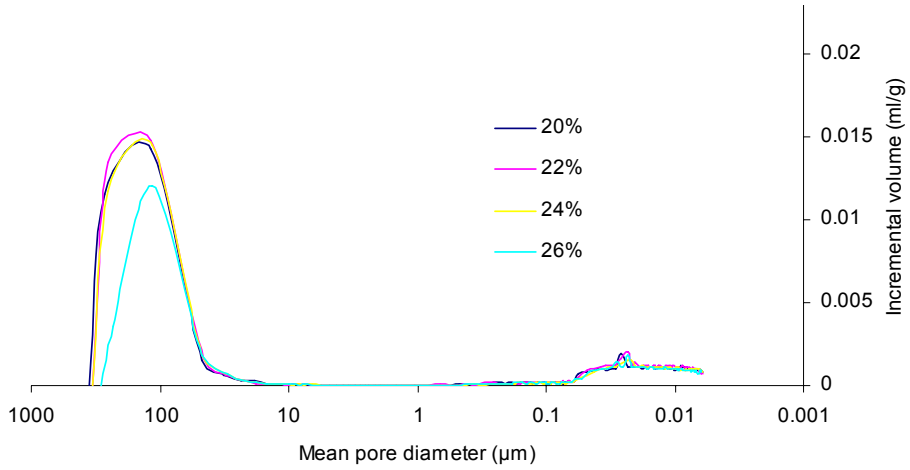


Figure 5.15. Pore size distribution of pellets with different coating levels of enteric polymer

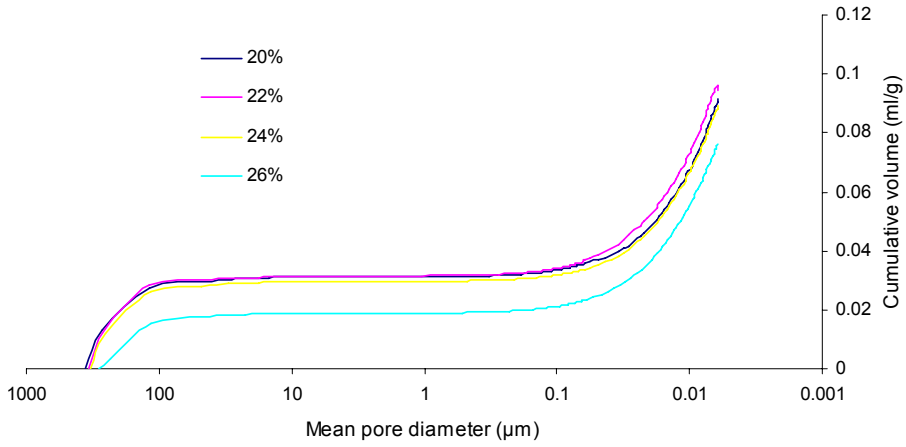


Figure 5.16. Cumulative intrusion volume vs. pore diameter of pellets with different coating levels of enteric polymer

In addition to the physicochemical properties of drug, the coating level can play an important role in determining the release rate of the drug from the coated pellets. The lansoprazole release in pH 6.8 from Eudragit L30 D-55 coated pellets decreased with increasing coating level because of the increased diffusion path length at higher coating level. The release profiles were sigmoidal in shape, with the lag phase presenting the gastric resistance of pellets, and did not differ significantly ($p<0.05$). As expected, pellets containing 20% of solid enteric polymer showed a faster release rate than those containing 26% of solid enteric polymer.

All four formulations of pellets containing from 20% (w/w) up to 26% (w/w) of solid enteric polymer showed release profiles which were according to the criteria stated in the USP (see Figure 5.17). USP is stating that no more than 10% release of lansoprazole in one hour in 0.1 N HCl and no less than 80% release after one hour in phosphate buffer pH 6.8 is allowed. Pellets containing 20% of solid enteric polymer showed highest and the fastest release in pH 6.8, with the drug release of 100.4% (Figure 5.17), while pellets containing 24% had the slowest and the lowest release (96.8%).

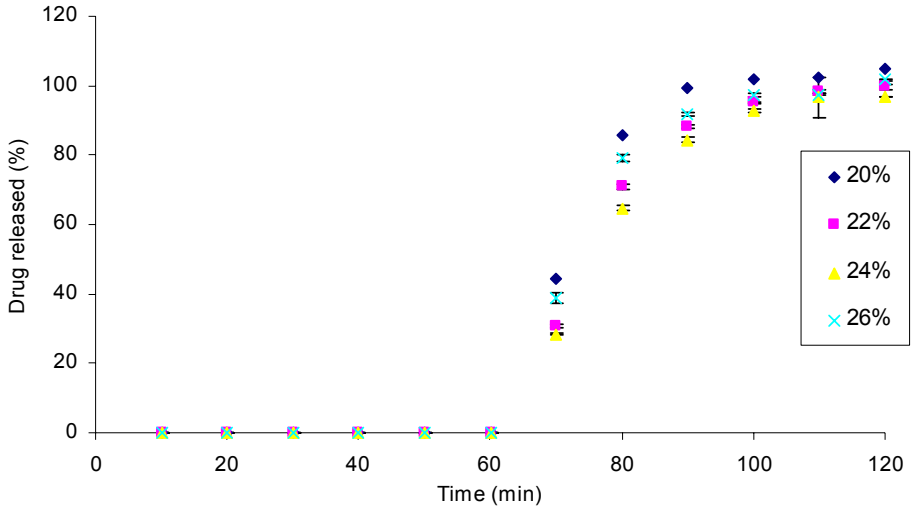


Figure 5.17. Effect of coating level on the lansoprazole gastric resistance in 0.1N HCl and dissolution in phosphate buffer, pH 6.8. The bars represent standard error of the mean (n=6)

Eudragit L30 D-55 belongs to a group of polymers which are only conditionally gastroresistant around or below pH 5. Since in-vivo stomach pH for PPI patients on a multiple dose regimen is higher (>pH 4) than the official pH in USP monograph for the enteric resistance testing, pellets were tested in the modified bio relevant media which better simulates the gastric environment. Gastric resistance of pellets in the modified acid media of pH 4.5 is presented in Figure 5.18. Gastric resistance ($t_{60min} \leq 10\%$) was achieved for all coating levels in the modified acid stage medium with pH 4.5. Release profiles were not significantly different ($p<0.05$).

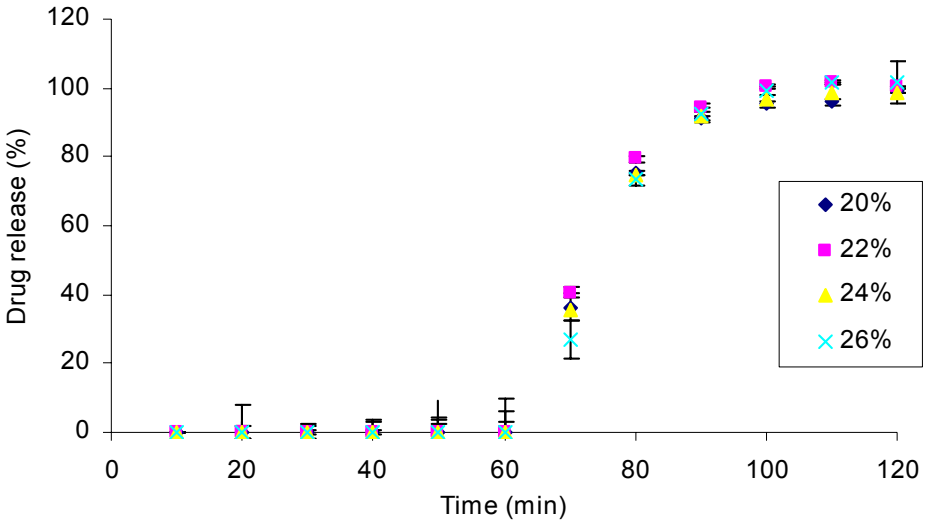
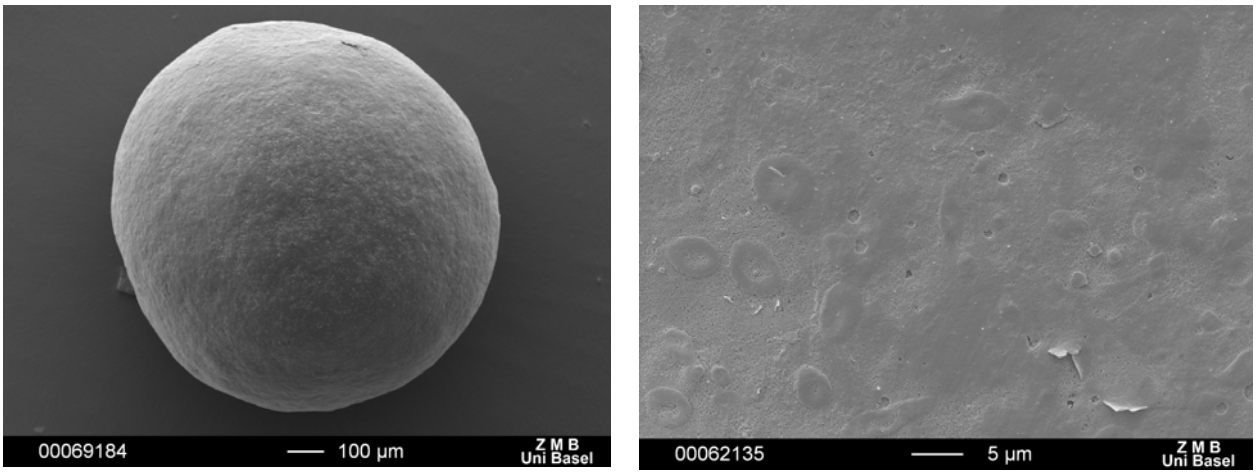


Figure 5.18. Effect of coating level on the lansoprazole gastric resistance in modified gastric media, pH 4.5 and dissolution in phosphate buffer, pH 6.8. The bars represent standard error of the mean (n=6)

From the results can be concluded that the porosity of pellets in the range of 8.6 to 10.8% did not play an important role in the gastric resistance of pellets in both tested acid stage media. For further studies coating level of 20% of enteric polymer was used for the reasons of time investment (reduced processing time) and lower costs.

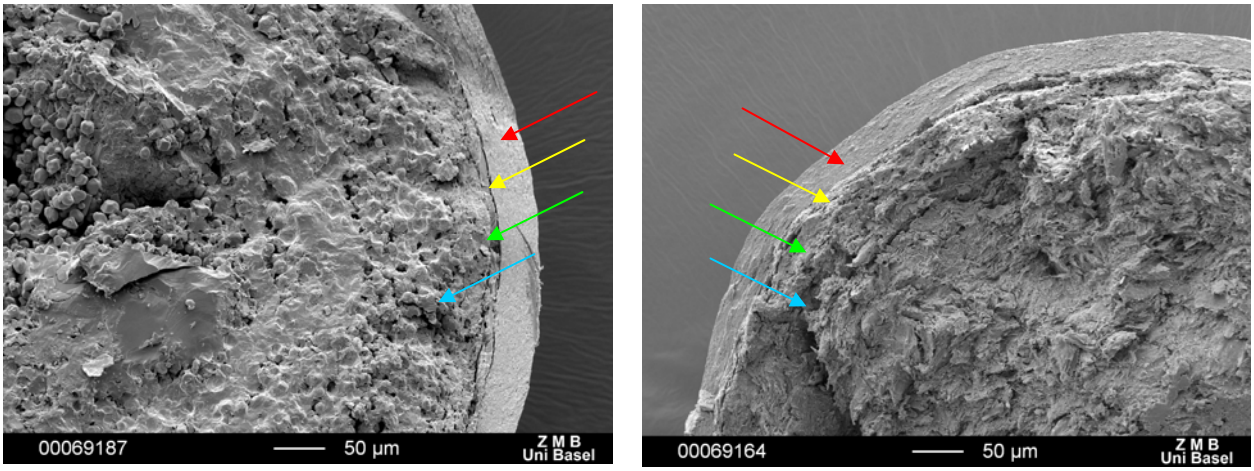
5.8. Properties of lansoprazole pellets prepared with solution/suspension layering

Observed visually, the pellets were spherical and intact in shape, and no obvious surface defects could be observed in either one of the process stages (Figure 5.19 and Figure 5.20).



a) b)

Figure 5.19. SEM photographs of lansoprazole pellets of a) surface from run 1 with magnification x70 and b) surface from run 1 with magnification x2000



a) b)

Figure 5.20. SEM photographs with magnification x200 of cross section of lansoprazole pellets with active, protective and enteric coating containing a) sugar as neutral core from run 1 and b) MCC as neutral core from run 6. Arrows point the layers.

It was observed that the pellets from runs 3, 4, 7 and 8 (Table 4.1) after application of the enteric coating have changed the color form yellowish white to light beige, which was not the case with the pellets from other runs. Color change from pale yellowish white to dark reddish brown with the decrease in lansoprazole content has been observed by Tetsuro et al., 1992, when lansoprazole was stored on 40, 50 and 60°C for 3 months. DellaGreca et al., 2006, have investigated the chemical behavior of lansoprazole in aquatic environment, but failed to characterized it, assuming that it consists of a mixture of very liable degradation products. Stroyer et al., 2006, investigated stability and discoloration of physical mixtures of the benzimidazole, omeprazole, and enteric polymers on 40°C and 75% RH for 1 month. Mixtures stored at ambient temperature over desiccant showed no discoloration, while mixtures stored at accelerated conditions showed discoloration at different extend. From the previously mentioned studies discoloration of lansoprazole in pellets could be connected with the absence of the protective coating, even though they contained a stabilizing agent. Present alkaline agents did not stabilize lansoprazole sufficiently and the degradation of lansoprazole under the influence of free carboxyl groups of enteric polymer occurred.

Because the neutral pellets were uniform in size it was expected that the distribution of layered pellets will also be narrow, since it is one of the requirements to have a good reproducibility of in vitro release. Increase in size for sugar pellets containing active, protective and enteric coating was approximately 16% (Table 5.7). For MCC neutral pellets, the same quantity of polymer resulted in smaller increase in size, around 10% to achieve pellets containing active, protective and enteric coating. Decrease in particle size of neutral pellets leads to the significant increase in the surface area available for polymer deposition, and therefore the same quantity of polymer applied on pellets gives layers of different thickness. Study of Wesdyk et al., 1990,

demonstrated that the film thickness of various size of beads coated in fluidized bed is not uniform and the larger beads received a thicker film than did the smaller beads.

It can be seen that the d(0.5) size (Table 5.7) for pellets with the same core, containing the same layers, were comparable, meaning that the applied layers were uniform in thickness (run 1 and run 5, run 3 and run 7 for sugar core pellets; run 2 and run 6, run 4 and run 8 for MCC core pellets).

Table 5.7. Particle size of lansoprazole pellets prepared with solution/suspension layering

Run	*d (0.1) ± RSD (%)*	*d (0.5) ± RSD (%)*	*d (0.9) ± RSD (%)*
1	838.63 ± 1.59	1125.52 ± 1.54	1490.09 ± 1.40
2	707.18 ± 0.21	961.93 ± 0.08	1326.59 ± 0.10
3	818.22 ± 0.36	1100.70 ± 0.35	1465.75 ± 0.30
4	701.08 ± 0.07	954.34 ± 0.06	1318.13 ± 0.09
5	852.64 ± 0.70	1143.82 ± 0.63	1513.14 ± 0.52
6	712.76 ± 1.25	969.04 ± 1.43	1333.87 ± 1.35
7	816.25 ± 0.02	1098.26 ± 0.004	1463.04 ± 0.01
8	698.79 ± 0.30	951.04 ± 0.24	1313.35 ± 0.19

Using Karl Fischer titration method, different levels of water content in the enteric coated pellets were determined, even though the same process parameters were used. From the Table 5.8 it can be seen that the pellets which contained MCC as neutral core had a higher water content than the pellets containing sugar as neutral core. Pellets from the run 6 had the highest water content of 3.8%, while pellets from the run 7 had the lowest of 1.9%.

Table 5.8. Properties of lansoprazole pellets prepared with solution/suspension layering

Run	*Intermediate porosity (%)*	*pH of slurry of pellets*	*Water content (%) (n=2)*	*True density (g/cm^3) ± RSD (%)*	*Porosity after coating (%) ± RSD (%)*
1	10	8.9	2.0	1.39 ± 0.02	8 ± 20.04
2	14	8.8	2.6	1.34 ± 0.18	13 ± 7.35
3	13	8.8	2.4	1.39 ± 0.04	8 ± 29.00
4	16	8.7	3.4	1.34 ± 0.10	12 ± 16.53
5	10	7.8	2.6	1.36 ± 0.00	8 ± 29.00
6	16	7.6	3.8	1.34 ± 0.05	12 ± 13.14
7	11	7.8	1.9	1.40 ± 0.02	8 ± 19.06
8	16	6.4	2.9	1.34 ± 0.03	14 ± 9.78

Difference in the true density of the pellets containing same composition, with and without protective coating, is observed only between the run 5 and run 7 (see Table 5.8). All other pellets had the same true density and no influence of protective coating on true density was

observed. Pellets containing sugar as a neutral core had a little higher values of true density (1.36 to 1.40 g/cm^3) than the pellets containing MCC (1.34 g/cm^3).

The release profiles of tested pellets were not significanlty different ($p<0.05$) and were sigmoidal in shape with the lag phase presenting the gastric resistance of pellets (see Figure 5.21), while the presence of lag phase in the phosphate buffer has not been observed. Small difference in the size of pellets and the thickness of the coatings did not have a significant influence on release of the drug. All tested pellet formulations showed similar release profiles, which were according to the criteria stated in the USP, see Figure 5.21. USP is stating that no more than 10% release of lansoprazole in one hour in 0.1 N HCl ($t_{60min}\leq 10\%$) and no less than 80% release after one hour in phosphate buffer pH 6.8 is allowed ($t_{60min}\geq 80\%$). After gastric resistance test all formulations showed immediate release of lansoprazole in pH 6.8. The slowest release of drug was observed with pellets from run 2 and run 6, both containing MCC as neutral core and protective coating. These findings were in agreement with the literature, as many publications asserted the advantages of preparation of solid dispersion by deposition of drug onto inert pellets in order to improve the solubility of poorly water soluble drug (Sethia and Squillante, 2004; Leuner and Dressman, 2006; Sun et al., 2007). Study of Chokshi et al., 2007, showed that preparation of drug in a form of solid dispersion also improves wettability of the drug, what in the case of lansoprazole plays an important role since Kristl and Vrecer, 2000, found that the contact angle of lansoprazole is 79.9°, indicating a low wettable substance. In addition, Zhang et al., 2008, confirmed the inhibition of lansoprazole crystalinity and presence of amorphous form in the PVP solid dispersion which improved a dissolution of the drug.

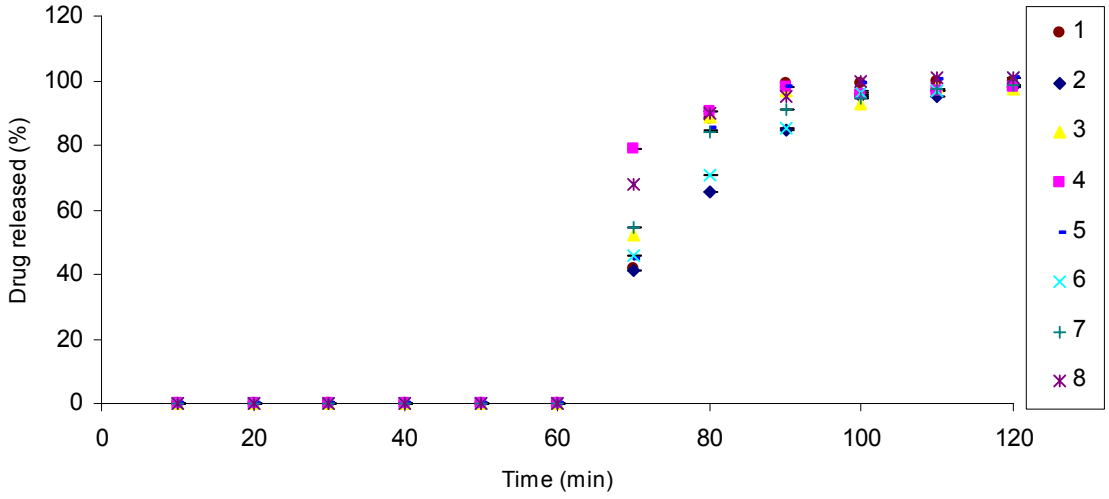


Figure 5.21. Gastric resistance of lansoprazole pellets in pH 1.2 and dissolution in pH 6.8. The bars represent standard error of the mean (n=6)

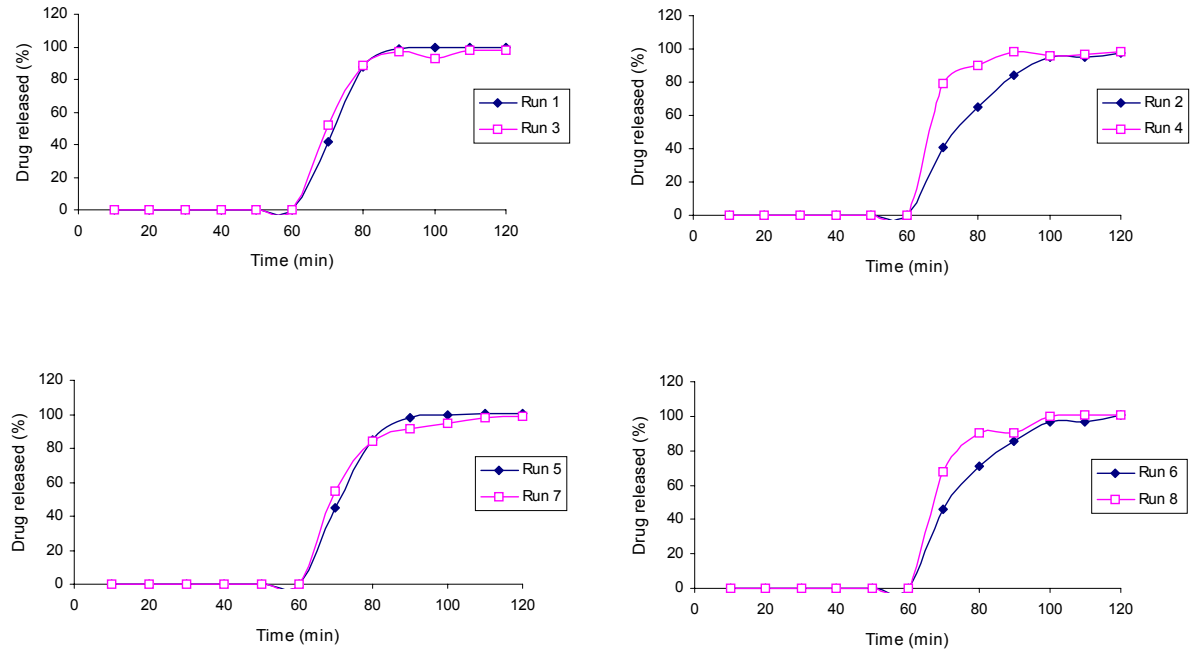


Figure 5.22. Influence of protective coating on dissolution of pellets

In the case of pellets containing sugar as a neutral core influence of HPMC coating on dissolution was not observed (Figure 5.22). Pellets with MCC core and HPMC coating (run 2 and run 6) showed a small delay in drug release in comparison to pellets without protective coating (run 4 and run 8). This finding was associated with the starch content in Suglets® (6% w/w), which moderately accelerated the dissolution processes.

5.9. Accelerated degradation stability testing of solution/suspension layered pellets

Accelerated degradation study and Arrhenius shelf-life prediction is used as a comparative technique in obtaining the information on the most important formulation parameter in obtaining stable lansoprazole pellets. Since the order of reaction had to be determined from the stability data, results were fitted to the zero and first order model, since those are the one which are likely to occur (Chapter 2.3). More in depth linear regression analysis has been carried out using Analysis Toolpak in Excel in 95% confidence interval.

To verify the validity of the kinetic model and to measure the linearity, correlation coefficient (R^2) and standard errors (or standard deviation of the residuals) were compared.

For all four temperatures there was a small difference in the correlation coefficient (R) and coefficient of determination (R^2) of the two models. The standard error or the standard deviation of the residuals, which is based on the deviation of the data points from the line, was in all cases higher in the case of zero order, and it increased with the increase in temperature. For

the lowest applied temperature of 30°C standard error for the zero order was in the range of 0.9 to 3.3, while for 60°C it was in the range of 10 to 25 for the zero order.

Analysis of variance of all tested models showed that all proposed relationships are statistically significant and the lowest significance F value ($p<0.05$ probability that the observed fit could be generated by random means alone) was obtained for zero order model. Coefficients obtained for intercept and variable models showed lower standard deviations, with lower p-value in the case of first order kinetics. The weighted residuals plots can be very useful in the evaluation of the chosen model. The basic approach is to look for a pattern in a plot and the best result is a plot with no discernible pattern (Bourne, 2006). The presence of trend could be explained with a small number of observations and in this case the residual analysis would represent only the experimental error. However, the model parameters taken into consideration were ambiguous and were not always optimal for the same order. For example, first-order model seemed to fit better to the stability data obtained on 55°C than the zero-order.

It has been reported that the most often degradation kinetics in solid dosage form appears to be a zero-order (Connors et al., 1986c; Connors et al., 1986b). Tested stability data showed that both models can be approximated by straight line kinetics and that it is hard to distinguish between the zero-order and the first-order kinetics. The resulting apparent zero-order model was considered to be the best descriptive for the given stability data and it was assumed to occur in the product since a high moisture content was present and that degradation of lansoprazole occurred only in the moisture layer.

The results obtained from stability studies that were performed on 8 formulations of lansoprazole pellets on 30°C, 40°C, 55°C and 60°C at 79% RH are illustrated graphically in Figure 5.23. The Figure 5.23 shows that the storage at 30°C, 40°C, 55°C and 60°C at 79% RH resulted in gradual decomposition of lansoprazole in all tested pellets.

Decrease in lansoprazole content followed apparent zero-order kinetics and apparent zero-order rate constants for each temperature were calculated. However, not many zero-order degradation kinetics exist in pharmaceutics, but some drugs in certain dosage forms, such as suspensions, follow zero-order kinetics. This approximation was in concordance with the study by Tabata et al., 1992, as it was hypothesized that the degradation content of lansoprazole would be proportioned to the product of the degradation rate constant (k) and the total solubility (S). He defined apparent zero-order degradation rates of drugs in the solid state can be expressed with following equation (Tetsuro et al., 1992):

$$-\frac{d(Lansoprazole)}{dt} \propto kxS \qquad \text{Equation 5.1}$$

Similar approach to solid-state degradation has been described by Connors et al., 1986c, that the degradation in solid state is a function of both the solubility of the drug and the amount of available solvent.

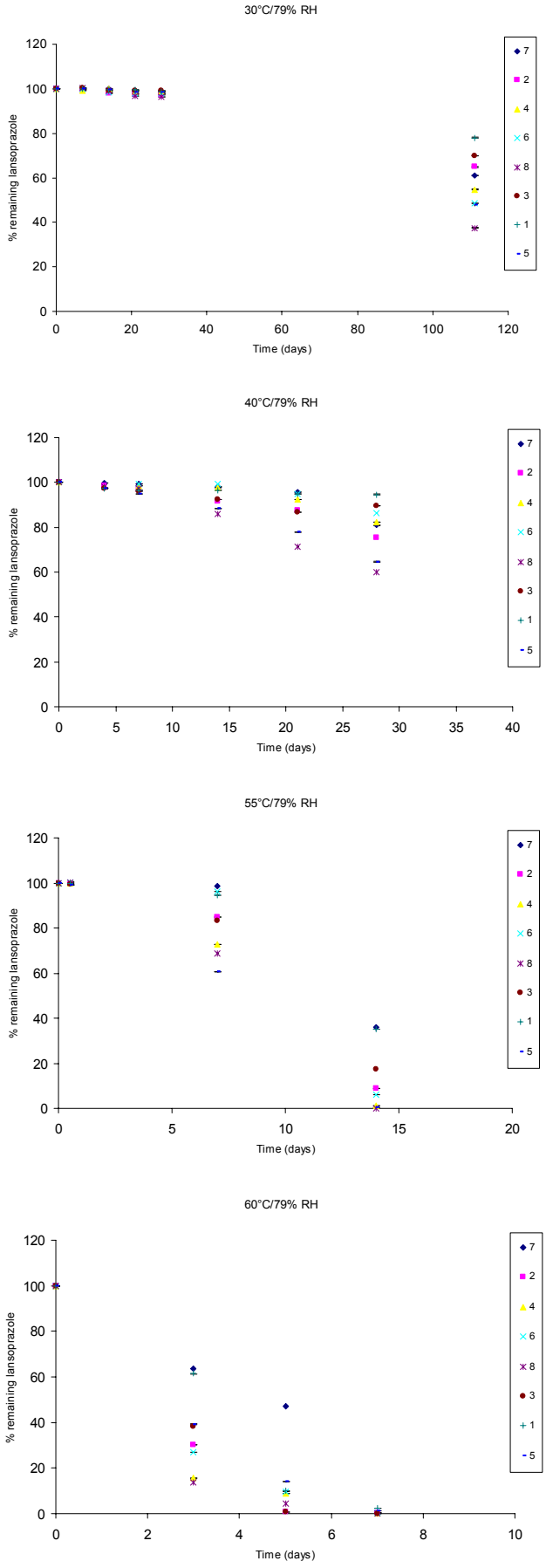
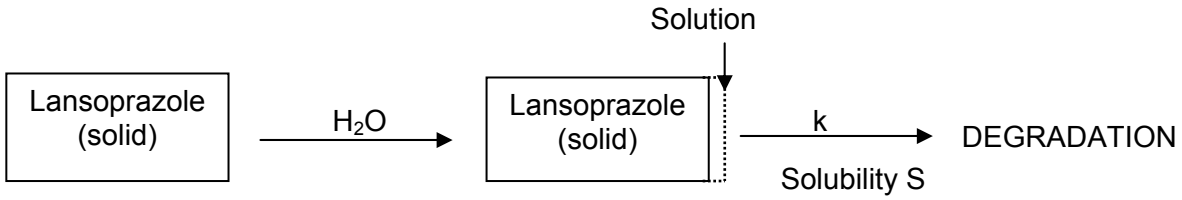


Figure 5.23. Decrease in lansoprazole content on temperatures of 30, 40, 55 and 60°C on 79% RH

Tabata et al., 1992, assumed that the degradation of lansoprazole only occurs in the dissolved fraction of drug. It was suggested that lansoprazole is unstable in the presence of liquids in which the drug is at least partly soluble.



The rate of decomposition was increased by storage of the pellets at high temperatures what is presented in the Table 5.9. The rate of lansoprazole decomposition (k) showed the maximum value when the pellets were stored at 60°C. The rate of decomposition was obtained from the slope, after plotting the data according to the apparent zero order kinetics.

Table 5.9. Effect of temperature and humidity on the rate of decomposition of tested pellet formulations

Trial	*T[°C]*	*k (M/day^{-1})*	*R^2*
Run 1	30	0.211	0.957
	40	0.164	0.845
	55	4.371	0.908
	60	14.996	0.914
Run 2	30	0.332	0.972
	40	0.831	0.937
	55	6.226	0.885
	60	14.76	0.884
Run 3	30	0.292	0.960
	40	0.431	0.815
	55	5.676	0.827
	60	15.04	0.914
Run 4	30	0.436	0.951
	40	0.833	0.961
	55	6.895	0.948
	60	14.028	0.817
Run 5	30	0.376	0.962
	40	0.584	0.716
	55	4.279	0.784
	60	13.586	0.958
Run 6	30	0.494	0.960
	40	0.587	0.917
	55	6.313	0.797
	60	14.238	0.890
Run 7	30	0.505	0.955
	40	1.287	0.967
	55	7.068	0.989
	60	14.293	0.949
Run 8	30	0.601	0.966
	40	1.519	0.967
	55	7.050	0.964
	60	14.178	0.801

The Arrhenius plots of the stability data at four storage temperatures was obtained by plotting the lnk versus the 1000/T for all tested formulations and it is presented in the Figure 5.24.

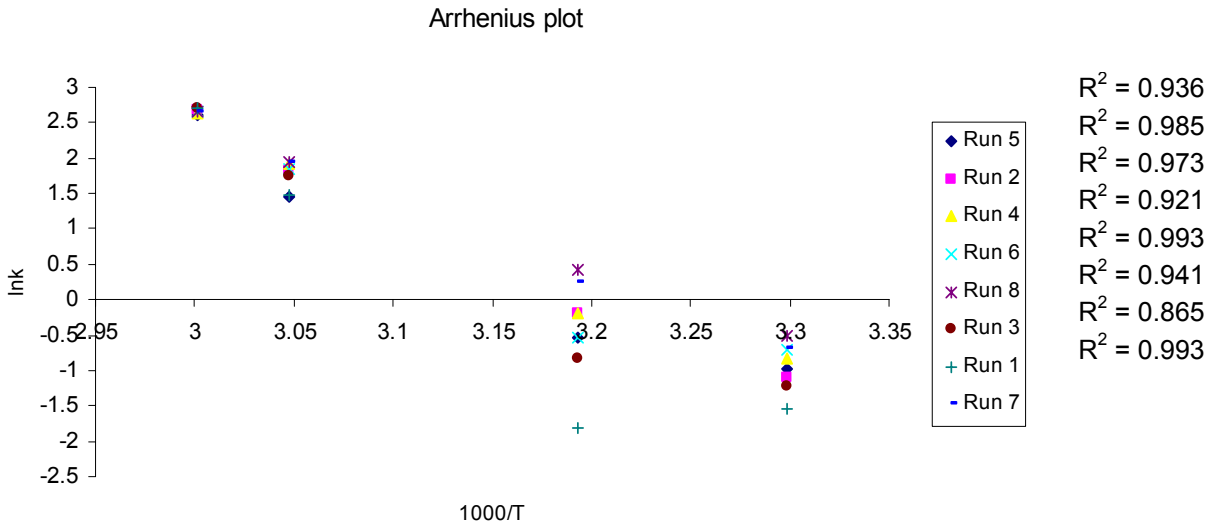


Figure 5.24. An Arrhenius plot showing the stability of 8 tested pellet formulations

By extrapolation, the k value at 25°C for each of the tested pellet formulations is calculated and presented in the Table 5.10. Apparent activation energy Ea can also be calculated from the slope of resulting line, according to the Equation 2.1.

Table 5.10. Rate of decomposition, predicted shelf-lives and half-lives at 25°C

Formulation	*Ea (J/mol)*	*k(Mday^{-1}) (n=3)*	*R^2*	*$t_{0.5}$(days)*	*$t_{0.9}$(days)*
Run 1	26.397	0.04338	0.865	1152.5	230.5
Run 2	16.731	0.13688	0.985	365.3	73.1
Run 3	20.312	0.08943	0.941	559.1	111.8
Run 4	15.062	0.11666	0.973	428.6	85.7
Run 5	16.702	0.13734	0.937	364.1	72.8
Run 6	15.062	0.16690	0.921	299.6	59.9
Run 7	11.837	0.24488	0.993	204.2	40.8
Run 8	9.829	0.31090	0.994	160.8	32.2

Linear regression can be extrapolated to the room temperature and thus half-life and shelf-life of the product at the room temperature can be predicted (see Table 5.10, Equation 2.7 and Equation 2.8). The visual assessment of discoloration of pellet surface was conducted during the whole testing period. The color change of pellets to brown on 55°C and 60°C was observed at the first testing interval, 0.5 days and 3 days, respectively. Pellets from the runs 4, 7 and 8 had a slightly lighter color than the pellets from other runs. Pellets on these temperatures have stick to the walls of the bottle and at the end of the testing period changed their color to black.

Pellets at temperature of 30°C and 40°C have only slightly changed their color during the whole testing interval to light beige.

Results presented in Table 5.10 show that calculated apparent activation energy for all pellets formulation lies between 10 J/mol and 26 J/mol. Such a low apparent energy of activation can be explained with very drastic experimental conditions on which pellets were exposed. In the study of Carstensen et al., 1987, on decomposition of aspirin in solid state in the presence of limited amounts of moisture, activation energy is calculated to be 18 kcal/mol. Pellets containing magnesium carbonate heavy in the combination with the sugar neutral pellets had better shelf-lives than the ones containing MCC neutral pellets. Pellets containing protective coating regardless of the type of neutral core had better shelf-lives than the ones without it. Longest shelf-life of 230.5 days was predicted for run 1 containing sugar (Suglets®) as neutral core, with protective coating and magnesium carbonate heavy as alkaline agent. The least stable formulation was run 8 (32.2 days), with microcrystalline cellulose (Etispheres®) as neutral core, with no protective coating and sodium dihydrogenphosphate as alkaline agent. Predicted shelf-life for this formulation was seven times lower than the predicted shelf-life for the pellets from run 1.

In the previous work of Tetsuro et al., 1992, it has been suggested that lansoprazole degrades when a proton attacks the sulfoxide in the structure and lansoprazole seems to be especially sensitive to such attack compared to the other members of the 2- (2-pyridylmethyl) sulfinyl-benzimidazole family of drugs. It has been suggested that stabilization of lansoprazole in the solid state can be achieved by using different pH adjusters which are capable of providing pH $\geq$ 7 when present alone in water in the core of pellets containing substituted benzimidazoles. Lansoprazole is unstable also under strongly basic conditions, but its degradation is minimized under weakly basic conditions. Therefore the degradation of lansoprazole in dosage forms can be minimized when formulated with stabilizing compounds suitable to produce such a weakly basic pH (Tabata et al., 1992). But not only suppression of proton attacks can stabilize lansoprazole. The mechanism of stabilization is also connected with the solubility of lansoprazole in the moisture adsorbed layer. In the strictest sense, the term pH is not defined in a solid system. For it to have a meaning there must be some water mediation (Carstensen, 2000). With addition of pH adjusters it is possible to control the pH of the microenvironment. Incorporation of pH adjusters has been utilized to maintain the micro-environmental pH in a range that will decrease drug solubility and improve stability during manufacture and storage.

Solubility of lansoprazole increases with increase in pH. In order to stabilize lansoprazole we need the material which will buffer the environment in the alkaline region and in the same time solubility of lansoprazole should be as low as possible. According to the study of Tabata et al., 1992; Kristl and Vrecer, 2000, solubility of lansoprazole increases slowly up to pH 9, but after pH 9 rapid increase in solubility is evident. DellaGreca et al., 2006 and Lagerstörm et al., 1984,

in their studies confirmed that degradation of LSP is accelerated in acid conditions while the drug is quite stable in solutions at pH 7 and 9. Tabata et al., 1992, proposed that region from pH 7 to pH 9 presents a suitable region for stabilization of lansoprazole in the dosage form.

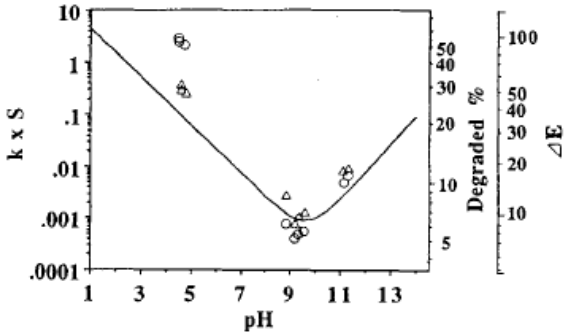


Figure 5.25. Relationship between pH and kxS of lansoprazole

The Figure 5.25 shows that the minimum degradation of lansoprazole occurs in the environment of pH 9 (Tabata et al., 1992).

Pellets containing magnesium carbonate heavy as alkaline agent had lower rate of degradation and better shelf-life prediction. Pellets with sodium dihydrogenphosphate as alkaline material had lower predicted shelf-lives. Measured pH of the slurry of pellets showed that with magnesium carbonate heavy it was possible to obtain microenvironment of approximate pH of 9, while using sodium dihydrogenphosphate pH of microenvironment was in the range of 6.4 to 7.8 (Table 5.8). Calculated apparent activation energy showed strong correlation of 0.748 with microenvironmental pH (measured pH of the pellets slurry). Obtained result was in conformance with the work of Tetsuro et al., 1992. They found a negative correlation between pH and LSP degradation rate constant in solution and that magnesium carbonate. This indicates that measurement of pH of slurry of pellets can help identifying possible problems in stability of lansoprazole.

Pellets containing sugar as a neutral core and the same alkaline material, showed lower porosity and lower rate constants, in comparison with pellets containing MCC as neutral core. Suglets® containing sucrose and maize starch, showed stabilizing effect on lansoprazole pellets, contrary to literature data. Tetsuro et al., 1992, conducted a compatibility study of lansoprazole with crystalline cellulose, sucrose and corn starch on 40°C and 75% RH for one week. Quantity of remaining lansoprazole is highest in the case of crystalline cellulose 99.7%, while in the case of sucrose and corn starch quantity of lansoprazole is 99.2% and 99.4%, respectively. One of the reasons for better stability of pellets containing sugar core could be that sugar is soluble in water and during the process surface of the core becomes sticky and incorporates the active drug forming a less porous layer. This is supported by the SEM pictures of pellets with sugar as

a neutral core (Figure 5.14). From Table 5.8 can be seen that the pellets with sugar neutral core had lower intermediate porosity, in range of 10% to 13 % compared to pellets manufactured with microcrystalline cellulose which had porosity from 14% to 16%. Negative correlation has been found between the calculated Ea and intermediate porosity of pellets (-0.518) leading to the conclusion that the higher the intermediate porosity the lower the Ea and lower predicted stability of pellets. Better stability of lansoprazole in pellets with sugar core could also be explained with the property of MCC to absorb water and retain it in the core of the pellet Fielden et al., 1992, resulting in the higher moisture content which can be seen in the Table 5.8. Pellets containing MCC core had higher moisture content than the pellets containing sugar core, enabling a closer contact of water with the drug.

Measurement of true density of pellets showed no difference between pellets with the same formulation and no influence of protective coating on density was observed. Only pellets from run 7 (without protective coating) showed higher density than pellets from run 5, which had the same formulation but no protective coating.

Calculated activation energy for pellets which contained protective coating was higher for pellets having the same formulations, but no protective coating. This difference was more pronounced in pellets containing MCC pellets as neutral core. Presence of protective coating in the case of sodium dihydrogenphosphate as alkaline material has significant influence on stability of lansoprazole pellets. In the case of pellets containing sodium dihydrogenphosphate as alkaline material and protective coating, with the same neutral core, showed lower rate constants and had higher shelf-life predictions in comparison with the ones without protective coating (run 5 vs. run 7 and run 6 vs. run 8).

Exposing lansoprazole pellets to RH of 79% resulted in protrusion of moisture and its adsorption on the surface, dissolution of lansoprazole in the formed moisture layer, probably resulting in hydrolysis of the dissolved lansoprazole. Since the solubility of lansoprazole increased with increase in pH and apparent activation energy depended on the solubility of the substance in the moisture layer, it would be expected to have a higher degradation of lansoprazole in pellets with magnesium carbonate heavy (pH 9) than with the sodium dihydrogenphosphate (pH 7). But if we take into account that lansoprazole degraded rapidly in the environment with pH lower than 7, pH of 9 is more favorable for stability. No correlation between the moisture content of starting pellets and stability of pellets was determined. This suggests that the total amount of water present in the system is not the driving force for the degradation of the drug. Instead only the water that comes in the contact with the drug and that is available for chemical reaction is the important parameter, what is in accordance with the study of Stroyer et al., 2006. Sugar core stabilized lansoprazole in a way of forming less porous active layer on the surface, disabling a contact of water and the active substance. Presence of the protective layer has been justified

since it increased the stability of lansoprazole acting as a physical barrier between the drug and the free carboxyl groups of enteric coating polymer.

Confirmation of the predicted shelf-life and applicability of Arrhenius equation in the prediction of stability of lansoprazole pellets should be confirmed with the actual stability data accumulated in time. However, performed stability study with Arrhenius prediction was helpful in obtaining information on the most important formulation parameters and the optimum formulation of lansoprazole pellets for stability.

5.10. Influence of coating level of enteric polymer on stability of pellets

Stability data at three storage temperatures (30, 40 and 55°C) of pellets with different quantity of enteric coating polymer (20%, 22%, 24% and 26%) are presented in Figure 5.26. Data were plotted according to the zero order kinetics, and further on, obtained slope values (*k*) were plotted in a form of natural logarithm versus 1000/T to obtain the Arrhenius plots (see Figure 5.27).

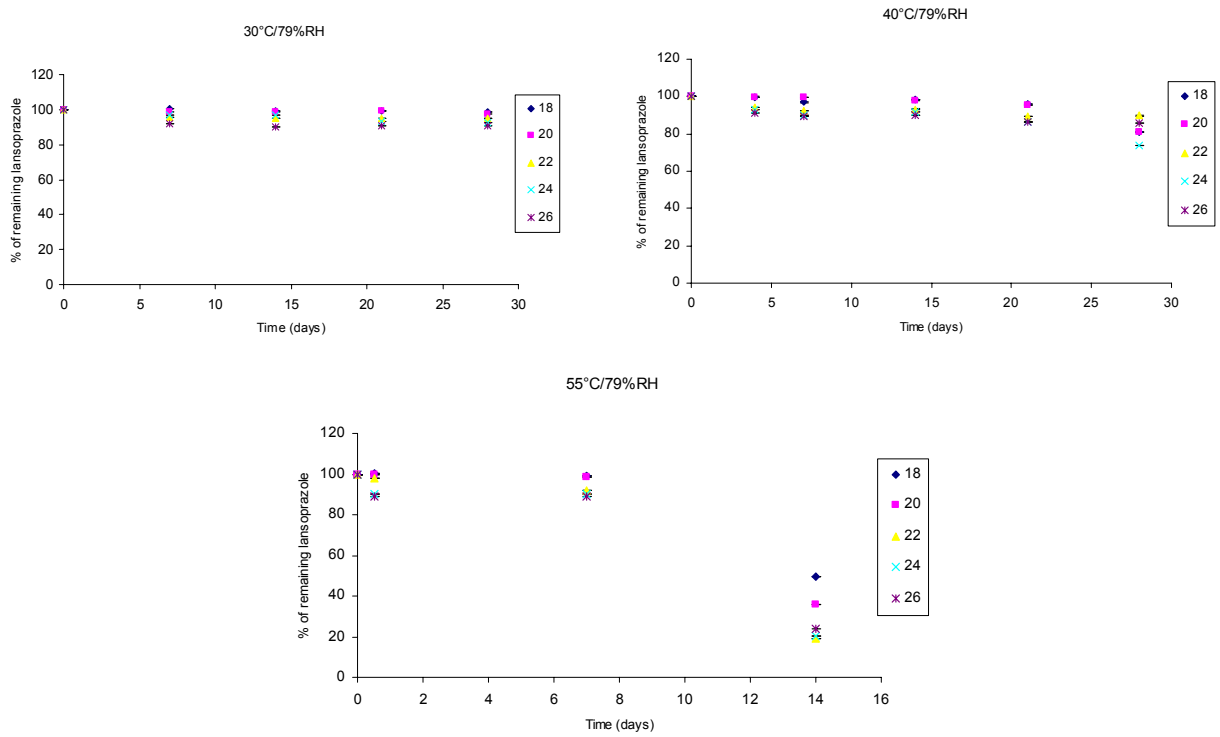


Figure 5.26. Decrease in lansoprazole content on temperatures of 30, 40, 55 on 79% RH in pellets containing different coating levels of enteric polymer Eudragit L 30 D-55

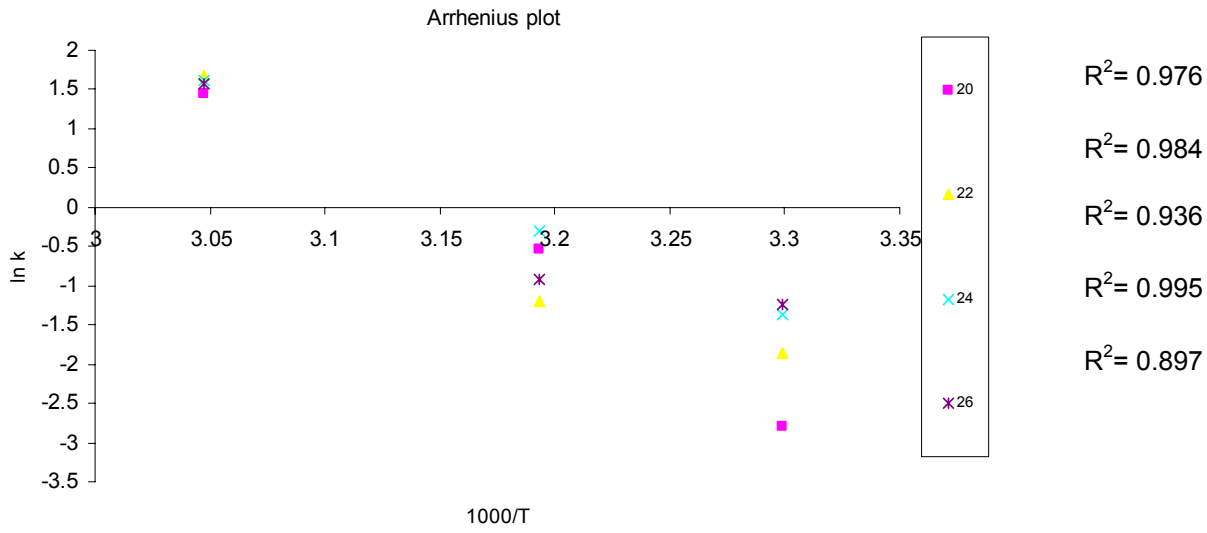


Figure 5.27. An Arhenius plot of pellets with different coating levels of enteric polymer

Higher coating levels of enteric polymer had a negative influence on the stability of tested pellets. From Table 5.11 can be seen that pellets which contained lowest percent of enteric coating polymer (20% w/w solid polymer) had highest calculated energy of activation and longest predicted shelf-lives, even though they showed highest porosity after coating. Obtained result supported a decision on coating lansoprazole pellets with 20% of solid enteric polymer.

Table 5.11. Rate of decomposition, predicted half-lives at 25°C and calculated energy of activation for pellets from run 5 with different coating levels of enteric polymer

Formulation	*k (Mday^{-1}) n=3*	*r^2*	*$t_{0.5}$ (days)*	*Ea (J/mol)*	*Porosity after coating (%)*
20	0.02923	0.984	1710.6	33.207	11
22	0.05086	0.936	983.1	28.761	11
24	0.12262	0.995	407.8	23.832	10
26	0.10888	0.897	459.2	23.010	9

5.11. Pellets prepared with direct pelletization

5.11.1. Optimization of pellet size using experimental design

In this study, the factorial design “with D optimization” was chosen (where D stands for determinant of the results matrix), which fits particularly to investigate the perimeter and the central point of the experimental domain. A Vertex-Centroid Design, quadratic; (D-opt.), was used to find the optimal levels for spray rate, rotor speed and drug load on chosen dependent variables, geometric mean diameter and moisture content. Figure 5.28 presents a Vertex-Centroid design points for the optimization procedure.

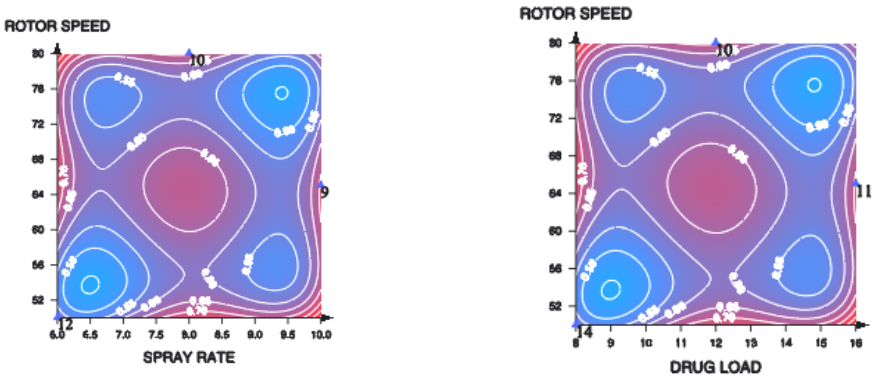


Figure 5.28. Vertex-Centroid design points

Preliminary investigation

The levels of different formulation and process variables were determined by a serious of preliminary experiments. Pelletization of powder mixture (see Table 4.9) with water, without the presence of binder, was found to be inadequate since no pellet formation occurred, even when the high spray rates were applied. In order to influence the bonding mechanism, second step of preliminary investigation included addition of solid HPC as a binder to the powder mixture and pelletization with water. Obtained pellets with spray rate of 8 rpm's had an average particle size of 206 μm and with the spray rate of 12 rpm's approximately 346 μm. Pelletization of powder mixture with the HPC (4% w/v) solution with the spray rate of 8 rpm's was found to be appropriate when the fluidizing air flow was set to 80 m^3/h and the inlet air temperature was 28°C. Resulting pellets had average particle size of 500 μm.

In the preliminary experiments different spheronization times in the range of 5 – 15 minutes were investigated since it is known that the sphericity of a pellet is a function of spheronization time. It was observed that spheronization beyond 8 minutes led to pellets adhesion to the walls of product container. As a part of preliminary investigation influence of amount of water soluble excipient on size and properties of pellets, was investigated. Fraction of pellets in size of 500 microns was tested on true density, porosity and dissolution to determine if the difference of 15% w/w) of water soluble excipient (lactose monohydrate) influences properties of obtained pellets. Difference in 15% of lactose monohydrate did not influence the true density of pellets. Pellets from the trial 2 had higher median particle size than the pellets obtained in the trial 1, even though the quantity of binder solution used in both trials was the same. Pellets from the trial 1 had median particle size of 448.2 μm while pellets from the trial 2 had median particle size of 531.0 μm. Observed difference could be assigned to lactose ability to absorb water increasing the pellet size, since pellets containing higher quantity of MCC tend to shrink after the removal of water as it was described by the "crystallite-gel" (Kleinebudde, 1994; Paterakis et al., 2002). Pellets from the trial 2 containing more lactose had higher porosity (15.4%) than the pellets from the trial 1 (13.0%). Since the quantity of water soluble excipient in the optimization

phase differed only 8% (w/w) in the formulations with the different drug loads, and the tested range was 15% (w/w), it was assumed that lactose will have no influence on pellet characteristics.

5.11.1.1. Response variable 1: Geometric mass mean diameter

The results for geometric mass mean diameter and moisture content at the end of binder addition phase of pellets produced according to the experimental design are listed in the Table 5.12. The geometric mean diameter by weight is determined using a procedure described in Chapter 2.8.1.

Table 5.12. Vertex-Centroid Design, quadratic; optimization

	Factor 1	*Factor 2*	*Factor 3*	*Response 1*	*Response 2*
Run	*A: Spray rate*	*B: Rotor speed*	*C: Drug load*	*GMD*	*Moisture content*
	rpm	*%*	*%*	*µm*	*%*
1	6	50	8	120.6	16
2	6	50	16	204.4	23
3	6	80	8	259.4	23
4	6	80	16	91.8	5
5	10	50	8	510.7	32
6	10	50	16	916.9	31
7	10	80	8	235.8	28
8	10	80	16	350.1	32
9	10	65	12	448.2	32
10	8	80	12	150.2	16
11	8	65	16	191.6	12
12	6	50	12	96.7	5
13	6	65	8	84.3	4
14	8	50	8	182.3	19

From the results it can bee seen that not all experiments resulted in pellets. In this study, only the size beyond 250 µm was referred to as pellets. Five experiments gave satisfactory results regarding the particle size (runs 3, 5, 6, 8 and 9), but not all pellets were spherical. Scanning electron microscopy photographs of pellets obtained in all trials according to the experimental design, in size range of 355 and 500 microns are presented in the Figure 5.29. Pellets obtained in trials 5, 6, 9, 12, 13 and 14 were considered most spherical ones from the visual point of view, since sphericity has not been calculated. Pellets with the smoothest surface were obtained in trials 5, 6, 8 and 9 (Figure 5.29) with high spray liquid addition rate. SEM photographs showed similar surface structure of pellets obtained in trials 1, 3, 10, 11, 12, 13 and 14 which seemed to be more fibrous and porous than the surface of the pellets obtained in other runs. These pellets were prepared using low and medium levels of spray rate. The run 2 seemed to have a mix of pellets with different surface characteristics, including fibrous and smooth surface pellets. Presence of pellets with different surface characteristics in run 2 can be accounted to a combination of low spray rate and low rotor speed which could lead to non-

uniform addition of binder liquid to the powder mass, which has been reported by Liew et al., 2000.

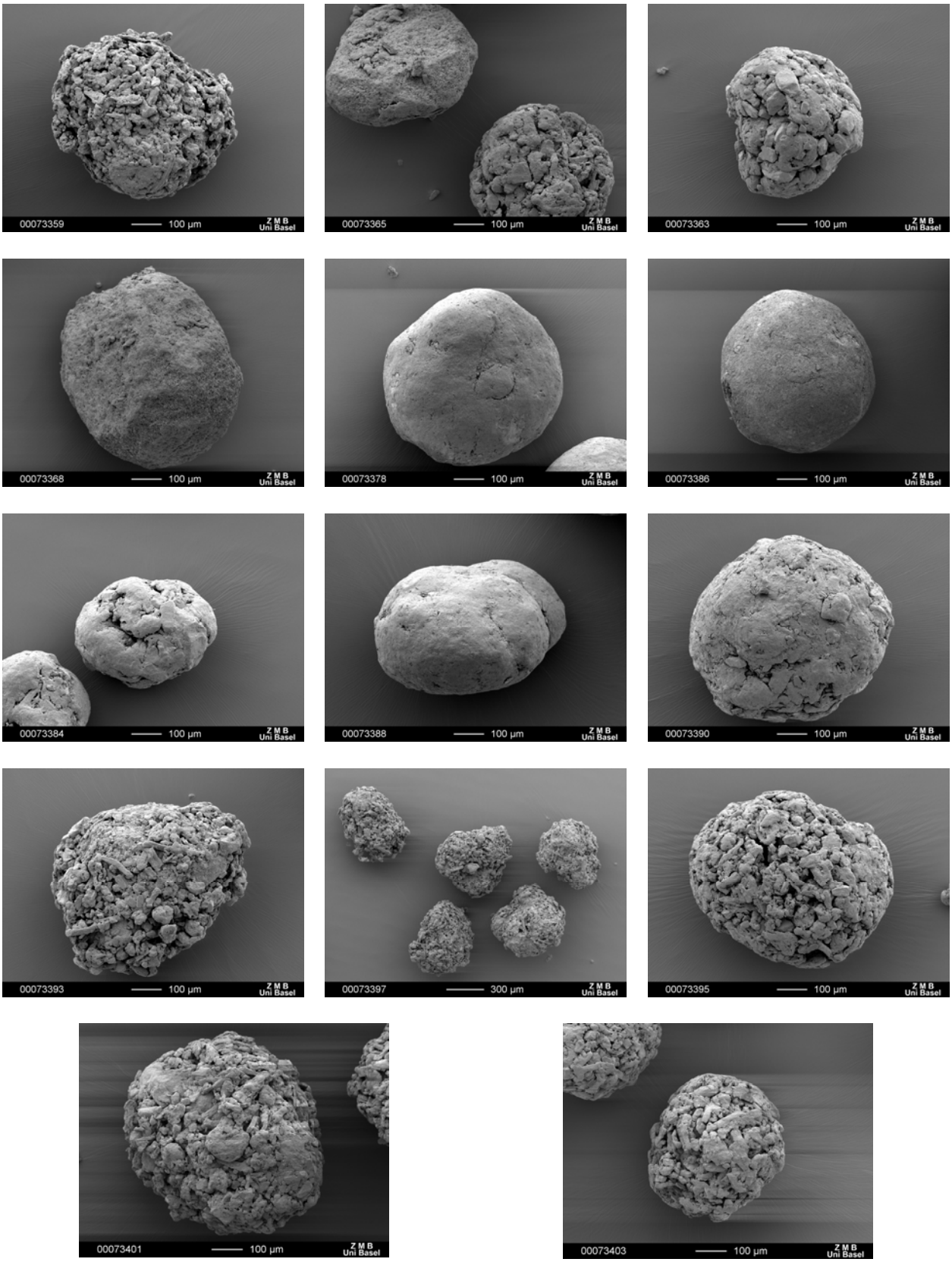


Figure 5.29. SEM photographs of pellets obtained with direct pelletization (from run 1 to run 14, respectively)

In this study, the whole set of data was analyzed by ANOVA using STAVEX 5.0 program and statistical significance of the main effects as well as the interaction effects was determined ($p<0.1$). Following second order polynomial equation was selected for estimating the response variables:

$$y = a_o + a_1x_1 + a_2x_2 + a_3x_3 + a_4x_1^2 + a_5x_2^2 + a_6x_3^2 + a_7x_1x_2 + a_8x_1x_3 + a_9x_2x_3 \qquad \text{Equation 5.2}$$

Where y is response, a_0 intercept, a_i coefficients computed from the responses of the formulations (the effect of the factor x_i), x_1 spray rate, x_2 rotor speed, x_3 drug load, x_ix_j interaction effect, x_i^2 curvature effect.

The estimated values of geometric mass mean diameter (GMD) and regression coefficients postulated by Equation 5.2 are presented in Table 5.13. In the left column, the single factors and their combinations are listed, whereas their influence on the response variables is expressed with the p-value. A p-value<0.1 indicates a significant effect of the factor on the analyzed response variable (Aicos, 1999).

Table 5.13. Model equation for geometric mean diameter

Parameter	Estimated	p-value	Regression coefficients	Factor description
Intercept	+ 144.383	0.0063	+ 929.8	intercept
* S	+ 175.909	0.0001	- 209.6	SPRAY RATE
* R	- 93.310	0.0008	- 8.182	ROTOR SPEED
* D	+ 56.051	0.0053	+ 14.19	DRUG LOAD
* S2	+ 107.992	0.0087	+ 27.00	SPRAY RATE square
* R2	+ 79.687	0.0242	+ 0.3542	ROTOR SPEED square
* D2	+ 0.383	0.9873	+ 0.02396	DRUG LOAD square
* S*R	-113.407	0.0006	- 3.780	SPRAY RATE * ROTOR SPEED
* S*D	+ 74.200	0.0029	+ 9.275	SPRAY RATE * DRUG LOAD
* R*D	- 69.190	0.0037	- 1.153	ROTOR SPEED * DRUG LOAD
Goodness of fit (R^2)				0.9934
Corrected goodness of fit (R^2_c)				0.9786

After the analysis has been carried out, corrected goodness of fit was R^2_c = 0.9786, meaning that the model has a very good fit. There was no evidence for non-normality of model deviations and ANOVA showed that the residuals are identically distributed for all levels of the factors.

The effects of the three factors on the geometric mass mean diameter were found statistically significant ($p<0.1$) what confirmed the application of optimization design (Table 5.13). According to the model, spray rate had the highest influence on the geometric mean diameter ($p<<0.1$). Many studies (Vecchio et al., 1994; Paterakis et al., 2002) have shown the importance of the spray rate in controlling the size of the pellets. The model showed that the rotor speed also had a crucial effect on the response variable with the p value<<0.1, which was in agreement with the findings of several previous studies of Rashid et al., 1999; Korakianti, 2002.

If the interactions are considered, most significant effect on the GMD had the interaction of spray rate and rotor speed ($p<<0.1$).

After the evaluation of the model has been carried out, plotting of response variable versus factors has been generated for the easier interpretation of the results. Surface plots of geometric mean diameter vs. spray rate (x-axe), rotor speed (y-axe) and drug load with the fixed value of 16% is presented in Figure 5.30.

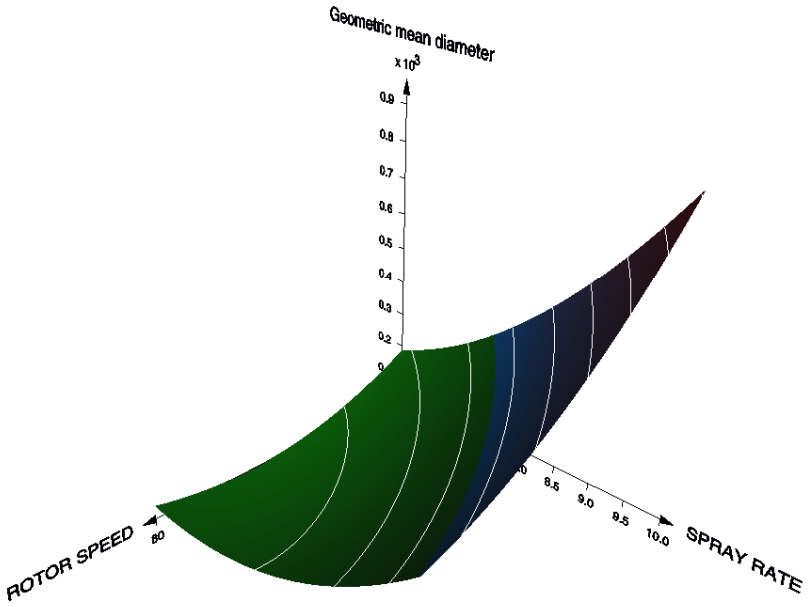


Figure 5.30. Surface plot of geometric mean diameter, spray rate (x-axis), rotor speed (y-axis) at fixed level of drug load to 16%

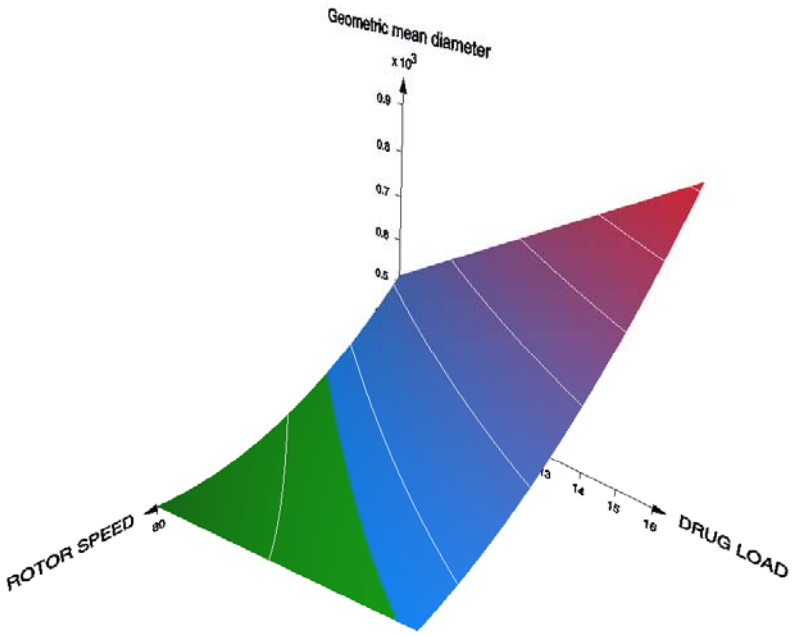


Figure 5.31. Surface plot of geometric mean diameter, drug load (x-axis), rotor speed (y-axis) at fixed level of spray rate (10 rpm)

Pellets with low geometric mean diameter have been obtained with low spray rate of the binder liquid and high rotor speed, while pellets with high geometric mean diameter have been obtained with high spray rate and low rotor speed. From the surface plot (Figure 5.31) it can be seen that combination of high spray rate and low rotor speed led to higher geometric mean diameter of pellets. Only trials with the spray rate of 10 rpm gave satisfactory geometric mean diameter of pellets, in the range of 300 – 1000 μm (run 5, 6, 8 and 9). Generally, it was expected that a higher liquid addition rate would result in larger agglomerates and lower porosity since there is shorter time for liquid to evaporate (Chapter 2.4.1.4) as it is described in the study of Kristensen and Schaefer, 2000; Paterakis et al., 2002. It was found by Menon et al., 1996, that granule growth is directly proportional to the spray rate and inversely proportional to the inlet air temperature.

The effect of the drug load on geometric mean diameter is found to be dependent on the rotor speed (Figure 5.31). Increase in the drug load, with the high rotor speed level (higher than 70%) at fixed level of spray rate, does not lead to an increase of the geometric mean diameter. Higher drug loading, in the combination with the low rotor speed led to a production of pellets with the higher geometric mean diameter (see Figure 5.31).

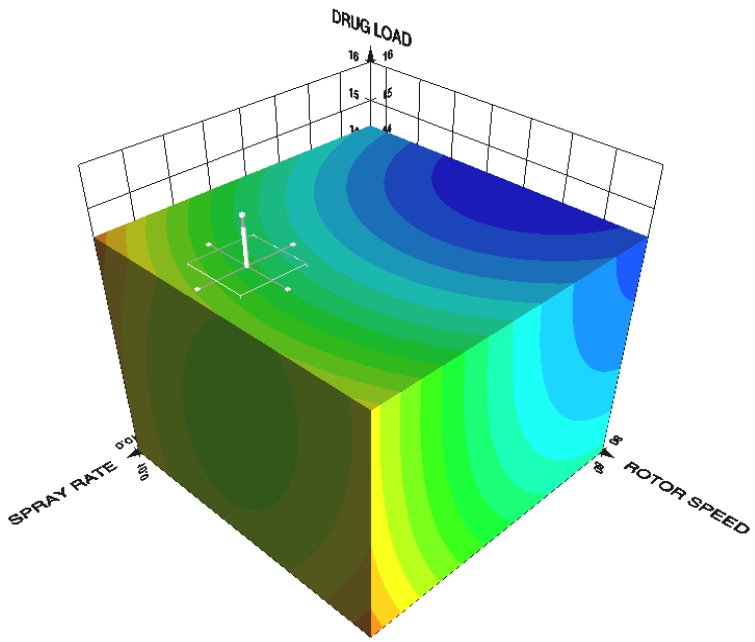


Figure 5.32. 4D contour plot of geometric mean diameter (as colour) versus several variables on the axes x, y and z

Combination of all three factors with the predicted value of geometric mean diameter is presented in a 4D contour plot (Figure 5.32). The spray rate level of 9 rpm‘s, rotor speed of 62% and drug load of 14% yields pellets with particle size of 408 μm with 20.8% moisture content.

As mentioned above, high rotor speed had a negative influence on the geometric mean diameter which could be accounted to a faster movement of powder bed leading to higher friction between the pellets and between the pellets and wall of the container, consequently, decreasing the size.

Global optimum for the geometric diameter was computed, but it was necessary to carry out a confirmatory experiment to check the correspondence between theory and practice. In order to evaluate the prediction power of the model in the 90% confidence interval, confirmatory experiments have been conducted.

Two confirmatory runs have been conducted. One in the proposed range of optimization, in order to establish whether the optimized predicted value of GMD lies in the confidence interval, and the other one in the chosen area (in the range size of 500 μm pellets) from the contour plots.

First confirmatory run, run no. 15, with the spray rate of 10 rpm, rotor speed of 60% and 12 % drug loading has predicted the value of 506 μm for geometric mean diameter of obtained pellets. The second confirmatory run estimated optimum of 914.5 μm, with the spray rate level of 10 rpm, rotor speed 50% and drug load of 16% (confirmatory run 16).

The results of the confirmatory experiments were 452 μm for the run 15 and 911 μm for the run 16. Experimentally obtained values for geometric mean diameter were within the confidence range so the predicted values have been confirmed.

5.11.1.2. Response variable 2: Moisture content

According to the Equation 5.2 estimated effects and p-values moisture content (MC) are presented in Table 5.14.

Table 5.14. Model equation for moisture content

Factor	Estimated effects	p-value	Regression coefficients	Factor description
Intercept	+ 11.5146	0.1960	+ 141.8	intercept
* S	+ 9.5326	0.0259	- 12.81	SPRAY RATE
* R	- 0.9079	0.7584	- 2.595	ROTOR SPEED
* D	- 0.5150	0.8609	- 1.838	DRUG LOAD
* S2	+ 4.0002	0.5478	+ 1.000	SPRAY RATE square
* R2	+ 5.2025	0.4418	+ 0.02312	ROTOR SPEED square
* D2	+ 2.2383	0.7323	+ 0.1399	DRUG LOAD square
* S*R	+ 0.0508	0.9877	+ 0.001694	SPRAY RATE * ROTOR SPEED
* S*D	0.9794	0.7669	+ 0.1224	SPRAY RATE * DRUG LOAD
* R*D	-2.4254	0.4759	- 0.04042	ROTOR SPEED * DRUG LOAD
Goodness of fit (R^2)				0.7773
Corrected goodness of fit (R^2_c)				0.2762

Goodness of fit for this variable was R^2 = 0.777, but the corrected goodness of fit for this variable was R^2_c = 0.276, what indicated a poor fit of the model.

The most significant effect on the moisture content had spray rate with the p-value less than 0.1 ($p<0.1$) and correlation of 0.786. Water content at the end of the liquid addition phase, as a resulting variable of process factors, had proved to be the critical one for the formation of pellets. Results correlated well with literature data, confirming that the pellets growth is facilitated by the increased deformability of the agglomerates caused by higher water content (Kristensen et al., 2000). Figure 5.33 shows that the water content at the end of liquid addition phase correlates with the pellet size (correlation 0.763). Higher water content led to the formation of bigger pellets.

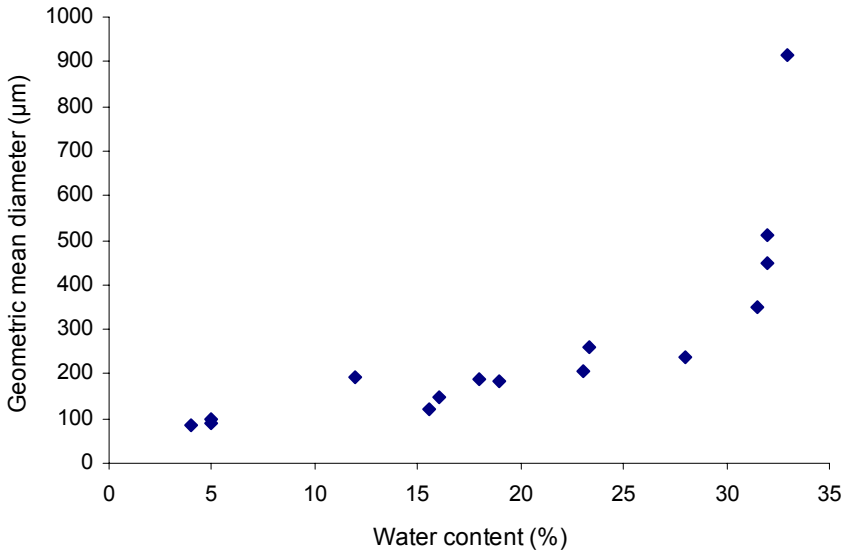


Figure 5.33. Correlation between the water content at the end of liquid addition phase and size of pellets

Surface plots of the moisture content, as dependant variable, are presented in the Figure 5.34 and Figure 5.35. As it is presented in the Figure 5.34 lower rotor speed and higher spray rate led to the higher moisture content. This corresponds to the result obtained for the geometric mean diameter discussed in Chapter 5.11.1.1.

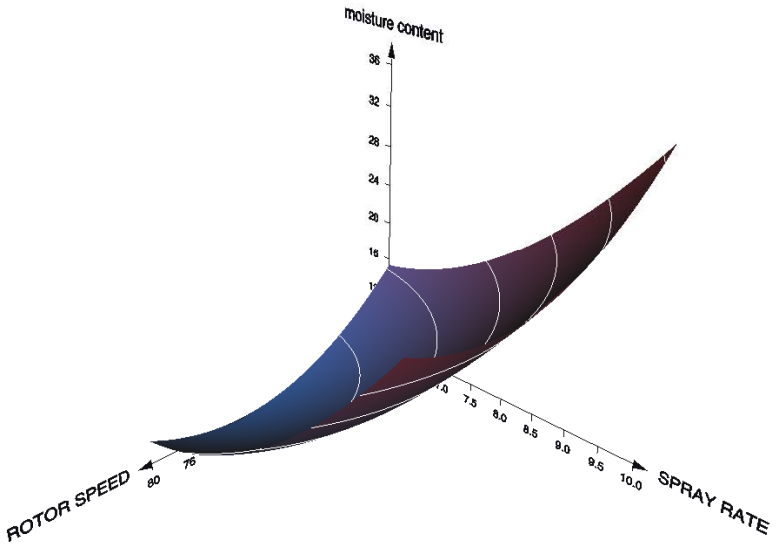


Figure 5.34. Surface plot of moisture content, spray rate (x-axis), rotor speed (y-axis) at fixed level of drug load to 16%

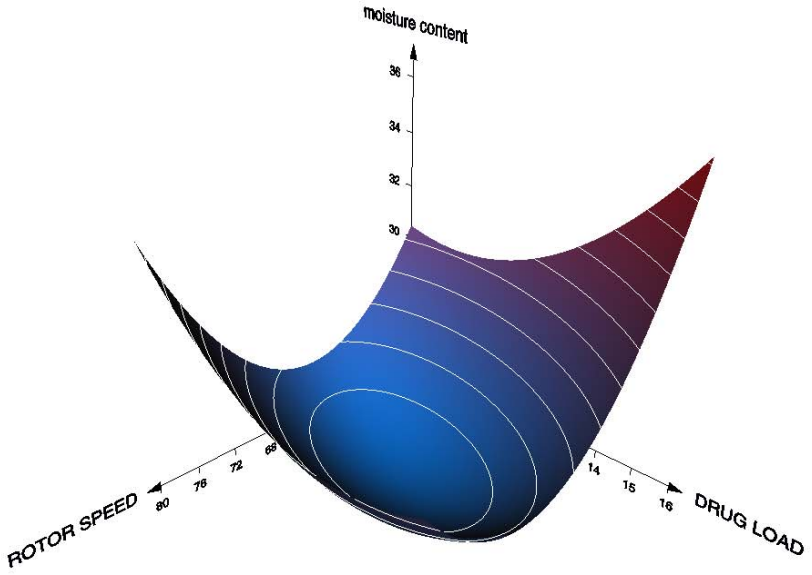


Figure 5.35. Surface plot of moisture content, drug load (x-axis), rotor speed (y-axis) at fixed level of spray rate (10 rpm)

A difference in the moisture content at the end of liquid addition was observed for the low spray rate applied and no connection with the rotor speed or drug load could be observed, while the higher spray rates showed no such differences. When the spray rate of 6 rpm's was applied, with the rotor speed of 50% and the drug loads of 8, 12 and 16%, measured moisture content was 16, 5 and 23%, respectively. The same inconsistency in the moisture content has been observed for the higher rotor speed of 80%, with the drug load of 8 and 16%. Measured moisture content was 23% for the drug load of 8% and 5% for the drug load of 16%. With the higher spray rates applied, 8 and 10 rpm's, inconsistency and big difference in the measured moisture content has not been observed. These findings led to a conclusion that there is a possibility of confounding of another factor which influences the moisture content of the powder bed. This influence has been found to be extreme in the case when the low spray rate is applied, while in the case of a high spray rate this influence is minor. Since the inlet air humidity was noncontrollable parameter and its effect on the moisture content of powder bed was not measured, lack of fit of the model could be explained and inlet air humidity can be assumed as a confounding factor. Further investigation on the influence of inlet air humidity on pellet properties is described in more details in Chapter 5.11.1.3.

5.11.1.3. Dissolution of pellets obtained by experimental design

Pellets obtained from the experimental design were tested on dissolution in phosphate buffer (procedure described in Chapter 4.5.7) in fraction size form 355 – 500 μm, in order to minimize the effect of pellets size on dissolution of drug. Figure 5.36 shows the release profiles in pH 6.8 of all tested pellets without being exposed to 0.1N HCl for one hour, since no enteric coating has been applied in this stage.

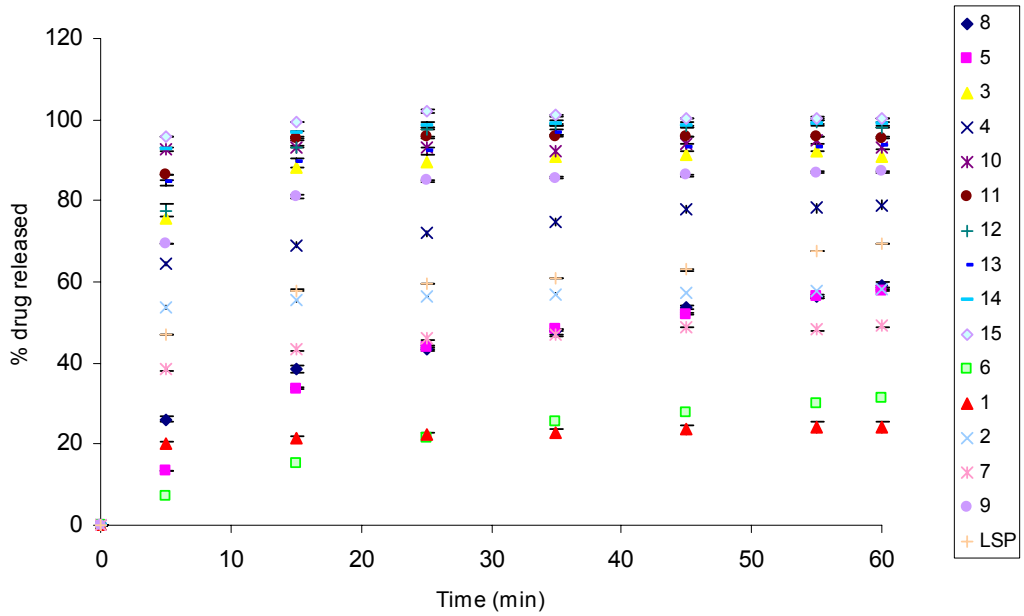


Figure 5.36. Dissolution profiles of pellets obtained using experimental design. The bars represent standard error of the mean (n=3)

Lowest drug release of 24% was observed with pellets from the run 1 prepared with the combination of low levels of all three factors (Table 5.12). The immediate release profile with the highest quantity of drug released (99%) was achieved with pellets from the run 14 prepared with spray rate of 8 rpm's, rotor speed of 50 % and drug load of 8%. From the Figure 5.36 it can be seen that obtained dissolution profiles could be categorized in two groups. First group consists of pellets which had incomplete or retarded release of lansoprazole in comparison to the lansoprazole powder (Figure 5.36, LSP). Run 1, 2, 5, 7 and 8 showed incomplete release of lansoprazole in phosphate buffer pH 6.8 (below 60%). Second group includes pellets from run 3, 4, 9, 10, 11, 12, 13 and 14, which showed a higher drug release than lansoprazole powder itself. Quantity of drug released in 60 minutes in pH 6.8 for second group was more than 80%.
From the results presented in the Figure 5.36 no valid conclusion could be drawn on the optimal settings of spray rate, rotor speed and drug load on the dissolution of pellets. Since a correlation of 0.507 between a date of production of pellets and dissolution in phosphate buffer pH 6.8 has been observed, and a negative correlation of 0.583 between moisture content at the end of liquid addition rate and dissolution, it was assumed that the differences in the dissolution

profiles could be assigned to the difference in the moisture content and with this to the inlet air humidity differences. In the case of runs with the spray rate of 6 rpm's, pellets with the lower moisture content at the end of liquid addition had better dissolution than the ones with the higher moisture content. In the case of pellets prepared with 10 rpm spray rate, dissolution varied slightly with the difference in the moisture content and in between runs, since there was a small difference in the moisture. It was assumed that incomplete release (or retarded release) is due to the higher moisture content of the bed during pelletization caused by the higher inlet-air humidity (noncontrollable process parameter as mentioned in Chapter 5.11.1.2). In order to confirm this assumption two trials have been conducted to simulate the effect of in-process bed humidity on the release. This assumption could also clarify the lack of fit of the model for the moisture content.

5.12. Properties and stability testing of pellets prepared with direct pelletization

Preparation of core pellets using direct pelletization and further coating gave pellets, as judged from visual examination and SEM pictures (Figure 5.37), which could generally be described as nonspherical.

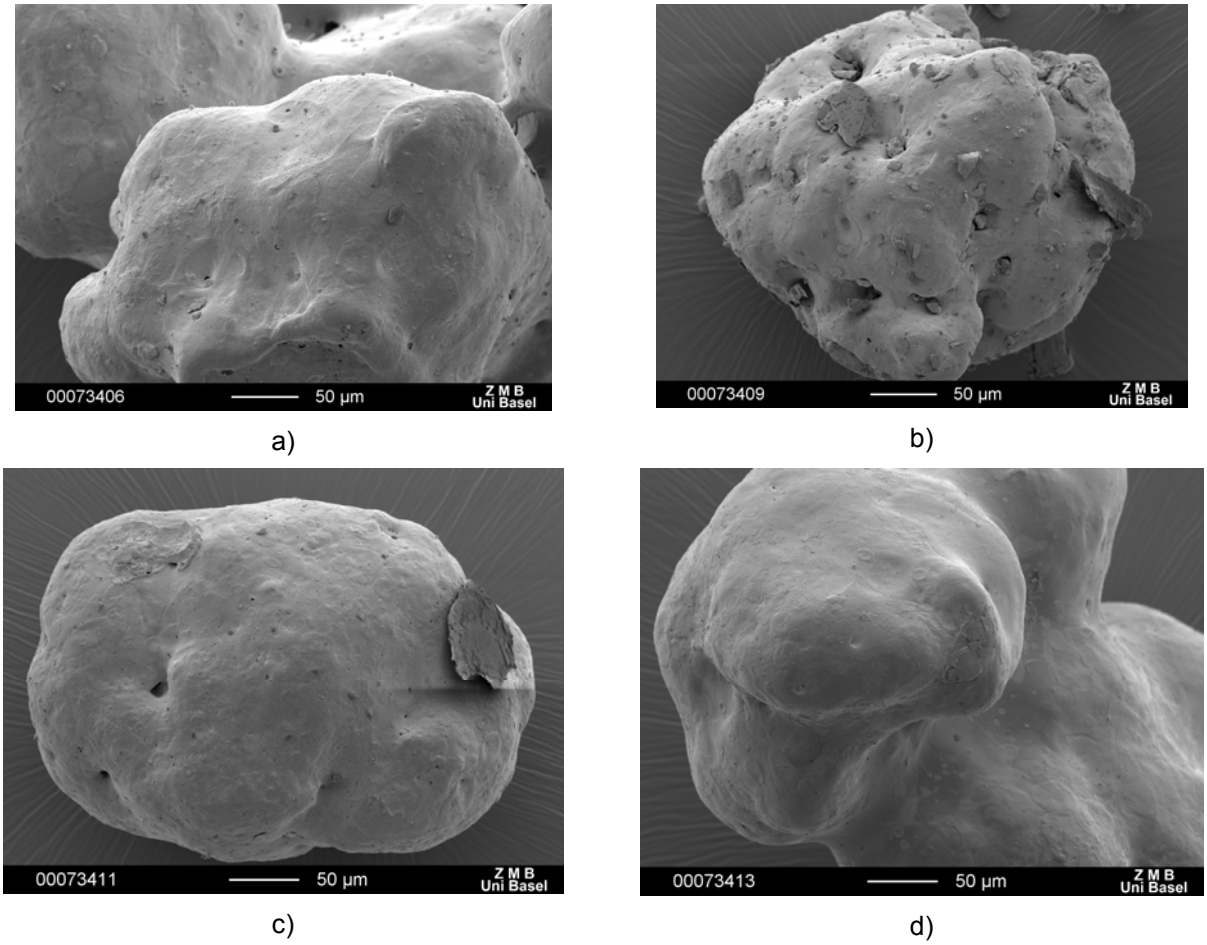


Figure 5.37. Scanning electron microscopy pictures of enteric coated pellets from direct pelletization with magnification x300: a) trial 1, b) trial 2, c) trial 3 and d) trial 4

The loss of sphericity of core pellets could be ascribed to the coating process parameters, since the process had to be interrupted for several times. As judged from the SEM pictures during the enteric coating process of all trials agglomeration of pellet units and deposition of material onto the surface occurred. Obtained pellets showed a higher porosity than the pellets obtained with solution suspension layering. Water content of coated pellets was in the range of 2.64 to 3.09%. pH slurry of uncoated pellets revealed that with magnesium carbonate heavy as alkaline agent it was possible to obtain microenvironmental pH 9.9, while with powder mixture (Balocel®) obtained pH was 8.2.

Table 5.15. Properties of enteric coated pellets obtained with direct pelletization

Formulation		*Porosity after coating (%)*	*True density (g/cm3)± RSD (%)*	*pH of slurry of pellets*	*Water content (%) ± RSD (%)*
Trial	1	13	1.42 ± 0.0027	9.9	2.64 ± 0.01
Trial	2	12	1.47 ± 0.0005	9.8	2.93 ± 0.13
Trial	3	10	1.44 ± 0.0004	8.2	3.09 ± 0.08
Trial	4	13	1.42 ± 0.0012	8.2	3.01 ± 0.07

The results obtained from forced stability studies that were performed on lansoprazole pellet formulations prepared with rotary processor (see Table 4.16 and Table 4.17) on 30°C, 40°C, 55°C and 60°C at 79% RH are illustrated graphically in Figure 5.38.

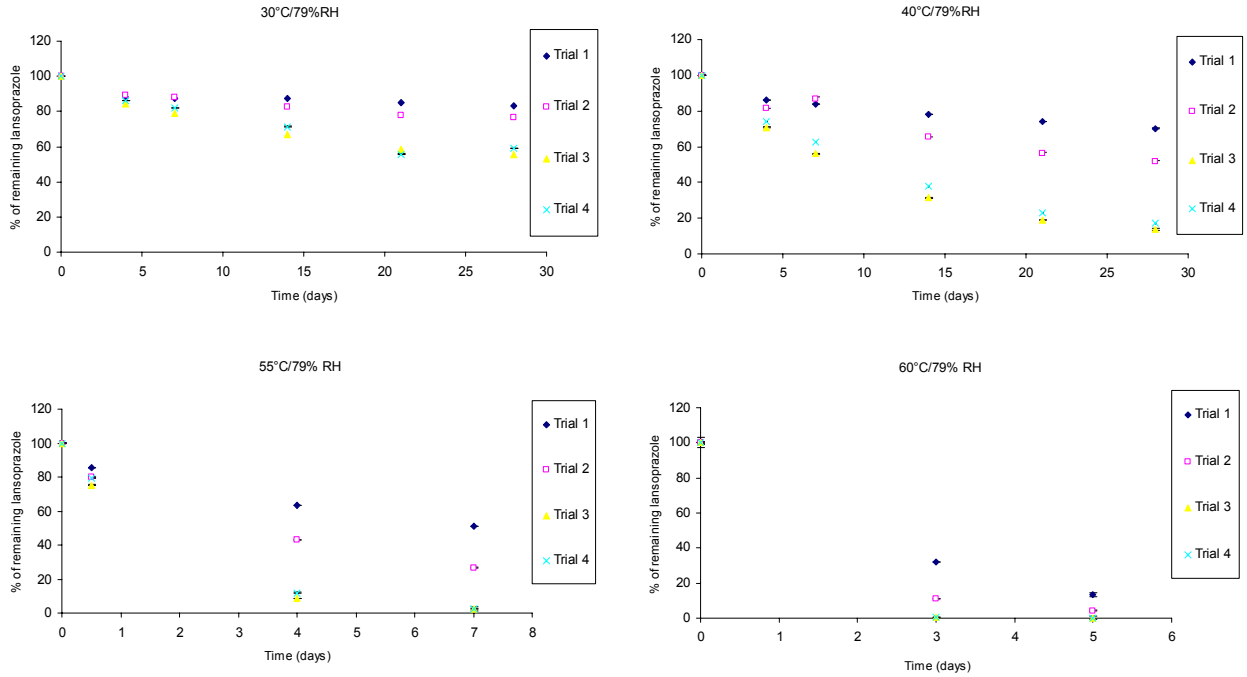


Figure 5.38. Decrease in lansoprazole content on temperatures of 30, 40, 55 and 60°C on 79% RH in pellets prepared by direct pelletization

Rapid decrease of lansoprazole in pellets obtained by direct pelletization was most pronounced at temperature of 60°C. Pellets from the trial 1 had the highest calculated apparent energy of activation with the predicted shelf life of 59.2 days. Pellets from the trial 1 contained a magnesium carbonate heavy as alkaline agent and protective coating. Pellets from the trial 3, without alkaline agent and without protective coating, had lowest stability with calculated shelf life of 12.2 days.

Table 5.16. Rate of decomposition of lansoprazole in pellets from direct pelletization, predicted shelf-lives and half-lives at 25°C

Formulation		*k(Mday^{-1}) (n=3)*	*R^2*	*$t_{0.5}$(days)*	*$t_{0.9}$(days)*	*Ea (J/mol)*
Trial	1	0.1689	0.937	296.1	59.2	104.9
Trial	2	0.3512	0.985	142.4	28.5	93.3
Trial	3	0.8218	0.973	60.8	12.2	76.5
Trial	4	0.8057	0.921	62.1	12.4	77.2

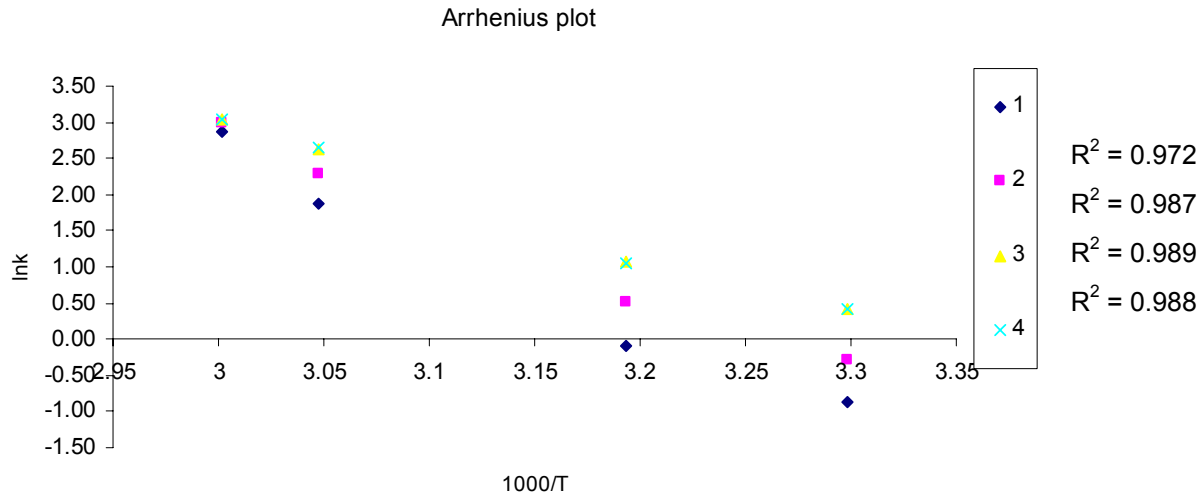


Figure 5.39. An Arhenius plot of pellets prepared with direct pelletization (run 09)

Dissolution profiles of tested pellet formulations (Table 4.16 and Table 4.17) with 20% of Eudragit L 30 D-55 (w/w calculated on solid polymer) are presented in Figure 5.40. The release data showed that application of 20% of solid enteric polymer was not equally suitable to obtain desired performance, as in the case of solution suspension layering, since the coating did not prevent a release of drug in pH 1.2. From SEM pictures (Figure 5.37) it is visible that the complete film formation on the surface has occurred.

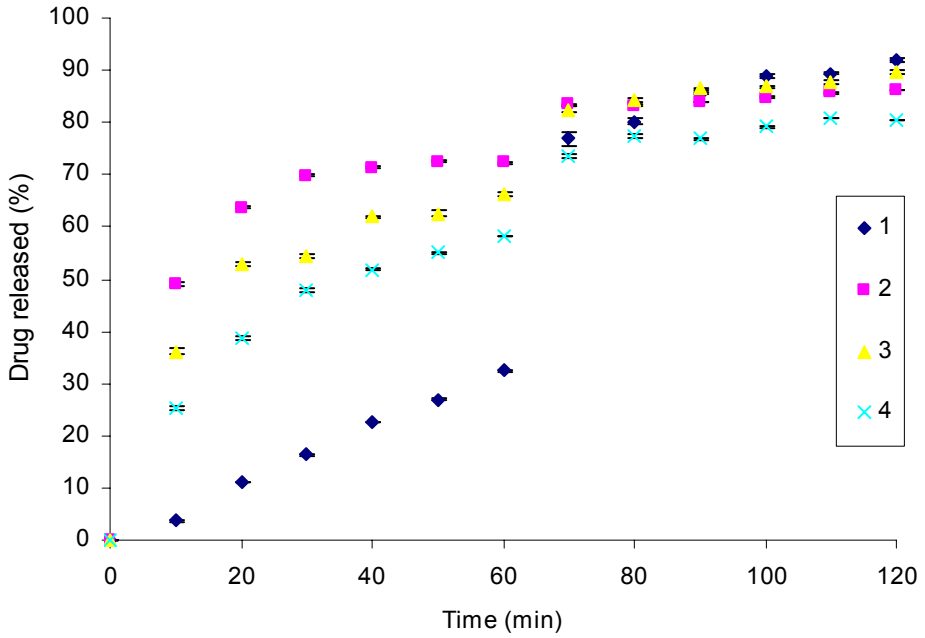


Figure 5.40. Gastric resistance in pH 1.2 and dissolution in pH 6.8 of enteric coated pellets from the run 9. The bars represent standard deviation of the mean (n=6)

The reason for low performance of enteric coating could be found in the smaller size of pellets and higher porosity, having a direct influence on surface area of pellets, which required a higher quantity of enteric polymer to achieve the same performance like in the case of solution/suspension layered pellets. This result is in line with literature data, since several studies reported that smoother pellets are consistently better and more homogeneously coatable than those featuring rough, irregular surface (Lehman et al. 1994; Beckert et al., 1996). Additionally, the interruption of the process for several times because of the nozzle blockage, could lead to a spray drying of dispersion droplets and deposition of material on the surface of pellets. Pellets from the trial 1 had slowest release in acid medium while pellets from trial 2, 3 and 4 showed an immediate release of drug. The study of Pisek et al., 2005, reported that preparation of lansoprazole pellets in rotary processor in MCC matrix system lowers the dissolution profile of lansoprazole and that the MCC matrix system represents a sustained release carrier system for the low soluble drugs if no disintegrants are added (Pisek et al., 2005). Contrary to these findings, in our study, we were able to obtain immediate release pellets of lansoprazole using MCC matrix system. This could be explained with the presence of sodium carboxymethyl cellulose (10.7% w/w in the pellet formulation) in Balocel® powder premix which could act, depending on the processing time and moisture content of the powder bed, as a disintegrants. Thoma and Bechtold, 1999, reported that pellets containing undercoat, which smoothes the core surface reducing the surface area, require a lower amount of gastric resistant dispersion polymer. In addition, the presence of subcoat was suggested to minimize a

diffusion of the drug into the coating, especially if the drug is soluble in the polymer (Felton, 2007). This explains lower drug release in acid media of pellets from trials 1 and 4 compared to trials 2 and 3. Optimization of the enteric coating process and determination of coating level necessary to obtain enteric resistance was not further conducted.

In conclusion, low stability prediction of the pellets obtained by rotor processor could also be attributed to a porous enteric coating layer and it's incapability to protect the drug from the moisture. Additional optimization of the enteric coating should be conducted in the further studies.

6. Conclusion and Outlook

In the pharmaceutical industry formulations nowadays are usually developed under high-time pressure on the basis of “trial and error” experiments which often result in a non-robust product Leuenberger and Lanz, 2005. They are variable and complex systems influenced not only by formulation parameters, meaning the properties of active substance and excipients, but also in the large number of processes involved in manufacturing.

The main purpose of the study was to investigate and gain understanding about the factors affecting the stability of lansoprazole delayed release pellets and the processes used. Pellets were prepared using two different pelletization techniques solution/suspension layering and direct pelletization in rotary processor. Arrhenius relationship has been introduced in the study, as a comparative technique for a prediction of stability of obtained pellets.

Thermal characterization of lansoprazole, as decomposing substance has proven to be very difficult. Immediate decomposition of LSP in a form of a sharp exothermic peak on DSC thermogram, which is unusual for decomposition processes, can lead to a false conclusion. Application of DSC in combination with TGA and HSM in investigation of LSP thermal behavior proved to be crucial in revealing the real nature of LSP. Using combination of DSC, TGA and HSM it was confirmed that the endothermic event obtained by DCS measurement corresponded to reported literature melting point range by O'Neill, 2006, (T_m=178 – 182°C) and exothermic event could be assigned to decomposition process. TG analysis revealed a weight loss prior to melting when low heating rates were applied, which was not connected with the loss of water, postulating the assumption of formation of eutectic with decomposing substances. HSM has revealed that the shift of the melting peak is caused by a formation of eutectic of LSP and its degradation products, leading to a shift of the peak with the increase in the heating rate. Even though lansoprazole melting point is highly influenced by heating rate (from 2.5°C/min to 40°C/min difference of 15°C), higher heating rates should be employed since they give lower variability of results. Applied heating rates beyond 20°C/min leave no space for formation of eutectic and shift of the melting peak became less pronounced. In conclusion, a very dynamic method and exactly standardized measurement conditions, particularly with regards to heating rate, (e.g., in DSC) have to be employed to enable reliable determination of a melting point of these decomposable substances.

It has been reported by some authors (Sethia and Squillante, 2004; Leuner and Dressman, 2006; Sun et al., 2007) that deposition of solid dispersions on the surface of neutral core improves solubility of poorly water-soluble drugs by formation of high-energy amorphous phase. Subsequent layering of initial neutral pellets in solution suspension layering technique has proven to be a lengthier process than the direct pelletization. Obtained pellets were uniform in size, with narrow size distribution and smooth surface. Neutral sugar pellets (Suglets®) proved

to be advantageous over MCC neutral pellets (Etispheres®), in terms of processing and stability of pellets. Pellets containing sugar neutral core had lower intermediate porosity and better shelf-life predictions than MCC pellets. Negative influence of intermediate porosity on stability of lansoprazole pellets had been found. Lower shelf-life prediction of pellets containing MCC neutral core can also be connected with the higher surface area, since MCC neutral pellets were smaller in size and had higher porosity, and contained a higher level of entrapped moisture. Porosity of enteric coated pellets was insufficient for the estimation of the drug stability in pellets, since porosity values in a range of 8% to 14% did not seem to have an influence on stability of pellets. Lowest tested amount of enteric polymer (20% w/w calculated on solid polymer) showed satisfying gastric protection of the drug and the best stability results, in comparison to pellets containing higher coating levels (22%, 24% and 26%). Application of 4% weight gain of HPMC as a subcoating to the active layered pellets slightly delayed the release of lansoprazole in phosphate buffer pH 6.8, which suggested that the subcoat did act as a barrier to prevent contact between the drug and free carboxyl groups of enteric polymer. The release profiles of pellets prepared by deposition of solid dispersion on the neutral core were not significantly different and the release of the drug was according to the USP requirements. A critical formulation parameter for stability of lansoprazole pellets was microenvironmental pH. It has been confirmed that the mechanism of stabilization of lansoprazole was not only suppression of proton attacks but also a limitation of its solubility in the moisture layer since it was found that in the pellets, lansoprazole degraded following apparent zero-order kinetics. Stability issues related to pH sensitivity of lansoprazole in the pellets formulation have proved to be closely connected with the microenvironmental pH and the presence of protective coating. Since lansoprazole degrades in acidic, neutral and strongly basic conditions and its solubility increases with increase in pH, incorporation of pH adjusters has been utilized to maintain the micro-environmental pH in a range that will increase drug stability during manufacture, and storage and keep the solubility at the lowest level. Even though the microenvironmental pH is not exactly defined in the solid system, it has been confirmed that the pH range of 9 to 10 leads to a significantly better stability results than the pH range of 6 to 7. When pH adjusters which are buffering in the weak basic or neutral range are used, protective coating has proven to be crucial for stability. The other reason for usage of protective coating is prevention of chemical interaction leading to instability of the active ingredient. The formulation with best shelf-life prediction (230.5 days) on 25°C on 79% RH contained neutral sugar pellets (Suglets®), magnesium carbonate heavy as buffering agent and protective coating.

Direct pelletization in the rotary processor as a multivariable process needs to be highly controlled and the application of experimental design techniques presented a useful tool for development of robust process. The influence of process variables like rotor speed, spray rate and inlet-air humidity should be taken into account in preparing acceptable MCC drug loaded

pellets, in terms of size, shape and dissolution. On the basis of the results of experimental design, combination of high spray rate with lower rotor speed had a positive effect on pellets size. Optimal formulation was predicted (90% confidence interval) by a model generated by STAVEX and combination of 10 rpm's spray rate, 50% rotor speed and 16% (w/w) drug load gave pellets in size of 911µm. The study has confirmed that moisture content at the end of liquid addition phase correlated with the size of the pellets and presents the most critical factor for the formation and size of the pellets. Higher moisture content at the end of liquid addition favored an increase in pellet size. Combination of process factors should be set at levels which promotes higher powder bed moisture in order to achieve bigger pellets. Moisture content up to 30% showed no significant influence on size increase, while presence of 30% moisture increased the size by twofold. Dissolution of pellets prepared by direct pelletization could be influenced by powder bed moisture content, especially in the case when swellable substances were used as excipients. Presence of MCC and sodium carboxymethylcellulose in Balocel® powder premix, which is known as a viscosity increasing agent with ability to absorb water, showed dissolution modifying properties depending on the powder bed moisture. Inlet-air humidity had a direct influence on the powder bed moisture when a low spray rate was used, which further influenced a dissolution of lansoprazole from pellets. The study confirmed that by changing one process parameter, inlet-air humidity, in rotary processor it was possible to obtain pellets from immediate to retarded release of the drug. This led to the conclusion that even though a comprehensive pre-experimental planning has been conducted, another property of pellets which was not investigated could be affected by another variable which could not be controlled. Screening design, prior to optimization, should be applied whenever it is possible and importance of inlet air-humidity should not be disregarded. In terms of formulation variables drug load and quantity of water soluble excipient plays an important role in pellet properties. Addition of 15% (w/w) of water soluble excipients to a powder mixture Balocel® changed the properties of pellets, like porosity and true density. Balocel® has shown good pelletization properties but the addition of binder solution was necessary to obtain pellets. Contrary to the literature data (Pišek et al., 2005) Pisek et al., 2005, it was possible to obtain immediate release lansoprazole pellets from the MCC matrix system. Obtained pellets showed a higher porosity than the pellets obtained with solution suspension layering.

The solution-layered pellets required a 20% weight gain of solid enteric polymer (Eudragit L30 D-55) to achieve a gastric resistance, whereas the direct pelletization pellets required a higher coating level of enteric polymer. The higher coating levels required for the direct pelletization pellets compared to the solution layered pellets to achieve gastric resistance was attributed to the higher surface area of pellets.

Solution suspension layered pellets showed better stability predictions than pellets obtained with direct pelletization. Lower stability results obtained for the direct pelletization pellets could

be accounted to the higher surface area and porosity, partially caused by incomplete enteric coating, leaving more space for moisture to penetrate into the core. This should not exclude the usage of rotary processor in production of lansoprazole pellets, since further optimization of enteric coating could give pellets with significantly better stability.

It can be concluded that when a pellet formulation of a low soluble micronized drug is considered, in terms of dissolution, preferred technique could be layering on neutral core. When the drug is layered onto the surface of neutral pellet, transformation of the drug to the high energy amorphous state leads to an accelerated dissolution. In the case of direct pelletization, when the drug is incorporated into the core of the pellets, dissolution profile depends on the composition of the pelletizing agent. When swellable substances are present in the powder premix, moisture content of the powder bed plays a major role in dissolution. With the higher moisture level, obtained by application of lower spray rates leading to a longer processing time, MCC and sodium carboxymethylcellulose swell incorporating a drug into the pellet, which results in a slow-down of dissolution rate.

On the basis of present results it can be concluded that both techniques have some advantages and disadvantages.

Even though the solution suspension layering, or the solid dispersion method, seems to be lengthier than the direct pelletization process, and with this more expensive, it has shown some advantages:

- More controllable and simpler process
- Less variability in the dissolution profiles
- Pellets more uniform in size and potency are obtained, since atomized droplets produce denser layer around the substrate particles
- Possibility of obtaining high potency pellets

The advantage which was observed in preparation of lansoprazole pellets with Balocel® in rotary processor was that it is possible to obtain immediate and prolonged release dosage form with the same formulation used, changing only one process parameter, inlet-air humidity. Further studies are necessary to confirm this assumption.

Although methods, as accelerated drug breakdown, cannot replace rigorous stability testing procedures on the product in which it is to be marketed, they do lead to a considerable saving of time at the product development stage. Applied accelerated stability testing can be used as a screening and comparative technique to obtain valuable information on factors influencing stability, as it has been done in this study.

It has to be kept in mind that there are few facts which are limiting the significance of the performed stability test and the prediction of product stability. Firstly, degradation products were not quantified during the stability testing only nondegraded lansoprazole present in the system was determined. Secondly, experiments were conducted in an open system with a high excess

of moisture, which will not be the case in the more realistic storage conditions. Furthermore, the assumption has been made that the reaction order does not change with the increase in temperature and that the operating mechanism will be the same at room temperature. Predicted shelf-lives were not confirmed with actual data accumulated in time.

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8. Appendix

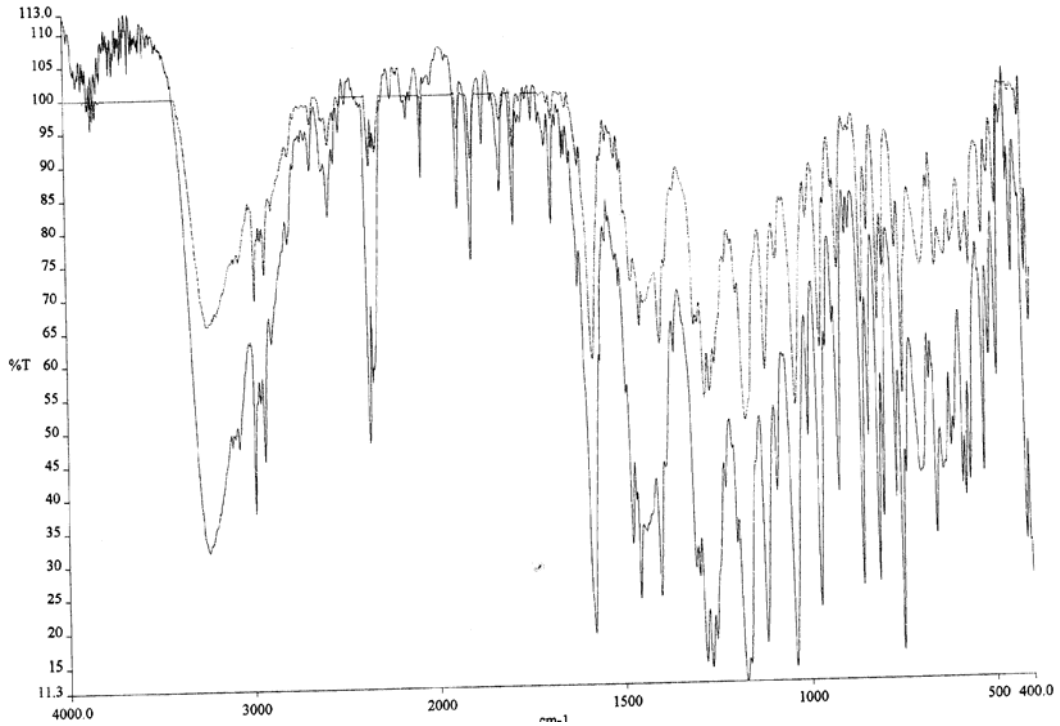

Figure 8.1. FTIR spectrum of lansoprazole and lansoprazole USP standard

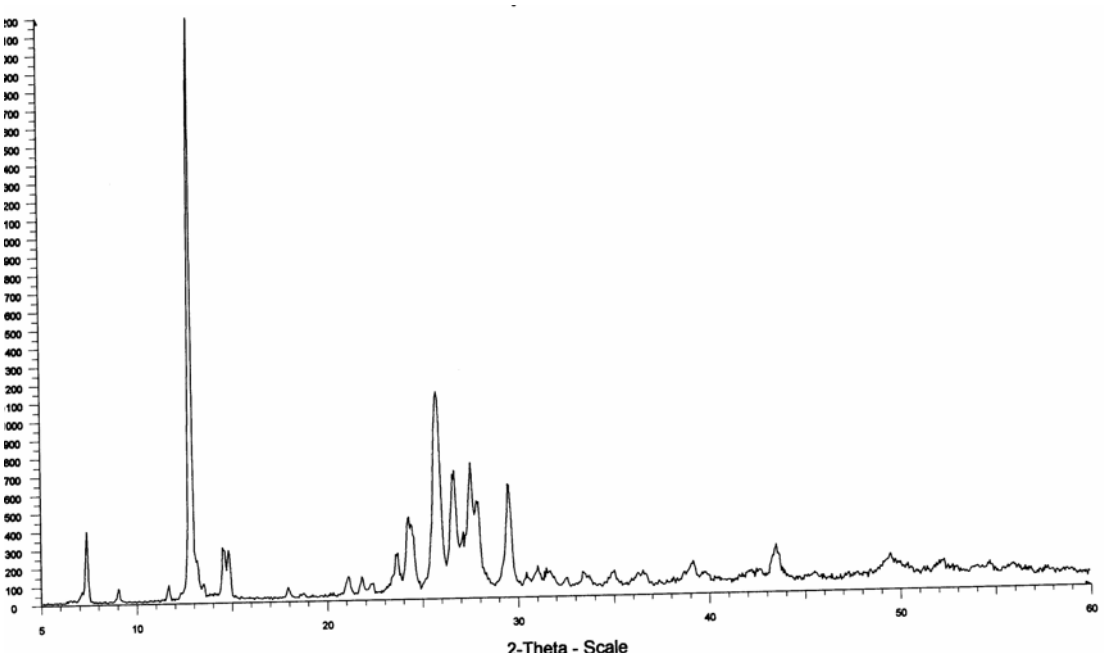

Figure 8.2. Powder X-Ray diffractogram of lansoprazole

Table 8.1. Results obtained for lansoprazole Cipla (n=3)

Endothermic event

Heating rate	Peak (°C) ± RSD(%)	Peak Height (mW) ± RSD(%)	Area (mJ) ± RSD(%)	Onset (°C) ± RSD(%)	Delta H (J/g) ± RSD(%)	End (°C) ± RSD(%)
2.5°C/min	174.39 ± 0.17	4.75 ± 5.18	226.29 ± 3.17	173.04 ± 0.06	89.08 ± 1.84	174.71 ± 0.19
5°C/min	180.70 ± 0.12	20.63 ± 19.12	577.7 ± 6.58	179.56 ± 0.25	229.62 ± 5.18	181.25 ± 0.12
10°C/min	184.33 ± 0.27	36.87 ± 0.27	592.53 ± 21.74	182.76 ± 0.38	236.61 ± 22.01	185.24 ± 0.26
20°C/min	188.42 ± 0.68	53.91 ± 17.39	432.47 ± 9.55	185.74 ± 1.12	169.61 ± 11.28	189.84 ± 0.95
30°C/min	189.52 ± 0.18	67.10 ± 5.82	454.97 ± 1.21	187.01 ± 0.15	181.50 ± 0.39	191.42 ± 0.17
40°C/min	190.85 ± 0.03	82.21 ± 3.79	416.32 ± 10.58	188.22 ± 0.09	166.56 ± 11.51	192.92 ± 0.07

Exothermic event

Heating rate	Peak (°C) ± RSD(%)	Peak Height (mW) ± RSD(%)	Area (mJ) ± RSD(%)	Onset (°C) ± RSD(%)	Delta H (J/g) ± RSD(%)	End (°C) ± RSD(%)
2.5°C/min	175.33 ± 0.14	-6.85 ± 2.24	-349.94 ± 2.75	174.75 ± 0.12	-138.46 ± 0.69	177.61 ± 0.25
5°C/min	181.68 ± 0.11	-17.44 ± 16.08	-718.78 ± 24.96	181.00 ± 0.11	-285.33 ± 23.59	185.07 ± 0.06
10°C/min	185.39 ± 0.23	-31.22 ± 9.15	-864.38 ± 18.55	184.18 ± 0.15	-345.16 ± 18.83	188.89 ± 0.49
20°C/min	191.49 ± 0.80	-32.69 ± 21.91	-582.72 ± 29.55	189.17 ± 0.65	-232.69 ± 32.53	198.00 ± 0.96
30°C/min	193.41 ± 0.15	-40.19 ± 2.39	-557.22 ± 4.67	190.53 ± 0.17	-222.36 ± 5.45	201.37 ± 0.08
40°C/min	195.41 ± 0.11	-52.09 ± 3.172	-621.73 ± 4.96	191.88 ± 0.01	-248.38 ± 5.08	204.36 ± 0.37

Table 8.2. Results obtained for lansoprazole USP standard (n=3)

Endothermic event						
Heating rate	Peak (°C) ± RSD(%)	Peak Height (mW) ± RSD(%)	Area (mJ) ± RSD(%)	Onset (°C) ± RSD(%)	Delta H (J/g) ± RSD(%)	End (°C) ± RSD(%)
2.5°C/min	172.64 ± 0.15	3.64 ± 04.23	149.91 ± 2.49	170.94 ± 0.62	56.41 ± 1.96	173.21 ± 0.09
5°C/min	178.46 ± 0.002	14.99 ± 12.30	654.14 ± 12.90	176.46 ± 0.16	258.5 ± 2.25	179.19 ± 0.04
10°C/min	181.56 ± 0.013	19.65 ± 13.96	255.01 ± 18.28	179.98 ± 0.08	100.79 ± 14.45	181.91 ± 0.08
20°C/min	184.66 ± 0.10	29.63 ± 3.54	227.17 ± 7.51	182.35 ± 0.15	89.56 ± 5.47	185.12 ± 0.18
30°C/min	186.54 ± 0.27	42.51 ± 23.97	267.14 ± 25.77	184.08 ± 0.42	103.97 ± 24.78	187.23 ± 0.41
40°C/min	189.52 ± 0.21	61.71 ± 4.07	464.51 ± 6.56	185.52 ± 0.13	192.75 ± 6.64	193.09 ± 0.24
Exothermic event						
Heating rate	Peak (°C) ± RSD(%)	Peak Height (mW) ± RSD(%)	Area (mJ) ± RSD(%)	Onset (°C) ± RSD(%)	Delta H (J/g) ± RSD(%)	End (°C) ± RSD(%)
2.5°C/min	173.86 ± 0.14	-4.32 ± 35.5	-209.20 ± 19.10	173.18 ± 0.17	-77.48 ± 21.76	176.78 ± 0.35
5°C/min	179.74 ± 0.03	-15.04 ± 12.36	-884.45 ± 7.82	178.66 ± 0.02	-349.56 ± 7.42	184.98 ± 0.73
10°C/min	188.43 ± 0.10	-9.89 ± 7.55	-179.97 ± 0.184	182.44 ± 0.18	-71.75 ± 5.75	186.01 ± 0.21
20°C/min	188.43 ± 0.10	-15.18 ± 4.90	-289.10 ± 2.01	186.16 ± 0.18	-114.11 ± 3.61	194.64 ± 0.39
30°C/min	191.67 ± 0.26	-18.16 ± 10.57	-260.92 ± 26.50	188.80 ± 0.41	-101.93 ± 27.65	194.03 ± 2.11
40°C/min	195.77 ± 0.003	-49.78 ± 8.30	-940.68 ± 5.81	191.09 ± 0.001	-390.29 ± 5.56	207.61 ± 0.81

Curriculum Vitae

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2003	State Exam in Pharmacy, Sarajevo, Bosnia and Herzegovina
2002	Since February 2002 in the pharmaceutical company “Bosnalijek d.d.”, Sarajevo, B&H; (Research & Development Department) A year of the practical trainee in Quality Control Department of Finished Products, in “Bosnalijek dd”, Bosnia and Herzegovina

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1996 - 2001	Pharmacy studies at the University of Pharmacy in Sarajevo, B&H
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