Development of a compressed bead-in-capsule drug delivery system for sustained release

L Esterhuizen-Rudolph

22140727

B.Pharm

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Supervisor: Dr J Viljoen

Co-Supervisor: Prof JH Hamman

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ñI know the plans I have for you,òdeclares the Lord, ñplans to prosper you and not to harm you, plans to give you hope and a future.ò

Jeremiah 29:11

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ABSTRACT

Multiple-unit pellet systems (MUPS) are solid oral dosage forms consisting of multi-particulates (e.g. coated and/or uncoated beads) compressed into mini-tablets or tablets; or they may be loaded into hard gelatine capsules. Mini-tablet-in-capsule dosage forms are well described in the scientific literature, but no information could be found on mini-MUPS-in-capsule dosage forms. The purpose of this study is to develop a mini-MUPS-in-capsule drug delivery system for sustained drug release.

Various bead formulations were prepared by extrusion-spheronisation containing different selected fillers (*i.e.* Avicel® PH 101, MicroceLac® 100; RetaLac®) and active ingredients (*i.e.* furosemide and pyridoxine) using a full factorial design. A portion of each formulation of beads was coated using a mixture of the film coating material Eudragit® L 100 and Eudragit® S 100 (50:50) in a rotating pan coater. Uncoated and coated beads were each compressed into mini-MUPS on a Korsch® single tablet press with a 6 mm diameter concave punch. Six mini-MUPS were loaded into a hard gelatine capsule (*i.e.* three containing coated beads and three containing uncoated beads) to form the mini-MUPS-in-capsule dosage form. Each mini-MUPS-in-capsule drug delivery system was formulated in such a way as to contain 60 mg of active ingredient in total (*i.e.* 10 mg per mini-MUPS unit).

The powder flow properties of the filler materials (*i.e.* Avicel® PH 101, MicroceLac® 100; RetaLac®) as well as the uncoated and coated beads were evaluated. Scanning electron microscopy (SEM) was used to evaluate the morphology of the powders, uncoated and coated beads as well as the optimum mini-MUPS formulations. The mini-MUPS formulations produced from the factorial design were evaluated in terms of physical properties and dissolution behaviour in order to obtain the optimum formulations for each model compound (furosemide and pyridoxine) and filler (Avicel® PH 101, MicroceLac® 100; RetaLac®) combination investigated in this study. Accelerated stability testing was conducted on the optimised mini-MUPS formulations over a six month period.

Neither the physical properties nor the dissolution profiles of the mini-MUPS were improved by film coating of the beads. All mini-MUPS-in-capsule drug delivery systems developed in this study illustrated pulsatile drug release. Therefore, different mini-MUPS-in-capsule drug delivery systems were developed with potential applications for modified drug release in the pharmaceutical industry.

KEY WORDS: Coating; Extrusion-spheronisation; Mini-MUPS-in-capsule system; Modified drug release; Multiple-unit pellet systems; Pulsatile release; Spherical beads.

UITTREKSEL

Meervoudige eenheidkraalsisteme (MEKS) is soliede orale doseervorme wat uit meervoudige deeltjies (bv. bedekte en/of onbedekte sferiese krale) saamgepers word om sodoende mini-tablette of tablette te vorm; of dit kan ook in harde gelatienkapsules gelaai word. Mini-tablet-in-kapsuul doseervorme word algemeen in die wetenskaplike literatuur beskryf, maar geen inligting kon gevind word rakende mini-MEKS-in-kapsuul doseervorme nie. Die doel van hierdie studie is om 'n mini-MEKS-in-kapsuul geneesmiddelaflewering-stelsel vir volgehoue geneesmiddelvrystelling te ontwikkel.

Verskeie kraalformulerings is ontwikkel deur middel van uitpers-sfeervorming van verskillende vulstowwe (*t.w.* Avicel® PH 101, MicroceLac® 100; RetaLac®) asook aktiewe bestanddele (*t.w*. furosemied en piridoksien) deur van ’n vol faktoriaalontwerp gebruik te maak. ’n Gedeelte van elke formulering krale is film-bedek deur gebruik te maak van ’n mengsel Eudragit® L 100 en Eudragit® S 100 (50:50) in ’n roterende panbedekker. Onbedekte en bedekte krale is afsonderlik saamgepers in ‘n Korsch® enkel tabletpers met ’n 6 mm deursnee konkawe stempel. Ses mini-MEKS is in ’n harde gelatienkapsule gelaai (*d.w.s* drie bevattende onbedekte krale en drie bevattende bedekte krale) om sodoende ’n mini-MEKS-in-kapsuul sisteem te vorm. Elke mini-MEKS-in-kapsuul geneesmiddelaflewering-sisteem is geformuleer om in totaal 60 mg aktiewe bestanddeel te bevat (*m.a.w.* 10 mg per mini-MEKS).

Poeiervloei eienskappe van die vulstowwe (*t.w.* Avicel® PH 101, MicroceLac® 100; RetaLac®) asook die onbedekte en bedekte krale is geëvalueer. Skanderings-elektronmikroskopie (SEM) is gebruik om die morfologie van die poeier, onbedekte en bedekte krale; asook die optimale mini-MEKS formulerings te evalueer. Die mini-MEKS formulerings vervaardig volgens die faktoriaalontwerp, is geëvalueer in terme van fisiese eienskappe en dissolusiegedrag ten einde die optimale formule vir elke model geneesmiddel- (furosemied en piridoksien) en vulstof- (Avicel® PH 101, MicroceLac® 100; RetaLac®) kombinasie in hierdie studie vas te stel en te ondersoek. Daarna is versnelde stabiliteitstoetse oor ’n tydperk van ses maande op die optimale mini-MEKS formules uitgevoer.

Die fisiese eienskappe en dissolusie-profiele van die mini-MEKS is geensins deur film-bedekking van die krale verbeter nie. Alle mini-MEKS-in-kapsuul geneesmiddelaflewering-sisteme ontwikkel in hierdie studie, illustreer pulserende geneesmiddelvrystelling. Verskillende mini-MEKS-in-kapsuul geneesmiddelaflewering-sisteme is dus ontwikkel met die gebruikspotensiaal vir gemodifiseerde geneesmiddelvrystelling in die farmaseutiese industrie.

SLEUTELWOORDE: Bedekking; Gemodifiseerde geneesmiddelvrystelling; Meervoudige eenheidkraalsisteme; Mini-MEKS-in-kapsuul sisteem; Pulserende vrystelling; Sferiese krale; Uitpers-sfeervorming.

CHAPTER 1
JUSTIFICATION, AIM AND OBJECTIVES

1.1 BACKGROUND

1.1.1 Mini-tablets

In the past few years, mini-tablets have become popular due to the fact that multiple-unit dosage forms are more effective in terms of drug delivery compared to that of conventional immediate release, single-unit dosage forms. Although mini-tablets have been defined as tablets with a diameter equal to or smaller than 1 to 3 mm (De Brabander *et al.*, 2000:195; Lopes *et al.*, 2006:93; Tissen *et al.*, 2011:164), formulation scientists have also referred to tablets with a diameter of 4 to 6 mm as mini-tablets (Gaber *et al.*, 2015:86; Goole *et al.*, 2008:311; Ishida *et al.*, 2008:47; Sujja-Areevath *et al.*, 1996:53). In this study, mini-tablets were prepared using a punch and die set with a diameter of 6 mm. These tablets are preferably called mini-tablets as they are designed to be loaded into a size 0 hard gelatine capsule.

1.1.2 Single-unit versus multiple-unit dosage forms

Administration of conventional single-unit, immediate release oral dosage forms, results in a rapid increase in the drug-blood plasma concentration followed by a direct decrease in the drug-blood plasma concentration due to elimination (*i.e.* metabolism and excretion). Therapeutic drug-blood plasma levels are therefore not sustained over long periods of time and these dosage forms only produce relatively short durations of action. Furthermore, these conventional immediate release oral dosage forms may cause patient compliance problems during longer treatment periods of chronic conditions due to the frequency that it should be taken to maintain therapeutic drug-blood plasma levels (Abdul *et al*., 2010:2-3; De Brabander *et al*., 2000:195; Ishida *et al*., 2008:46).

To prevent fluctuations in blood plasma concentrations and reduce repeated dosing associated with single-unit, immediate release drug delivery systems, extended release multiple-unit dosage forms have been developed (Rajabi-Siahboomi *et al.*, 2013:623). Multiple-unit dosage forms (*e.g*. beads filled into hard gelatine capsules or mini-tablet-in-

capsule systems) have many advantages over single-unit immediate release dosage forms, for example reduced adverse effects, less frequent dosing regimens, improved bioavailability, sustained blood concentrations and improved cost-effectiveness in terms of drug therapy (Abdul *et al.*, 2010:3; Ishida *et al.*, 2008:46).

Powder mixtures, granules or beads can be directly compressed to form mini-tablets, which can then be filled into hard gelatine capsules in order to compile mini-tablet-in-capsule systems (De Brabander *et al.*, 2000:195). These mini-tablet-in-capsule systems can incorporate incompatible drugs in separate mini-tablets, which are then combined in one dosage form resulting in a lower potential for dose dumping compared to single-unit dosage forms (Tissen *et al.*, 2011:164).

1.1.3 Modified release dosage forms

The concept of “modified release dosage forms” refers to dosage forms that are capable of producing drug release at a desired rate. It also includes dosage forms that can deliver the drug at pre-determined time points and/or to target drug delivery at specific sites in the gastrointestinal tract (McConnell & Basit, 2013:551-555). For delayed release dosage forms, a lag time exists between the point that the medicine is administered and when the drug becomes pharmaceutically available for uptake into the blood. A type of dosage form exhibiting delayed release is for example enteric coated tablets (McConnell & Basit, 2013:551).

Formulation of sustained release dosage forms can be demanding and involves consideration of the physico-chemical properties of the drug, pharmacokinetic properties of the drug, route of administration, disease state to be treated and the type of dosage form (Rajabi-Siahboomi *et al.*, 2013:623). Techniques used to achieve sustained drug release from solid oral dosage forms include embedding the drug in a polymeric matrix and/or coating the dosage form with a film through which the drug slowly diffuses (McConnell & Basit, 2013:551; Porter, 2013b:615-616). Matrix systems are monolithic drug delivery systems composed of a drug dispersed throughout a solid medium consisting of excipients. Matrix type tablet drug delivery systems can be produced by direct compression of a drug mixed with excipients such as polymers. An insoluble matrix system is prepared when a drug is dispersed in a matrix of water-insoluble polymers or waxes (Rajabi-Siahboomi *et al.*, 2013:627-629). Drug release rates from insoluble polymer matrix systems depend on the number and size of pores in the matrix, as well as the tortuosity of the matrix (McConnell & Basit, 2013:556-564).

1.1.4 Bead preparation by extrusion-spheronisation

Pellets/beads are defined as “small, free flowing, spherical particles manufactured by the agglomeration of fine powders or granules of drug substances and excipients using appropriate processing equipment.” Beads used for pharmaceutical applications are generally between 0.5 and 1.5 mm in size (Torrado & Augsburger, 2008:510). Beads can be prepared by different techniques, which include drug layering, cryopelletisation, spray drying, compression, spherical agglomeration and extrusion-spheronisation (Supriya *et al.*, 2012:44). Using extrusion-spheronisation as the technique to prepare beads has several advantages over other bead preparation techniques, which include:

- Regulation of bead size within a narrow particle size distribution,
- Production of high density beads with relatively low friability,
- Obtaining beads with exceptional surface characteristics for relative easy coating,
- Loading high levels of drug without producing exceptionally large beads,
- Combination of two or more drugs in the same dosage unit,
- Optimisation of the physical characteristics of the beads (Agrawal & Naveen, 2011:72; Sakr & Alanazi, 2013:594).

1.1.4.1 Film coating of beads

Film coating is a process used to apply a thin uniform polymeric film onto the surface of the substrate, for example on tablets or beads. In this study, film coating was applied to beads in order to delay and/or extend drug release.

Methacrylic acid - methyl methacrylate copolymers (Eudragit® L 100 and Eudragit® S 100) are polymeric coating materials that should not rupture when compression forces are applied to beads (Torrado & Augsburger, 2008:520). Film coating with these polymers offer many advantages, which include increased minimal core weight (2-3%) of tablets/beads; increased process productivity and output, as well as increased formulation flexibility and improved resistance to coat chipping (Porter, 2013b:614).

1.1.5 Multiple-unit pellet systems (MUPS)

Multiple-unit pellet systems (MUPS) are solid oral dosage forms consisting of multi-particulates (*e.g.* beads or granules) compressed into a tablet or loaded into a hard gelatine capsule. Two types of modified release MUPS tablets are possible, namely:

- MUPS consisting of coated beads or
- MUPS consisting of uncoated matrix type beads (Abdul *et al.*, 2010:3; Reddy *et al.*, 2011:1).

Although mini-tablet-in-capsule systems are well described drug delivery systems (Abdul *et al.*, 2010:3), no information on mini-MUPS-in-capsule systems could be found in the scientific literature. This study attempted to develop this unique drug delivery system as illustrated in Figure 1.1.

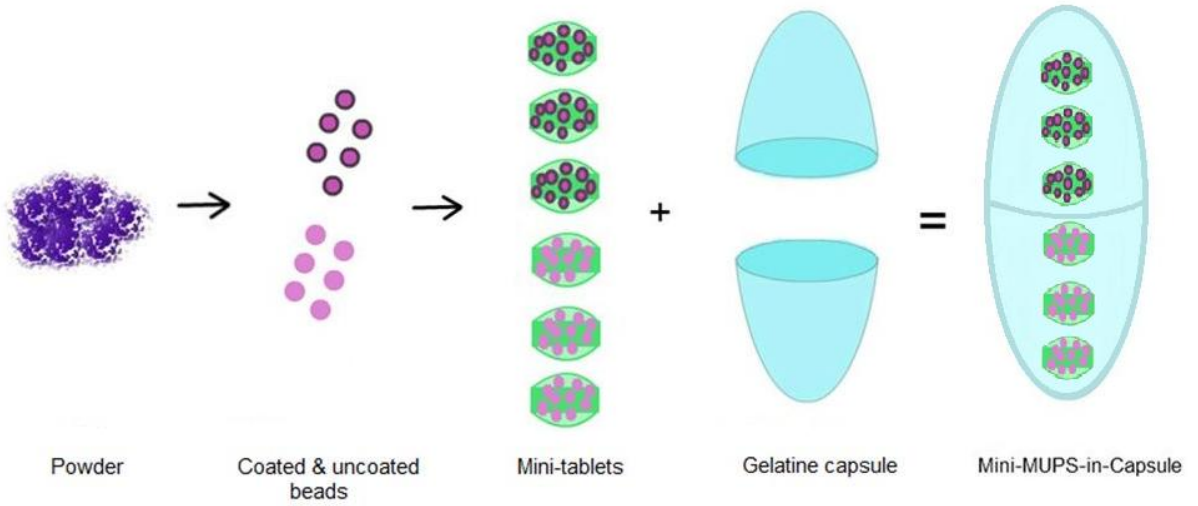


Figure 1.1: Schematic diagram illustrating the mini-MUPS-in-capsule system developed in this study

1.2 RESEARCH PROBLEM

The compliance of patients on chronic medication can sometimes present a problem, due to frequent dosing of immediate release conventional dosage forms over an extended period of time. Furthermore, fluctuations in blood plasma levels, especially in the case of a skipped dose, can cause side-effects or periods where no therapeutic effect is achieved. There is a need to optimise drug release and to maintain blood plasma levels over relatively long time periods, within specified limits by administration of the lowest number of doses. Formulation of a novel mini-MUPS-in-capsule drug delivery system may contribute towards achieving desired drug release, which may ultimately improve delivery from solid oral dosage forms.

1.3 JUSTIFICATION

Sakr and Alanazi (2013:581) stated that tablets remain the preferred choice of dosage form for administering medicines orally. Conventional tablets with an immediate drug release mechanism usually exhibit a short duration of action. Furthermore, these conventional oral dosage forms cause patient compliance problems during longer treatment periods of chronic conditions, resulting in increased side effects or therapeutic failure (Abdul *et al.*, 2010:2-3; De Brabander *et al.*, 2000:195; Ishida *et al.*, 2008:46).

Rajabi-Siahboomi *et al*. (2013:623) described extended release multiple-unit dosage forms (*e.g*. beads filled into hard gelatine capsules) as a solution to overcome the fluctuations in drug-blood plasma concentrations experienced with conventional solid oral dosage forms. Modified release dosage forms also reduce the number of doses needed to maintain therapeutic drug levels in the blood. Multiple-unit dosage forms are more beneficial compared to single-unit dosage forms in terms of more uniform drug delivery with less inter- and intra-subject variation (Abdul *et al.*, 2010:3; Ishida *et al.*, 2008:46; McConnell & Basit, 2013:551-555).

A relatively new type of solid oral dosage form has been developed in order to create modified drug release, namely a multiple-unit pellet system (MUPS). MUPS tablets are compressed from uncoated and/or coated beads or other multi-particulates (Abdul *et al.*, 2010:3; Reddy *et al.*, 2011:1).

MicroceLac® 100 and RetaLac® are fairly new types of lactose co-compressed fillers. Predominately, MicroceLac® 100 is designed for the direct compression of tablets, having advantages such as excellent flowability and compactibility (Meggle, 2014a:2-3). RetaLac® containing hypromellose and lactose (1:1) is the first co-compressed excipient designed for dry granulation and direct compression of modified release formulations. This filler can also be used in extrusion-spheronisation processes. To our knowledge these fillers have not previously been used in the manufacture of beads and MUPS. In this study products produced form these co-compressed fillers will be compared to those of the golden standard; Avicel® (Meggle, 2014b:3-4).

1.4 AIM

The aim of this study is to develop and evaluate mini-MUPS-in-capsule drug delivery systems as novel multiple-unit, solid oral dosage forms for sustained drug release.

1.5 OBJECTIVES

To achieve the aim of the study, the following objectives are set:

- Optimise bead formulations prepared by means of extrusion-spheronisation with three different fillers namely, Avicel® PH 101; MicroceLac® 100 and RetaLac® using a full factorial design of experiments.
- Film coat a portion of each of the three optimised bead formulations with a mixture of Eudragit® L 100 and Eudragit® S 100 using a rotating pan coater.
- Evaluate the morphology of the filler powders, uncoated and coated beads.
- Determine the flow properties of the fillers and each uncoated and coated bead formulation including the angle of repose, Hausner ratio, Carr's index, critical orifice diameter, particle size, particle size distribution and flow rate; as well as the physical properties of the beads including the friability.
- Prepare mini-MUPS by means of direct compression of the bead formulations (both uncoated and coated beads were compressed separately) and to evaluate their physical properties including mass variation, hardness, diameter, thickness, tensile strength, friability, disintegration, drug content and dissolution studies.
- Evaluate swelling and erosion of optimised mini-MUPS prepared from uncoated beads and mini-MUPS prepared from coated beads.
- Evaluate the morphology of the uncoated and coated mini-MUPS.
- Prepare and evaluate each mini-MUPS-in-capsule system formulation in terms of dissolution behaviour.
- Conduct accelerated stability testing for six months on each optimised uncoated and coated mini-MUPS formulation.

CHAPTER 2
ORAL MODIFIED RELEASE DOSAGE FORMS

2.1 INTRODUCTION

Lately, an improved understanding exists regarding disease; science and safety associated with pharmaceuticals. Based on this knowledge, various possibilities of advanced drug delivery systems have been developed regardless the route of administration. The purpose of drug delivery systems, however, has not changed. Dosage form design and development has two considerations, namely that drugs must be targeted to release at a specific site and/or time point in the body in therapeutically efficient quantities, and secondly pre-determined drug release rates (McConnell & Basit, 2013:551; Rajabi-Siahboomi *et al.*, 2013:623). Scientific literature states that tablets and capsules still remain the preferred choice of oral dosage forms, as patients then have total control of drug administration and possibilities of regimen flexibility. An additional advantage is increased patient compliance (Pinto, 2010:45; Riss *et al.*, 2007:78; Sakr & Alanazi, 2013:581).

An enormous amount of research has been conducted on modified release oral drug delivery systems in recent years. Modified release oral drug delivery systems may contain a single or a combination of various types of immediate release; delayed release and/or extended release components. By merging these various modified release components within multiple-unit dosage forms (*e.g.* beads and/or mini-tablets filled into capsules or compressed into multi-unit pellet systems (MUPS) or layered tablets), a vast spectrum of delivery systems can be created with numerous drug release properties (Rajabi-Siahboomi *et al.*, 2013:623). Various dosage forms with modified drug release mechanisms are shown in Table 2.1 as revised from Qiu and Zhou (2011:30).

Multiple-unit modified release dosage forms are more effective when compared to conventional single-unit immediate release dosage forms. The administration of an immediate release dosage form results in a spiked drug-blood concentration, followed by a relatively rapid decrease in the drug-blood concentration over time. This oral dosage form exhibits a relatively short duration of action as it does not sustain therapeutic blood levels over an extended time period.

Table 2.1: Various dosage forms of modified release systems

		Multiple-unit dosage form types					
		Monolithic tablet	Monolithic capsule	Layered tablet	Multi-units in capsule	Coated tablets	Multi-particulates
Modified release systems	Delayed						
	Pulsatile						
	Matrix						
	Reservoir						
	Osmotic						

To reduce the blood concentration fluctuations as well as repeated dosing, which is associated with immediate release dosage forms, modified release dosage forms have been developed (Abdul *et al.*, 2010:2-3; Ishida *et al.*, 2008:46; Rajabi-Siahboomi *et al.*, 2013:623).

This chapter focuses on the various types of oral modified release drug delivery systems, their mechanisms of action utilised in creating the specific drug release (section 2.2) and the manufacture of multiple-unit dosage forms (section 2.3).

2.2 MODIFIED RELEASE DRUG DELIVERY SYSTEMS

2.2.1 INTRODUCTION

Since the late 1800's, formulation of oral modified release dosage forms have been a developing study field to create a dosage form that can overcome gastrointestinal (GI) tract drawbacks. From 2006 until 2013 the quantity of oral modified release dosage form patents that were filed increased dramatically as pharmaceutical industries constantly want to improve medicine product performance (McConnell & Basit, 2013:551).

Modified drug release from a dosage form aims at delivering drugs at a desired rate and/or at pre-determined time points or specific target sites in the GI-tract (McConnell & Basit, 2013:551-555). Numerous drug release modification mechanisms can be used in different types of dosage forms as shown in Figure 2.1. Modified release drug delivery systems are either related to the type of formulation used (*e.g.* beads, granules and/or mini-tablets) or the manufacturing process (*e.g.* extrusion-spheronisation, tableting, film coating and/or encapsulation) (Porter, 2013a:580-581; Rajabi-Siahboomi *et al.*, 2013:623).

Immediate release drug delivery systems are often formulated as monolithic dosage forms that have to disintegrate within the 15 min time slot established by the British Pharmacopoeia (2015).

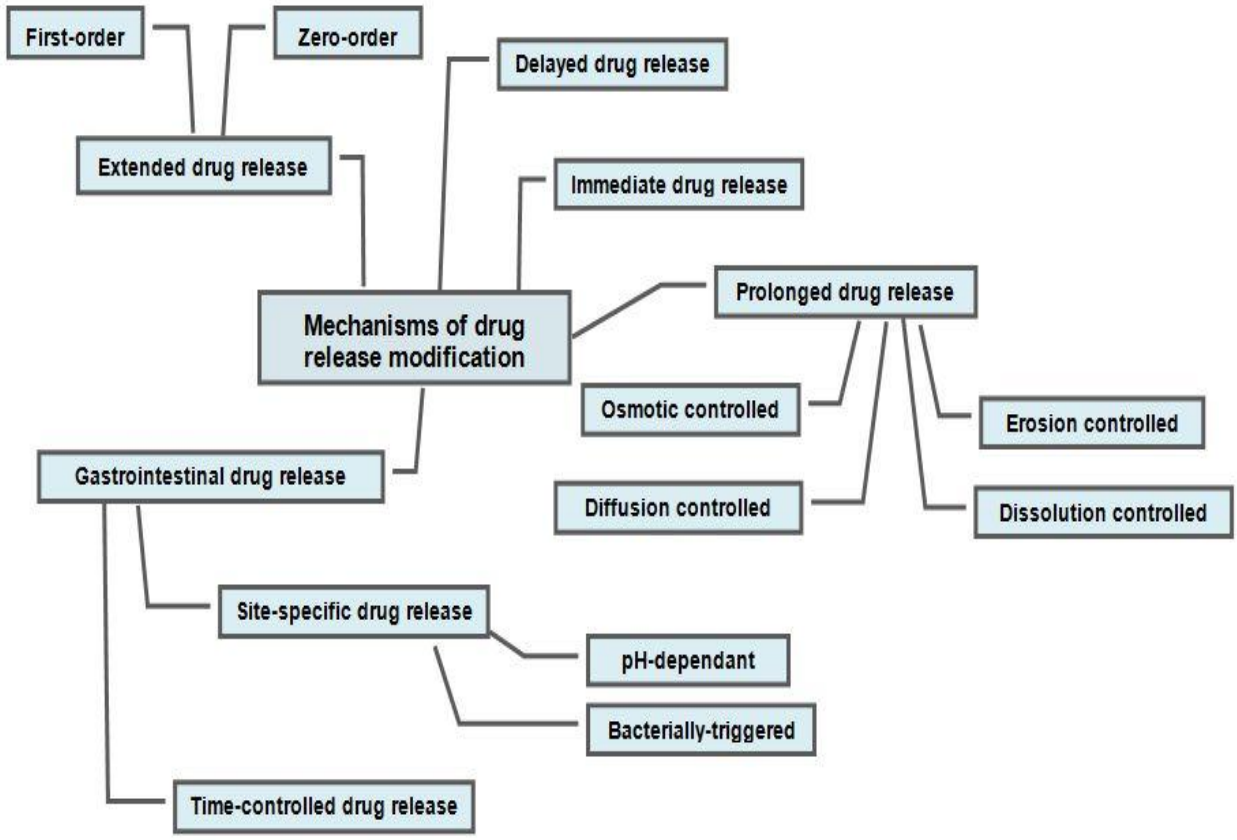


Figure 2.1: Types of modified release drug delivery systems and mechanisms of drug release modification (McConnell & Basit, 2013:551-552; Qiu & Zhou, 2011:23-30; Rajabi-Siahboomi *et al.*, 2013:623-631)

Furthermore, immediate release tablets and capsules are designed to be free of any rate-controlling qualities such as time-specific coatings or other formulation techniques (Mahato, 2007:156). On the other hand, modified release dosage forms are designed to provide sustained release, which has benefits over immediate release drug delivery systems, namely:

- Drug-blood levels are kept in the therapeutic range for a longer time period,
- Drug-blood levels can be kept relatively constant overnight time at non-dosing periods,
- Chronotherapy is made possible,
- Patient compliance is increased,
- Reduction in side effects can be obtained,
- Site-specific treatment in the GI-tract can be obtained (McConnell & Basit, 2013:551-552).

2.2.2 TYPES OF MODIFIED RELEASE DOSAGE FORMS

Modified release dosage forms are mainly divided into two categories, namely delayed drug release dosage forms and extended drug release dosage forms with numerous sub-categories as shown in Figure 2.2 (McConnell & Basit, 2013:551-552; Qiu, 2009a:469; Rajabi-Siahboomi *et al.*, 2013:623-631).

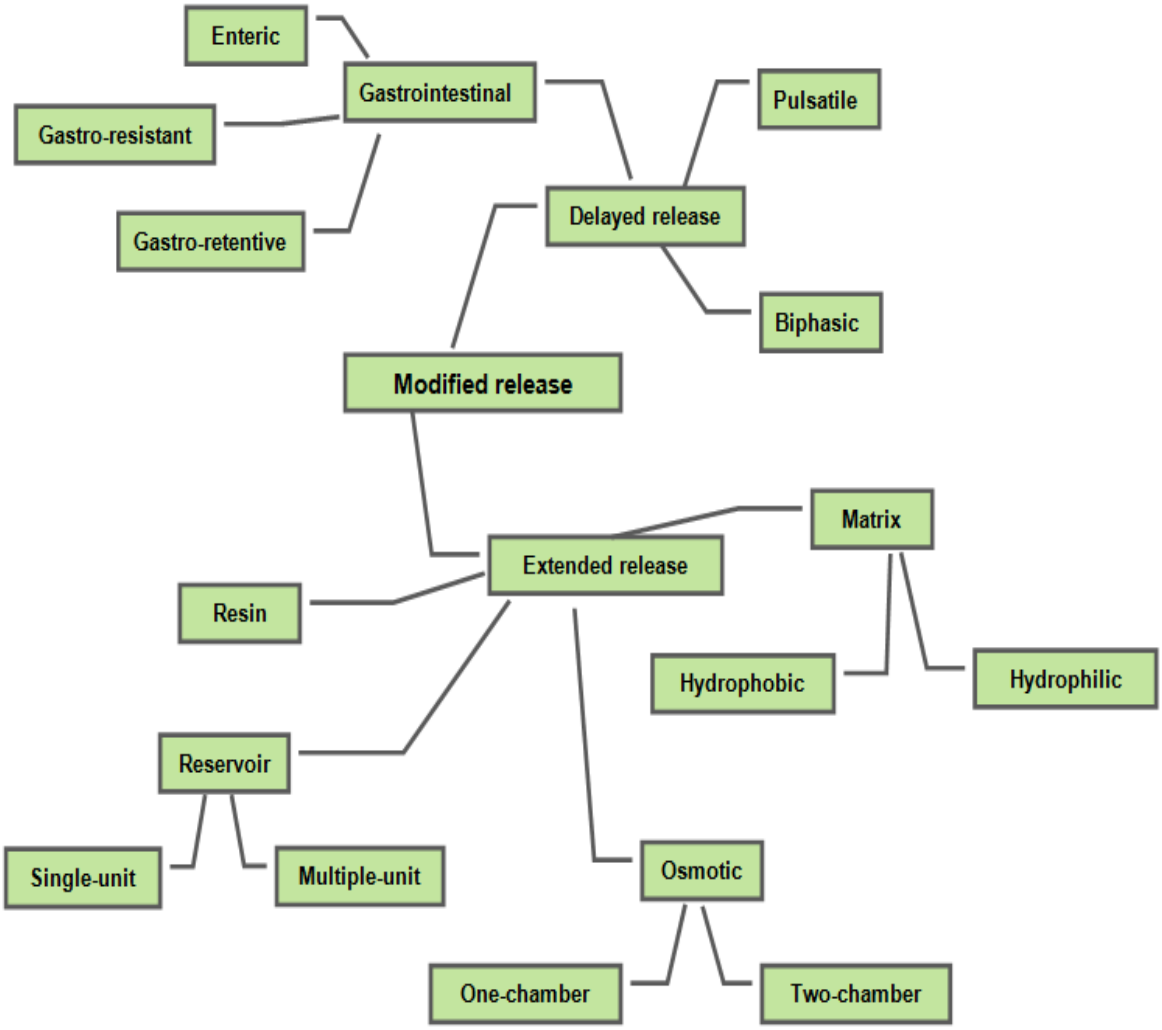
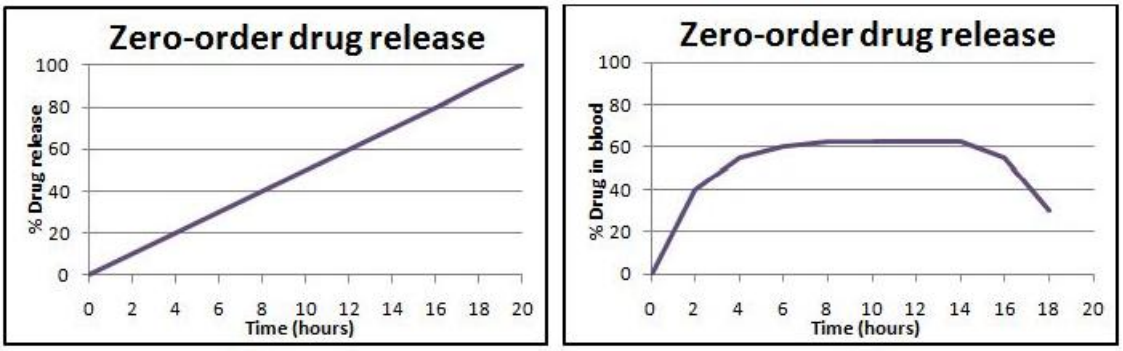


Figure 2.2: Modified drug release exposition (McConnell & Basit, 2013:551-552; Qiu, 2009a:469; Rajabi-Siahboomi *et al.*, 2013:623-631)

To provide the desired drug release profile, modified release systems must be altered to create a non-monolithic system with combined immediate release, delayed release and extended release properties. Mostly drug release profiles follow a zero-order or first-order drug release profile (Figure 2.3 and 2.4) (Qiu, 2009a:481).

A dosage form approaching zero-order drug release kinetics is independent of the concentration of reactants and thus has a constant drug release rate (Figure 2.3.a).

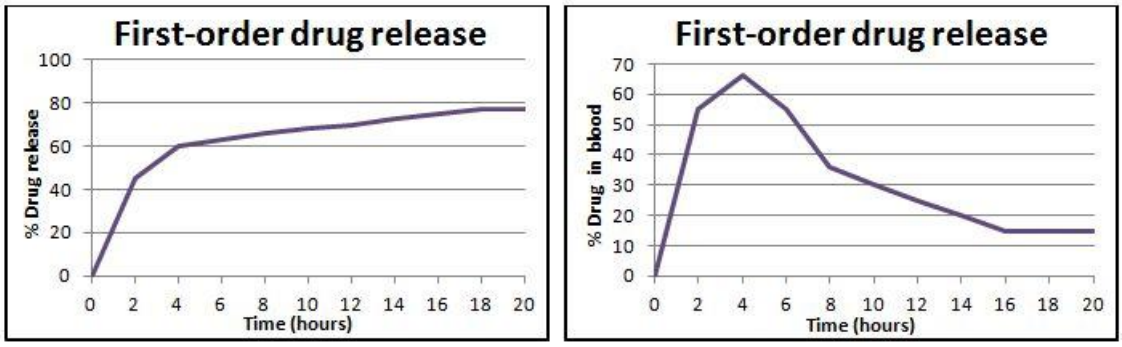
Drug release kinetics with a zero-order reaction typically occurs with drugs in suspension and/or controlled drug release dosage forms (Lund, 1994:213; Mahato, 2007:60-61; Pugh, 2013:119). Figure 2.3.b shows that during *in vivo* zero-order drug release, the drug-blood concentration builds up over time to a stable level that is maintained over an extended period of time (McConnell & Basit, 2013:557).



(a) **(b)**

Figure 2.3: Zero-order drug release profiles: **(a)** % drug release as a function of time; **(b)** % drug in blood as a function of time (McConnell & Basit, 2013:557)

First-order drug release can be defined as change in drug concentration as time changes (Figure 2.4.a), thus the drug concentration decreases exponentially over time (Figure 2.4.b) and do not maintain a constant drug-blood concentration (Mahato, 2007:61-63; McConnell & Basit, 2013:557; Pugh, 2013:116-117).



(a) **(b)**

Figure 2.4: First-order drug release profiles: **(a)** % drug release as a function of time; **(b)** % drug in blood as a function of time (McConnell & Basit, 2013:557)

Today in South Africa numerous pharmaceutical industries manufacture various types of modified release drug delivery systems as seen in Table 2.2 (Snyman, 2015:1-58).

Table 2.2: Summary of various modified release systems currently on the South African pharmaceutical market

Extended release			**Sustained release**		**Bi-layer**
Adalat®	Jurnista®	Venlor®	Tramazac®	Theoplus®	Tarka®
Alfuwin®	Klacid®	Wellbutrin®	Flomax®	TramaHexal®	**Delayed release granules**
Cardura®	Pexola®	Xatral®	Fortfen®	Tramal®	
Carzin®	Seroquel®	Concerta®	Illovex®	Ultipot®	Paser®
Ciprobay®	Vaticol®	Efegen®	Isoptin®	VeraHexal®	**Film coated**
ClariHexal®	Glucophage®	Efexor®	Ketoflam®	Voltaren®	Betaprofen®
Controlled release		**Long acting**	Adco-Vascard®	Anafranil®	Singulair®
Aropax®	Epilizine®		Adco-Zildem®	Augmentin®	Avigra®
Enablex®	Imdur®	Elantan®	Detrusitol®	Monicor®	Betacor®
Epilim®	Navalpro®	Inderal®	Nifedalat®	Wellbutrin®	**Beads**
Reminyl®	Tambocor®	Obex	Xanor®	Beniprosin®	Rinex®
Tegretol®	Tilazem®	Ritalin®	Calcicard®	Bezachole®	Topamax®

Prolonged release		**MUPS**		**Modified release**	
Advagraf®	Requip XL®	Losec®	Diaglucide®	Klarithan®	Stilnox®
Lyrinel®	Risperdal®	Nexiam®	Diamicron®	Omsal®	Uromax®
Oxycontin®	Zithromax®	Trustan®	Diaran®	Rantral®	Vercef®

2.2.3 DELAYED RELEASE SYSTEMS

Qiu and Zhou (2011:23) described delayed release as drug release that does not follow directly after drug administration, but rather occurs at a time period afterwards. A lag time therefore exits between a patient taking the medicine and when the drug is detected in the blood. In order to treat a diversity of illnesses, a variety of delayed release dosage forms are necessary to accommodate the different drug-blood profiles required. Numerous delayed release oral drug delivery systems consist of coated dosage forms *e.g.*, tablets, beads and MUPS, wherein single or multiple film layers are used to create a specific mechanism of delayed drug release and are explained below (Maroni *et al.*, 2013a:372).

2.2.3.1 Film coating

Porter (2013a:567) defined coating as a manufacturing process of layering the dosage form surface with a coating material. Dosage forms are coated to acquire specific characteristics such as modified drug release or to assist in product identification. Furthermore Porter (2013a:567) described that currently, pharmaceutical industries use one of the following three coating processes:

- Film coating,
- Sugar coating,
- Compression coating.

Film coating is a popular way to modify drug release as the coating material is applied to the outer layer of the dosage form. According to Bashaiwoldu *et al.* (2011:340), a film coating must thoroughly enclose a dosage form and remain intact throughout production, packaging and handling of the final product. Film coating is fairly flexible and a relatively wide range of products can be coated namely beads, granules, mini-tablets, tablets and even capsules (Torrado & Augsburger, 2008:520).

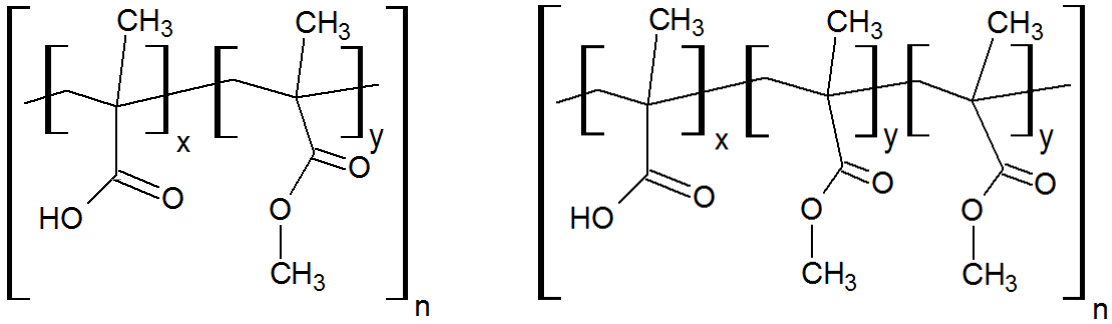
Various advantages exist for coated pharmaceutical dosage forms, including: protecting the drug from environmental factors *e.g.* moisture and light; taste masking; some patients swallow coated dosage forms (tablets) easier; creating modified release dosage forms with desired drug release kinetics; ensuring easy product identification, handling and improved esthetical product qualities, as well as improving dosage form appearance (Mahato, 2007:11; Porter, 2013a:567; Porter, 2013b:615).

According to Porter (2013a:568), film coatings can be divided into immediate release film coatings and modified release film coatings. Immediate release film coatings are water-soluble, whereas modified release film coatings can further be divided into enzymatically-degradable film coatings, pH-sensitive film coatings, pressure-sensitive film coatings and time-dependent film coatings as described by Maroni *et al*. (2013a:373). The following mechanisms of modifying drug release can be achieved by film coated dosage forms:

- A network of capillaries can be filled with dissolution media through which a drug is transported,
- Drug diffusion through a homogenous film barrier,
- Drug transport (*e.g.* diffusion) through a hydrated swollen film,
- Drug transport through cracks, flaws and imperfections within the coating of the matrix (Qiu, 2009a:477).

Various types of polymers can be used in the film coating process. Each polymer has unique characteristics needed in creating different drug release rates. Immediate release film coatings, for example, must utilise a polymer with a high solubility as rapid drug dissolution is crucial. In order to create modified release dosage forms, the following polymers can be employed: cellulose derivates *e.g.*, hypromellose (HPMC); methyl methacrylate copolymers; methacrylic acid copolymers and phthalate esters (Porter, 2013a:570,572-573).

In this study, the Eudragit® L 100 and Eudragit® S 100, anionic poly(methacrylate) polymers were utilised to apply a film coating of which the chemical structures are shown in Figure 2.5. The difference between the two chemical structures involves the ratio of the carboxyl groups to the ester groups. For the Eudragit® L 100 the ratio is approximately 1:1 whereas the ratio for in the Eudragit® S 100 structure is approximately 1:2 (Evonik, 2015:1).



(a) **(b)**

Figure 2.5: The chemical structure of poly(methacrylate) polymers. **(a)** Eudragit® L 100; **(b)** Eudragit® S 100

In 1955 Eudragit® L 100 and Eudragit® S 100 were the first anionic polymeric coatings established and implemented in the pharmaceutical industry to modify drug release from dosage forms. Presently, different types of poly(methacrylate) coatings exist, with various activity in time-controlled drug release in the GI-tract. Site-specific GI drug release can be acquired by adjusting the type of film coating used in order to obtain drug release once the pH in the GI-tract regulate. Poly(methacrylate) film coatings can be used in medicinal products to establish the following functions (Nollenberger & Albers, 2013:461-462,465-467):

- Taste masking,
- Moisture protection,
- Delayed drug release and/or,
- Extended drug release.

Prior to the coating process, the coating solution must first be prepared in such a way to exhibit the correct properties. Depending on the polymer or polymer combinations used, drug release occurs between pH-values 5.5 and 7.0 (Mahato, 2007:155; Nollenberger & Albers, 2013:465-468). In addition to the polymers, other excipients are added to the coating solution to assist in the film formation and physical properties of the final product. Anti-adherents such as talc are added to reduce film tackiness and bead agglomeration, whereas a plasticiser (*e.g.*, triethyl citrate or TEC) is added to reduce brittleness and increase flexibility of the film (Felton, 2010:104).

2.2.3.2 Pan coating process

Various coating machines and techniques can be employed to coat solid oral dosage forms such as beads, granules, multi-particulates, mini-tablets as well as tablets (Porter, 2013a:578, 581). Certain parameters need to be managed during the pan coating process which includes:

- Inlet process air temperature,
- Exhaust air flow temperature from the coating pan,
- Process flow rate to the coating pan,
- Pump speed and the suspension spray rate,
- Speed of the coating pan rotation (Porter *et al.*, 2009:783).

The pan coating setup includes a round pan rotating on an inclined axis. The pan's rotating movement causes the dosage form (*e.g.* beads) to appear frequently before the spray gun, while rotating. Heat is constantly blown onto the sprayed beads, allowing them to dry. Post coating, drying or curing is sometimes needed for complete film formation to occur. This curing process takes approximately 2 h at 40ºC (Evonik, 2012), but can be done under accelerated conditions for faster drying. If no curing process exist after coating, film merging will be incomplete and drug release will be affected over time (Felton, 2010:107,109-110).

2.2.3.3 Site-specific release systems

GI delayed drug release systems can be divided into enteric, gastro-resistant and gastro-retentive systems, depending on where the desired drug release must occur in the GI-tract. A variety of GI drug release mechanisms exist and the following drug release mechanism terms can be used interchangeably: "time-controlled drug delivery" and "site-specific drug delivery" (Nayak *et al.*, 2010:2; Pinto, 2010:50; Qiu, 2009a:481; Steubel *et al.*, 2006:501).

Qiu (2009a:481) classified the concepts of “time-controlled drug delivery” and “site-specific drug delivery” under the term “delayed drug delivery”. Pinto (2010:45-50) on the other hand, went into more detail describing time-controlled drug release as a drug delivery system influenced by the dosage form delivery system with drug release persisting 3-4 h after leaving the stomach. This author described site-specific drug release as a drug delivery system influenced by environmental factors such as pH or enzymes present in the GI-tract. Advantages of time-controlled oral drug delivery systems over immediate release drug delivery systems are:

- Limits drug-blood concentration fluctuations over a prolonged time period offering optimal therapeutic effects and a decrease in side-effects,
- Decrease in the daily dosage administration frequency resulting in increased patient compliance (Steubel *et al.*, 2006:501).

Steubel *et al.* (2006:501) explained that gastro-retentive site-specific drug delivery systems can offer an advantage over standard controlled release drug delivery systems. This is due to the fact that the gastric residence time (GRT) of the dosage form is prolonged, thus increasing the bioavailability in the stomach and upper small intestine.

2.2.3.3.1 Enteric release systems

Qiu and Zhou (2011:26) defined enteric drug release systems as dosage forms for which drug release is delayed, up until the dosage form progressed through the stomach and entered the small intestine. This delayed action can be achieved through film coating of the oral dosage form as described previously (section 2.2.3.1). An enteric film coated solid oral dosage form, therefore predominantly remains intact while progressing through the stomach where dissolution occurs when the small intestines are reached (Porter, 2013b:615).

2.2.3.3.2 Gastro-resistant release or colon specific drug delivery systems

McConnell and Basit (2013:551); as well as Qui and Zhou (2011:26), respectively defined gastro-resistant or colonic drug release as a type of delayed release system that ensures drug release in a specific pH-environment, or until the colon is reached to deliver immediate or controlled drug release in the large intestine. Drug release mechanisms for colonic drug delivery include site-specific targeting where pH, gut bacteria (microbiota), pressure or time-dependant polymeric film coatings (Table 2.3) are used to control drug release until the colon is reached (Maroni *et al.*, 2013a:372; McConnell & Basit, 2013:551). Colon-specific drug release is predominately used in the systemic treatment of inflammatory bowel diseases, for example, Crohn’s disease and ulcerative colitis (McConnell *et al.*, 2008:154).

Table 2.3: Mechanisms for gastro-resistant or colonic drug release (Maroni *et al.*, 2013a:372-374,380,382,387-389; McConnell *et al.*, 2008:154-155)

Type of coating	Mechanism
pH-sensitive	Both enteric- and acidic-soluble coatings can be utilised as pH-sensitive coatings as it starts dissolving as the specific pH site is approached. Enteric coatings, Eudragit® L/S 100, dissolve at pH 5.5 and 7.0, respectively. Acidic-soluble coatings can dissolve directly or indirectly due to change in pH caused by microbial fermentation processes within the colon.
Microbiota-activated	Naturally occurring polysaccharides (pectin, amylase, chitosan) or synthetic azo polymers have been utilised as film coatings or within matrix systems. These materials are activated by bacteria in the GI-tract to ensure drug release through enzymatic breakdown of the coating. Drug release of matrix dosage forms starts with breakdown of the coating surrounding the matrix followed by swelling of the matrix core.
Pressure-sensitive	Coating ruptures due to elevated intra-luminal pressure generated within the large intestine due to contraction of smooth muscle and compaction of solids, resulting in deformation of the solid dosage form resulting in drug release.
Time-dependent	Time-dependent coatings must be able to withstand exposure to gastric fluids, GI-tract bacteria and changes in pH. Accordingly, enteric coatings are used in order to delay drug release. Drug release then occurs through timed erosion, rupture or permeabilisation of the coating.

2.2.3.3.3 Gastro-retentive systems

Steubel *et al*. (2006:501) defined gastro-retentive dosage forms as a controlled drug release system with a prolonged GRT in the stomach (Goole *et al.*, 2008:310). Due to the short GRT of general extended release dosage forms in the stomach and upper intestine, gastro-retentive systems have been developed to eliminate this limitation. In order to prolong the GRT of the dosage form, the following gastro-retentive systems have been developed:

- Bioadhesive drug delivery systems adhering to mucosal surfaces in the stomach,
- Floating drug delivery systems controlled by the dosage form material density, allowing the dosage form to float on gastric fluids,
- Swelling drug delivery systems rapidly increase in size, blocking the passage through the pylorus sphincter (Pinto, 2010:47-48; Steubel *et al.*, 2006:501-506).

All types of the gastro-retentive drug release systems have a site-specific type of drug release mechanism in order to ensure drug release in the stomach and upper small intestine (Steubel *et al.*, 2006:501). Goole *et al*. (2008:310) stated that a multiple-unit floating system provided more efficient and sensible protection against early and unpredicted gastric emptying compared to single-unit systems. These multiple-unit dosage forms furthermore decrease the possibility of damage to the mucosa and created more reproducible GRT with improved drug dispersion through the GI-tract (Goole *et al.*, 2008:310).

2.2.3.4 Other delayed release systems

Several drug release mechanisms depend on an initial drug release component (loading dose) as part of the modified release dosage form. Combining an immediate release component with a delayed release component; modified release dosage forms with a programmed drug release, such as pulsatile and biphasic drug release can be manufactured (Long & Chen, 2009:327; Rathod *et al.*, 2014:545-547). These types of systems have the following combined advantages of immediate and delayed or extended drug release systems:

- Improved patient compliance due to less frequent dosing possible for medication of various acute and chronic conditions,
- Administration of solid oral dosage forms with liquid medication qualities and improved stability,
- Dosage forms can be designed with a desired drug release profile for optimised treatment (Long & Chen, 2009:327; Qiu, 2009a:481; Rathod *et al.*, 2014:545-547).

An immediate release component is essential for this type of modified drug release system. This component ensures rapid onset of action in addition to maintain drug release over an extended time period. From taking the medication immediate release components are generally required to release approximately 85% of the drug dose within 30 min (Long & Chen, 2009:327; Rathod *et al.*, 2014:545-547).

2.2.3.4.1 Pulsatile release systems

Pulsatile drug release systems are formulated for drug release to occur in a time-controlled manner where an initial dose is followed by a lag time before the next part of the dose is released, which can be repeated several times. The majority of oral pulsatile release systems are coated with a polymer to act as the drug release-controlling agent (Maroni *et al.*, 2013b:362; Qiu & Zhou, 2011:27).

A pulsatile drug release system has a non-monolithic and multi-cargo drug release drug-blood profile as it is compiled using a combination of immediate release and delayed release mechanisms. The delayed release mechanism of pulsatile release systems contain site-specific and/or time-controlled drug delivery mechanisms to ensure that the drug is released at a specific area in the GI-tract; or at a predetermined time. Pulsatile drug delivery systems have the following advantages: improved chronotherapy; natural endogenous secreting patterns can be resembled; and tolerance-induced drug therapy is improved because constant levels can cause receptor down-regulation (Qiu, 2009a:483).

2.2.3.4.2 Biphasic release systems

Biphasic drug release systems consist of an immediate release element added to a delayed release or extended release element in one dosage form for modified drug release. This system offers an initial rapid increase in the plasma concentration followed with extended drug release in order to decrease repeated dosing (Qiu & Zhou, 2011:27; Gaber *et al.*, 2015:87). Qui (2009a:483) described the non-monolithic biphasic or bimodal release system as one of the most commonly used modified release drug delivery systems with the following advantages:

- An immediate release-extended release combination dosage form that produce a rapid onset of action with extended drug release,
- Improved patient compliance due to optimised dosing schedules for chronotherapy drugs,
- Avoiding drug tolerance due to constant drug levels at a receptor site,
- Can subdue problems associated with non-linear pharmacokinetics, first-bypass metabolism and pharmacokinetic and/or pharmacodynamic reduced bioavailability and/or altered drug and/or metabolite metabolism (Qiu, 2009a:483; Qiu & Zhou, 2011:27).

2.2.4 EXTENDED RELEASE SYSTEMS

Extended release drug delivery systems predominantly maintain drug-blood and -tissue levels within certain limits for a prolonged time, thus reducing dosage frequency when compared to immediate release dosage forms (Ding *et al.*, 2005:939; McConnell & Basit, 2013:551).

Terms such as “sustained release”, “prolonged release”, “extended release” and “controlled release” have been used interchangeable in the literature to describe extended release

dosage forms (Alderborn, 2013:523; Qiu, 2009a:469). Lund (1994:208) for example, described sustained drug release as a delivery system that regulates drug release at a slow rate over a pre-determined time period. Ratnaparkhi and Gupta Jyoti (2013:11) defined sustained release as extended drug release over a specified time period, not depending on time.

Controlled release is described by various researchers as constant drug delivery at a pre-determined rate and/or location over a controlled period of time (Ding *et al.*, 2005:940; Lund, 1994:208; Mahato, 2007:155; Rajabi-Siahboomi *et al.*, 2013:624; Ratnaparkhi & Gupta Jyoti, 2013:12). This specialised extended type of drug release systems accomplish improved patient compliance as therapeutic blood levels are sustained and tend to diminish side effects occurring with multiple dosage regimens taken per day. Alderborn (2013:523), on the other hand, explained that "controlled drug release" is a misleading term, stating that all oral dosage forms, regardless of their formulation and use, should release a drug in a controlled and reproducible manner. Furthermore, Alderborn (2013:523) stated that no acceptable definition for "prolonged release" exists and that this term is subject to debate.

One or multiple mechanisms of drug release modification can be used to achieve extended drug release. Some of these modifications include (Porter, 2013b:615; Qiu, 2009a:471):

- Drug diffusion through pores in a barrier, channels or gel layer,
- Diffusion and/or erosion and dissolution due to system swelling,
- Osmotic pressure causing drug release.

Formulation of extended release dosage forms can be demanding and involves exceptional consideration of the physical-chemical (drug pKa; stability; solubility and molecular size) and biological (drug half-life; therapeutic index; dose size; absorption window and plasma concentrations) properties of the drug as this can affect the drug delivery system (Ratnaparkhi & Gupta Jyoti, 2013:13-14).

Controlling the rate and degree of drug release can fundamentally be divided into two mechanisms, namely: diffusion controlling drug release and dissolution controlling drug release. Extended release drug delivery can be enabled using the following drug release modification mechanisms: GI-fluid diffusion into the dosage form; dissolution of the dosage form releasing the drug; dissolving of a rate-controlling membrane enabling drug release (reservoir systems); hydration of the dosage form through swelling and/or dissolution (erosion) and diffusion of dissolved drug out of the dosage form through osmosis (Maderuelo *et al.*, 2011:5; McConnell & Basit, 2013:556; Rajabi-Siahboomi *et al.*, 2013:627,629-630).

2.2.4.1 Diffusion controlled drug release

Diffusion controlled drug release can be divided into matrix systems and reservoir systems, depending on the area of the release unit in which drug diffusion occurs. The rate-controlling step of this prolonged release system is through dissolution of the drug followed by diffusion out of the dosage form into the surrounding medium. During the whole diffusion process, the dosage form must stay intact. Diffusion controlled drug release follows two steps as described by Alderborn (2013:523-524):

- GI-fluid penetrates the dosage form in order to dissolve the drug. This establishes a concentration gradient between the interior and the exterior of the release unit that drives drug diffusion,
- The dissolved drug is then released into the surrounding medium via diffusion through the pores of the release unit or surrounding membrane (Alderborn, 2013:523-524; Qiu, 2009a:473).

Qui (2009a:473), on the other hand described diffusion controlled drug release from swellable systems by means of the following steps:

- GI-fluid penetrates into the matrix while lowering the polymer glass transition temperature,
- The polymer swells slowly and untwine followed by the drug dissolving in the gel section,
- The drug diffuses through the swollen gel section and is released into the external medium.

2.2.4.2 Dissolution controlled drug release

The rate-limiting step for dissolution controlled drug release is the drug dissolution in the GI-fluid rather than diffusion through the core matrix. Drugs, fillers and/or any other ingredients that are sparingly-soluble in fluids create a perfect dissolution controlled drug release system. Slow-dissolving coating can also be used in order to obtain prolonged drug release. Dissolution controlled drug release can be described by the following steps:

- GI-fluid surrounding the dosage form starts dissolving the outer membrane of the dosage form,
- The drug starts dissolving as it becomes more exposed to the GI-fluid (Alderborn, 2013:525).

2.2.4.3 Matrix release systems

Matrices have been described as monolithic systems composing of one or a combination of drugs entrapped in one or several release-controlling materials (Alderborn, 2013:523; Rajabi-Siahboomi *et al.*, 2013:626-630). Qiu (2009a:471) simplified the definition of a matrix drug release system as a drug release system consisting of a homogenously mixed drug in a rate-controlling material(s). Monolithic matrix materials can be directly compressed into tablets or mini-tablets resulting in an effortless, inexpensive manufacturing process (Kim, 2000:13; Riss *et al.*, 2007:78). Other advantages include simple up-scale of production processes; more dependable drug delivery as the system is capable of accommodating both low and high drug loading and consequently resulting in better patient compliance (Colombo *et al.*, 2008:433; Qiu, 2009a:472).

The mechanism of drug release occurs through drug erosion and/or diffusion of the drug through the pores in the dosage form or through the swollen gel front (Alderborn, 2013:523-525; Qiu, 2009a:471). The following formulation factors have an effect on the release rate of matrix systems:

- Drug amount incorporated in the dosage form,
- Release unit's porosity and the length of pores present in the dosage form,
- Solubility of the drug and excipient materials (Alderborn, 2013:525).

Furthermore, matrix release systems can be divided into hydrophilic and hydrophobic matrix systems, as previously shown in Figure 2.2.

2.2.4.3.1 Hydrophilic matrix systems

Hydrophilic or soluble matrix systems are defined as dispersed drug particles in a hydrophilic polymeric matrix (*e.g.* hydroxypropyl methylcellulose (HPMC) and microcrystalline cellulose). Drug release mechanisms and kinetics are predominantly determined by the solubility of the drug as well as the swelling and erosion of the formed polymer gel layer as shown in Figure 2.6 (Maderuelo *et al.*, 2011:2,5; Rajabi-Siahboomi *et al.*, 2013:627,629-630). Table 2.4 lists the disadvantages and advantages of hydrophilic matrix systems.

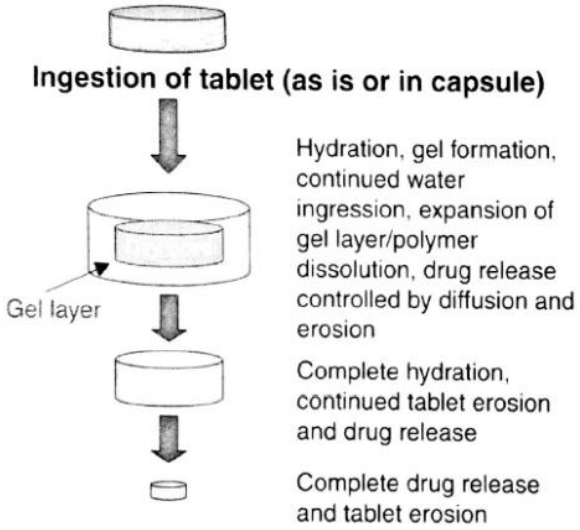


Figure 2.6: Schematic illustration that demonstrates the mechanism of drug release from hydrophilic matrix systems (Qiu, 2009a:472)

Table 2.4: Advantages and disadvantages of hydrophilic matrix systems (Qiu, 2009a:490)

Advantages	Disadvantages
Low and high drug loading is possible as drugs and materials can be used with various properties. Decrease in production time and cost as general manufacturing equipment is used. Drug release kinetics and profiles can be modified to suit the need. Multiple-unit dosage forms are possible.	Drug release is generally sensitive to test conditions. Dosage strength of single-unit systems is less flexible to adjustment. Relatively difficult due to complex processes/formulations (*e.g.*, layered or compression coated dosage forms).

2.2.4.3.2 Hydrophobic matrix systems

McConnell and Basit (2013:556-564) termed hydrophobic or insoluble matrix systems as a matrix comprising water-insoluble polymers or waxes with a drug dispersed within. The drug release rate of an insoluble polymer matrix depends on the rate of permeation of GI-fluid through matrix pores into the core for drug dissolution to occur as shown in Figure 2.7 (Rajabi-Siahboomi *et al.*, 2013:627-628). Table 2.5 lists the advantages and disadvantages of hydrophobic matrix systems.

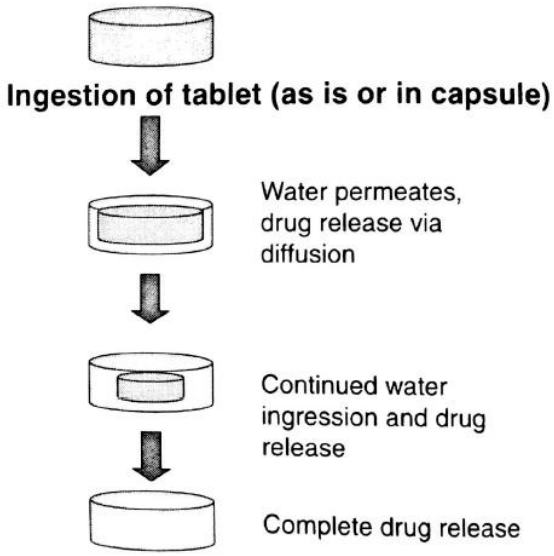


Figure 2.7: Schematic illustration that demonstrates the mechanism of drug release from hydrophobic matrix systems (Qiu, 2009a:472)

Table 2.5: Advantages and disadvantages of hydrophobic matrix systems (Qiu, 2009a:490)

Advantages	Disadvantages
Soluble drugs and materials can be used for low to high drug loading. General manufacturing equipment is used. Drug release kinetics and profiles can be modified to suit the need. Multiple-unit dosage formulations are possible.	Unsuitable for low solubility drugs and materials. Non-zero order drug release. Tendency for incomplete drug release exist. Drug release is generally sensitive to test conditions. Dosage strength of single-unit systems is less flexible to adjustment. Increased process/formulation difficulty as different drug release formulations are possible *e.g.*, layered or compression coated dosage forms.

2.2.4.4 Erosion controlled drug release

Erosion controlled drug release is a type of prolonged release system consisting of a matrix normally formed by tableting and can be described as a single-unit system. The matrix can consist of different pharmaceutical substances for example, waxes or polymers such as hydroxyethyl cellulose. Erosion is the time controlling step during drug release from the

matrix in which the drug is dispersed (Figure 2.8). Drug release from erosion controlled drug release systems can be described by the following steps:

- The dispersed drug in the matrix starts to dissolve from the surface of the bead or tablet.
- Furthermore, the dispersed drug is exposed to the GI-fluid, where the drug will mix and dissolve completely (Alderborn, 2013:525-526).

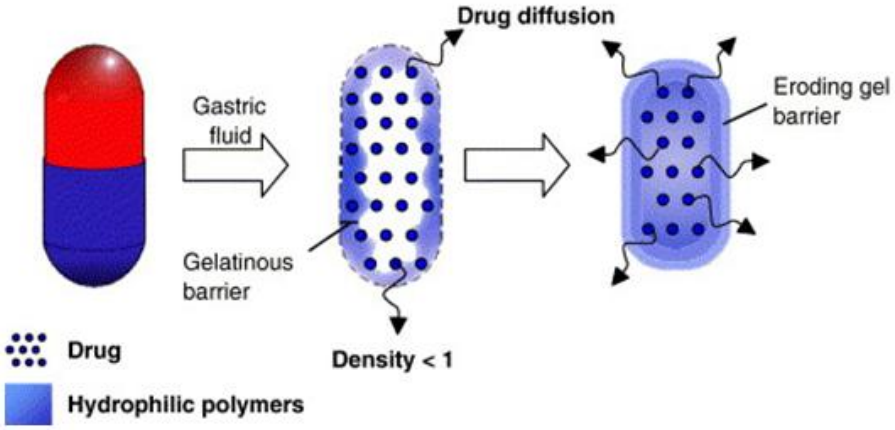


Figure 2.8: Schematic illustration of the mechanism of drug release by means of dosage form erosion (Bardonnet *et al.*, 2006:3)

2.2.4.5 Resin release systems

Jeong and Park (2008:26) stated that many studies have shown that ion-exchange resins can be used in drug delivery systems. An ion-exchange resin was defined by Jeong and Park (2008:26) as an electrostatic interaction of ions in solution and ion-exchange resins without alterations of resin properties and/or structure. Qiu (2009a:480) defined ion-exchange resins as “non-absorptive polymeric insoluble particles” incorporated with acidic or basic groups in duplicate on the polymer chain that can form ionic complexes with oppositely charged drugs. Both Jeong and Park (2008:26) and Qiu (2009a:480) explained that the drug bounded to the resin can be released into the system when interchanged with suitable charged ions in a favourable pH-environment in the GI-tract. Furthermore, one of the two groups may be neutralised when a favourable pH-environment is reached, thus removing the charge and releasing the drug (Jeong & Park, 2008:26; Kim, 2000:187; Qiu, 2009a:480). The advantage of ion-exchange resin drug delivery systems compared to other monolithic matrix systems is that high drug loading will not lead to faster drug release (Kim, 2000:188). Researched have showed that ion-exchange resins can be incorporated into hydrophilic matrix tablets in order to extend the drug release rate (Qiu, 2009a:481).

2.2.4.6 Reservoir release systems

Reservoir systems consist of a drug containing core surrounded by an enteric polymeric coating or a rate-controlling membrane. Drug release is limited by the polymer layer surrounding the active drug core. Usually reservoir systems are formulated in dosage forms such as pellets, beads, mini-tablets and tablets (Qiu, 2009a:477; Rajabi-Siahboomi *et al.*, 2013:627-630). Advantages of reservoir systems include; modifying drug release rates through various polymer types, concentrations and zero-order drug release. A couple of disadvantages also exist for these systems, namely:

- Potential of dose-dumping toxicity when the membrane is compromised,
- Increased manufacturing costs, and
- Difficulty in compounding high molecular weight systems (Ratnaparkhi & Gupta Jyoti, 2013:15).

Drug release transpires through diffusion of the drug through a thin water-soluble polymer film or membrane (± 5-20 µm in thickness) as GI-fluid enters the membrane surrounding the release unit. Reservoir systems are divided into single- and multiple-unit extended release systems as described below (Qiu & Zhou, 2011:25).

2.2.4.6.1 Single-unit systems

A simple diffusion system contains a water-insoluble polymer coating surrounding the drug core. In a single-unit system the drug is entrapped inside a single dosage form such as a tablet or capsule (Abdul *et al.*, 2010:3). The drug release mechanism depends on the diffusion rate of the drug through the coating layer (Rajabi-Siahboomi *et al.*, 2013:627-630).

2.2.4.6.2 Multiple-unit systems

Various drug release kinetics are possible with multiple-unit reservoir systems as multi-particulates with different release mechanisms and/or dose strengths can be incorporated into one dosage form, without requiring a new formulation. Multiple-unit reservoir systems are mostly used to minimise the risk of dose-dumping (Qiu, 2009a:477-478; Qiu & Zhou, 2011:25). Table 2.6 displays the advantages and some disadvantages of multiple-unit reservoir systems.

Table 2.6: Advantages and disadvantages of multiple-unit reservoir systems (Qiu, 2009a:490)

Advantages	Disadvantages
Reduced possibility for local irritations. *In vivo* performance is uniform. Safe in paediatric and geriatric use with simple dose adjustments *e.g.,* single formulations accommodating multiple strengths. Modified drug release kinetics and profiles already exist (biphasic, pulsatile and colonic). General manufacturing equipment is used.	Only highly soluble drugs and materials can be utilised. Drug loading is limited. Drug release is generally sensitive to test conditions. Manufacturing process development and scale-up are challenging.

2.2.4.7 Osmotic release systems

Alderborn (2013:526) defined osmosis as the flow or movement of solvent from a system with a low concentration of solute to a system with a high concentration of solute. The two systems or compartments are divided by a semi-permeable membrane, which allows flow of the solvent but not of the solute. Two types of osmotic release systems exist namely, one-chamber elementary osmotic pump (EOP) system (Figure 2.9.a) and a two-chamber push-pull osmotic pump (PPOP) system (Figure 2.9.b) (Qiu, 2009a:478). The difference between the two types of osmotic chamber systems is that the EOP system is for molecules with a limited solubility range of approximately 50-300 mg/ml to achieve zero-order and complete drug release, whereas the PPOP system was designed to accommodate less soluble molecules and/or higher drug loading (Verma *et al.*, 2002:8,10).

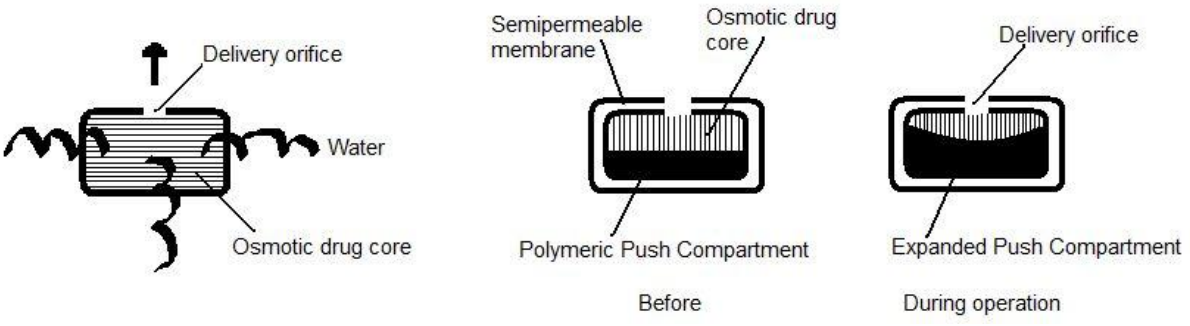


Figure 2.9: Different types of osmotic systems: **(a)** Elementary osmotic pump system; **(b)** Push-pull osmotic pump system (Qiu, 2009a:478)

Furthermore, the osmotic systems are prepared from osmotic agents located in the coated drug core of the bead. Drug release occurs through an opening (*i.e.* the delivery orifice) situated in the coating layer due to an osmotic pressure gradient generated as GI-fluid permeates into the drug core (Rajabi-Siahboomi *et al.*, 2013:627-630). Drug dissolution occurs within the drug core unit as the increased fluid volume in the unit increases the internal pressure, forcing the dissolved drug out into the surrounding medium to become available for absorption (Alderborn, 2013:526-527). Qiu (2009a:478) stated that this type of system is related to the reservoir drug release systems but contains osmotic agents in addition to the drug.

2.3 MULTIPLE-UNIT DOSAGE FORMS

2.3.1 INTRODUCTION

Multiple-unit dosage forms are primarily formulated as drug particles, crystals or beads, for example granules (irregular or spherical) or pellets incorporated within a capsule; filled into a sachet or pressed into a tablet or mini-tablet (Lund, 1994:212; Gaber *et al.*, 2015:86; Porter, 2013a:579-580). These multiple-unit dosage forms can be coated; and in comparison with immediate release dosage forms (tablets) have the following advantages: reduced adverse effects, toxicities and frequent dosing regimens; improved bioavailability and illness management; sustained blood concentration levels, and overall cost-effectiveness of drug therapy (Abdul *et al.*, 2010:3; Ishida *et al.*, 2008:46; Kim, 2000:3-4).

2.3.2 BEADS

According to Torrado and Augsburger (2008:510-512), different terms exist for solid particle systems used in pharmaceutical products. Different multiple-unit dosage forms exist, which include beads, pellets, granules, microspheres, microcapsules and aggregated particles. Torrado and Augsburger (2008:510-512) defined pellets or beads as “small, free flowing, spherical particles manufactured by the agglomeration of fine powders or granules of drug substances and excipients using appropriate processing equipment”.

Every so often the terms “pelletisation” and “granulation” have been used interchangeably. Granulation is a size enlargement process producing agglomerates ranging from 0.1 to 0.2 mm in size with relatively high porosity (20-50%); whereas pelletisation is a size enlargement process producing agglomerates (pellets) with a fairly narrow size range of 0.5 to 2 mm (Supriya *et al.*, 2012:43-44). Supriya, *et al.* (2012:44) defined pelletisation as an

agglomeration process for the conversion of fine powders or granules into “small, free flowing, spherical or semi-spherical units referred to as pellets”. To form pellets, beads or granules, several primary particle bonding mechanisms must be in place, namely:

- Adhesion and cohesion electrostatic forces on the surface of the powder particles,
- Interfacial forces in a mobile film form as extra liquid (binder liquid) is added to the powder mixture and distributed between the particles,
- Solid bridges form through partial melting, hardening binders and/or crystallisation of the dissolved substances as the solvent evaporates,
- The van der Waals forces existing between particles increase the final pellet, bead or granule strength (Aulton & Summers, 2013:473-475).

Certain advantages and disadvantages exist regarding pellets as a dosage form (Table 2.7); however, compressing these pellets or beads into tablets or mini-tablets creates a new type of dosage form, namely multiple-unit pellet systems or MUPS (section 2.3.3) (Bashaiwoldu *et al.*, 2011:340; Supriya *et al.*, 2012:43-44).

Table 2.7: Advantages and disadvantages of pellets as dosage forms (Bashaiwoldu *et al.*, 2011:340; Supriya *et al.*, 2012:43-44)

Advantages	Disadvantages
Improved product appearance, flow properties and uniformity of size with a narrow distribution; High bulk density and drug loading capacity without producing comprehensively larger particles and when formulated as modified release dosage form it’s less susceptible to dose-dumping and thus decreasing side effects.	Encapsulating beads in the final dosage form is more expensive; Possible coating damage can occur during the compression of beads into tablets to form a multiple-unit pellet system (MUPS).

2.3.2.1 Manufacturing methods: Pelletisation techniques

Various types of pelletisation techniques exist including balling; compression; globulation; freeze pelletisation; cryopelletisation; drug layering and extrusion-spheronisation. Extrusion-spheronisation is a multi-faceted process utilised in pellet/bead manufacture, exhibiting advantages over other pelletisation techniques (Supriya *et al.*, 2012:43-44,46).

2.3.2.1.1 Extrusion-spheronisation

Extrusion-spheronisation is getting increasingly popular as a technique to produce beads. The key elements in preparing beads by means of extrusion-spheronisation include dry blending, wet granulation, kneading, extrusion, spheronisation and drying (Sakr & Alanazi, 2013:594). Wet granulation follows after the binder liquid is slowly added in a controlled manner to the drug-filler powder mixture. Afterwards the wet granulate is forced through an extruder with pre-determined parameters to form homogenous, cylindrical extrudates. The powder mixture should have plastic properties ensuring the shaping and cohesive forces needed to acquire the non-friable, self-lubricating "rod shaped extrudate" prior to spheronisation (Müllers *et al.*, 2013:626). The extrudate is converted into consistently sized beads by means of a device called a spheroniser. This multi-bowl spheroniser (Caleva®, England) consists of a stationary cylinder with a smooth wall and a grooved rotating disc. Frictional and centrifugal forces generated with the rotating base plate spheronise and densify the extrudate to form beads. Extrusion-spheronisation has advantages over other techniques of bead preparation such as:

- Ability to regulate bead size within a narrow particle size distribution,
- Produce high density, low-friability beads,
- Exceptional surface characteristics for coated beads,
- Homogenous coating can be formed onto the beads,
- Ability to include higher levels of drug without producing exceptionally larger beads,
- The combination of two or more drugs in the same dosage unit,
- The inclusion of modified physical characteristics of beads,
- Production of exudates at normal temperatures without the decomposition of drugs due to high temperatures and the continuous manufacturing of heat-sensitive drug extrudates without temperature stress (Agrawal & Naveen, 2011:72; Müllers *et al.*, 2013:626; Sakr & Alanazi, 2013:594).

2.3.3 MULTIPLE-UNIT PELLET SYSTEMS (MUPS)

Reddy *et al.* (2011:1) defined MUPS as a single dosage form with the combined quality features of both modified release pellets in capsules and extended release/delayed release tablets. Thus, MUPS can be multi-particulates contained in a hard gelatine capsule forming MUPS-capsules or compressed multi-particulates to form a tablet or mini-tablet known as MUPS-tablets or mini-MUPS (Reddy *et al.*, 2011:1).

Although mini-tablets have been defined as tablets with a diameter equal to or smaller than 1 to 3 mm (De Brabander *et al.*, 2000:195; Lopes *et al.*, 2006:93; Tissen *et al.*, 2011:164), formulation scientists have also referred to tablets with a diameter of 4 to 6 mm as mini-tablets (Gaber *et al.*, 2015:86; Goole *et al.*, 2008:311; Ishida *et al.*, 2008:47; Sujja-Areevath *et al.*, 1996:53).

Mini-tablets are predominately manufactured through direct compression using powder, beads or granules, which can be filled into hard gelatine capsules in order to compile a multiple-unit delayed release/extended release dosage form (De Brabander *et al.*, 2000:195; Gaber *et al.*, 2015:86). Mini-tablet systems combine the advantages of multi-particulates with the more established manufacturing processes of tableting (Rajabi-Siahboomi *et al.*, 2013:630). Although tablet compression is a general dosage form manufacturing process, researchers have an "increased interest" in the compression of pellets (Lopes *et al.*, 2006:93).

Different kinds of MUPS can be made due to the fact that MUPS can be formulated with different excipients to form different systems for example, MUPS containing matrix beads (section 2.2.4.3) or MUPS containing coated (reservoir) beads (section 2.2.4.6). Advantages that MUPS have to offer compared to conventional modified release tablets and pellet filled capsules are:

- Once disintegration of the tablet occurs, beads are distributed uniformly inside the GI-tract defined by the bead size; increasing drug absorption, bioavailability and decrease potential local irritation of the mucosa,
- Uniform drug absorption and dissolution contribute towards controlled pharmacological action,
- Incomplete drug release and dose-dumping are decreased,
- Smaller tablet size increase patient compliance compared to capsules (Reddy *et al.*, 2011:1-2).

2.3.3.1 Manufacturing methods: MUPS tablets

Natoli *et al*. (2009:725); Sakr and Alanazi (2013:581) concurred that tablets remain the preferred choice in terms of the type of dosage form for administering medicines orally by professionals and patients alike. Natoli *et al*. (2009:725) also stated that in the pharmaceutical industry compressed tablets are one of the "most efficient" processes for single dose, dosage form manufacture.

Various models and types of tableting machines exist, where the single-punch design is the simplest. Compression of a tablet is accomplished through a single-punch machine following these steps: powder or granules from the feed shoe fills the die cavity directly when it aligns; all the powder or granules contained in the fill shoe retract and drag all excess granulation into the cavity; compression occurs when the upper punch is lowered into the die cavity containing the powder or granules; the tablet is ejected by the lower punch when the upper punch retracts from the cavity while the feed shoe returns to fill the die cavity for the following tablet compression; the compressed tablet is forced from the platform. Tablet weight can be determined through varying the volume of the cavity through adjustment of the lower punch (Sakr & Alanazi, 2013:595).

Mini-tablets as a modified release drug delivery system are becoming more important for the administration of incompatible drugs in a single dosage form as well as the oral administration of medication to paediatrics (Tissen *et al.*, 2011:164).

Avicel® (microcrystalline cellulose or MCC) is ordinarily considered an essential excipient in the manufacture of pellets/beads by means of extrusion spheronisation. Microcrystalline cellulose is generally recognised as an easily compressed material; however, the beads obtained from this excipient do not deform or brake easily as the beads are very hard (Torrado & Augsburger, 2008:514).

MUPS tablets can be prepared by compressing either uncoated or film coated beads. Coated beads in the formulation must be able to withstand compaction forces in order to compact into a tablet without breaking the coating. Torrado and Augsburger (2008:517) found that compaction of the coated beads led to the transformation of drug release. However, extended drug release qualities were not lost and the release kinetics was still useful for extended drug release. Bashaiwoldu *et al*. (2011:352) found that the rigidity of all pellet types increased through film coating, but the mechanical properties and thickness of the film were found to be less important during tablet compaction. During compaction film thickness influenced the amount of damage to the coating which in turn influenced the dissolution profiles of the drugs. Essential considerations in compaction of coated beads still able to maintain the wanted drug release, are: type of coating agent used as well as the coating amount, particle size, other excipients used, compaction forces whilst tableting and the tablet porosity after compaction (Torrado & Augsburger, 2008:517-525).

2.3.4 MINI-MUPS-IN-CAPSULE SYSTEM

Previously, pellets or beads as multi-particulate systems were usually contained in hard gelatine capsules (Ando *et al.*, 2007:99), but more recently mini-tablet-in-capsule dosage forms became popular (Ishida *et al.*, 2008:52; Reddy *et al.*, 2011:1). Li and Zhu (2004:381,389) developed a multi-functional multiple-unit oral dosage form containing versatile mini-tablets in a hard gelatine capsule with combined drug release behaviours. Ishida *et al*. (2008:52) on the other hand developed a controlled release mini-tablet with both immediate release and sustained release properties inserted into a single capsule.

In this study, the encapsulated mini-MUPS will consist of immediate release beads as well as coated beads to accomplish delayed drug release; thus forming a multiple-unit dosage form with different release properties. Several mini-MUPS can be contained in a capsule, each with a different dose, content and release profile. The inclusion of immediate release or uncoated beads enables the development of fast acting dosage forms with optimal pharmacokinetic properties for rapid release. Different delayed release pharmacokinetic properties can be included to ensure an optimal drug dosage form (Ishida *et al.*, 2008:47). Mini-MUPS are produced from the advantages that matrix systems and multiple-unit dosage forms offer, namely: well established production techniques *i.e.,* direct compression and extrusion-spheronisation. With an encapsulated mini-MUPS, drug doses can easily be modified. Figure 2.10 displays a schematic illustration of a mini-MUPS-in-capsule system (Riss *et al.*, 2007:78).

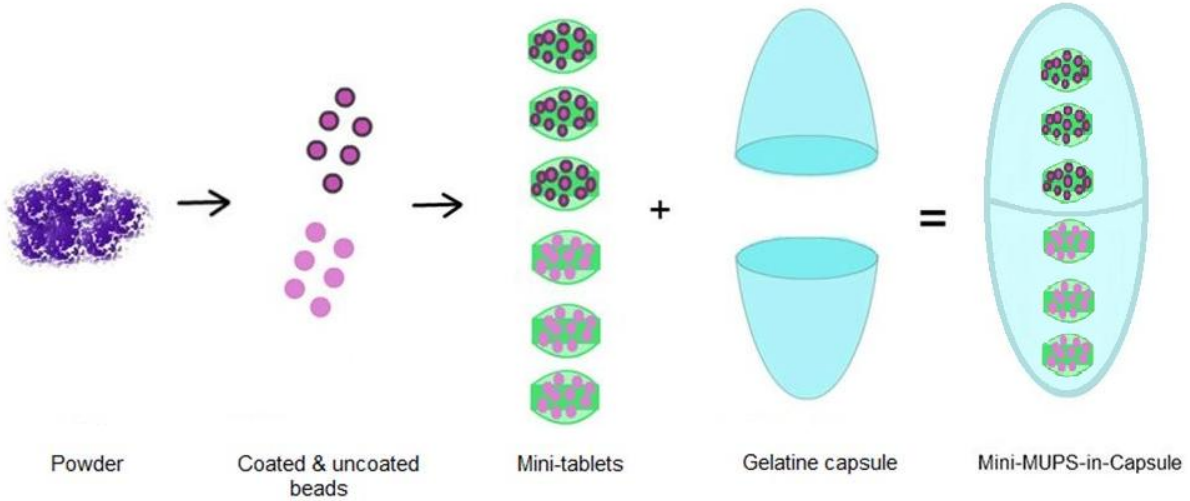


Figure 2.10 Illustration of the mini-MUPS-in-capsule system developed in this study

Advantages of the compaction and encapsulation of mini-MUPS compared to conventional modified release tablets and/or pellet-filled capsules include:

- Beads in the mini-MUPS will be rapidly released from the stomach into the small intestine with drug release as the pH-level increases. This will expectantly establish more uniform drug absorption and increased bioavailability,
- Dissolution of the coating combination of Eudragit® L 100 and Eudragit® S 100 will most likely establish sustained drug release and a decrease in the dose-dumping effect (Reddy *et al.*, 2011:1-2).

2.3.4.1 Manufacturing of mini-MUPS-in-capsule systems

In order to manufacture mini-MUPS various processes, as described throughout this chapter can be utilised. Beads can be manufactured by pelletisation techniques (section 2.3.2.1); and different types of film coating (section 2.2.3.1) can be used to produce coated beads to enable drug release at various sites in the GI-tract. Subsequently, tableting of the uncoated, coated or a mixture of uncoated and coated beads can be utilised to produce mini-MUPS. Once all the above-mentioned processes are completed, the mini-MUPS can be encapsulated to produce a mini-MUPS-in-capsule dosage form.

2.3.4.1.1 Capsules

Mainly two types of hard gelatine capsules exist, type A obtained from pork skins by acid processing and type B acquired from bones and animal skins through alkaline processing. Hard gelatine capsules contain two compartments; one fitting over the other, thus completely surrounding the drug formulation (Sakr & Alanazi, 2013:604).

One of the advantages of capsules is that it can contain a number of different drug release systems *e.g.,* beads, granules, mini-tablets and powders (Mahato, 2007:166). Other advantages include: capsules are tasteless, easily filled either extemporaneously or in large quantities. Patient compliance is increased due to easiness of drug administration as capsules are more easily swallowed than tablets. In practise capsules can provide single drug or multiple drugs administered in a single dosage form. This flexibility of dosage regimens are a huge advantage over tablets (Sakr & Alanazi, 2013:604).

2.4 SUMMARY

In recent years the amount of research conducted on modified release oral drug delivery systems were exponential. As modified release dosage forms can contain single- or multiple-units together with immediate release, delayed release or extended release drug components, various drug delivery systems can be created with numerous drug release

characteristics. Tablets remain the popular choice for the administration of medicine orally, but more recently mini-tablet manufacture became more popular due to incorporating incompatible drugs into one dosage form. The production of oral modified release drug delivery systems has various advantages compared to conventional oral dosage forms such as a decrease in side effects and an increase in duration of action and patient compliance. Utilising the multi-particulates of uncoated and/or coated multi-particulates into mini-tablets, MUPS are created with modified drug release.

Various oral modified drug release systems currently exist on the market. With research underway in order to improve and develop current and future modified release dosage forms; a dosage form can be developed with their own mechanism of drug release modification and manufacturing processes. The possibilities are endless for the manufacture of oral modified release systems as numerous advantages discussed in this chapter are known. Creating a mini-MUPS-in-capsule modified release system can have unlimited advantages for the pharmaceutical industry.

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 INTRODUCTION

In order to develop unique mini-MUPS-in-capsule drug delivery systems, various excipients were investigated in this study. Different tablet filler materials (*i.e.* Avicel® PH 101; MicroceLac® 100 and RetaLac®) were used to manufacture beads containing the water-insoluble and water-soluble model compounds furosemide and pyridoxine, respectively.

This chapter focuses on the various materials used in all the manufacturing processes and the different methods utilised to develop and evaluate the mini-MUPS-in-capsule systems. These methods include ways to determine and compare the flow properties of the pure filler powders and uncoated and coated bead formulations; the evaluation of the physical properties and dissolution behaviour of the compressed uncoated and coated beads, dissolution behaviour of the mini-MUPS-in-capsule systems, as well as accelerated stability testing for a period of six months.

3.2 MATERIALS

The materials utilised in this study, together with their batch numbers and manufacturers are summarised in Table 3.1. All of the materials were of analytical grade and were used as supplied.

3.2.1 Selection of model compounds

The model compounds were specifically chosen in this study to determine how a water-insoluble drug (*i.e.* furosemide) and a water-soluble drug (*i.e.* pyridoxine) are released from the prepared mini-MUPS-in-capsule systems.

Table 3.1: Materials, batch numbers and manufacturers of materials used in this study

Material	Batch number	Manufacturer
Acetone	020507AO	Rochelle Chemicals, Johannesburg, South Africa
Avicel® PH 101	60839C	FMC BioPolymer, Ireland
Cyclohexane	7338	ACE Chemical Enterprises, Johannesburg, South Africa
Ethanol (99%)	120315ET	Rochelle Chemicals, Johannesburg, South Africa
Eudragit® L 100	B120603009	Evonik Industries, D-64293, Darmstadt, Germany
Eudragit® S 100	B120705005	Evonik Industries, D-64293, Darmstadt, Germany
Furosemide	M110401	Warren Chem Specialties, Johannesburg, South Africa
Isopropyl alcohol	BX240	Allied Signal Inc., Burdick & Jackson, Muskegon, USA
Kollidon® VA 64	93520356P0	BASF The Chemical Company, Ludwigshafen, Germany
Magnesium stearate	21203	Warren Chem Specialties, Cape Town, South Africa
MicroceLac® 100	022-96887	Meggle, Wasserburg, Germany
Pyridoxine hydrochloride	H898a-1312209	Warren Chem Specialties, Cape Town, South Africa
RetaLac®	L416300	Meggle, Wasserburg, Germany
Talc	18654	Sigma-Aldrich, Johannesburg, South Africa
Triethyl citrate	445571/1 40703249	Fluka Chemika, Sigma-Aldrich GmbH, Switzerland

Furosemide is a high-ceiling diuretic, commonly used in the treatment of cardiac, hepatic or renal oedema and mild to moderate hypertension in patients with renal impairment (Rossiter, 2014:152-153). Given the narrow window of absorption in the gastrointestinal tract (GI) for the weak acid furosemide (pKa 3.9), absorption occurs mostly in the proximal part of the GI-tract. Due to the spontaneous agglomeration and cohesive properties of the drug particles, as well as poor solubility, poor wettability and the highly lipophilic character of furosemide, drug absorption is controlled by the dissolution rate in the GI-tract (Boopathi *et al.*, 2013:5053; de Kock, 2005:56-57). Approximately 60-70% of a furosemide oral dose is absorbed with a half-life ($t_{½}$) of 0.5-1 h (Rossiter, 2014:152-153). According to the British Pharmacopoeia (2015) furosemide is practically insoluble in water and freely soluble in solutions of alkali hydroxides, thus the rate of absorption occurs faster once the drug enters the small intestine (BP, 2015; Laulicht *et al.*, 2011:314).

Pyridoxine, or more commonly known as vitamin B_6, can be used in the management of vitamin B deficiency; isoniazid-induced peripheral neuropathy, idiopathic sideroblastic anaemia, and in nausea and vomiting as a symptom of premenstrual syndrome. In

combination with other vitamins and minerals it is also used as a multivitamin taken daily (Rossiter, 2014:92-93). It primarily functions as a coenzyme in the synthesis and breakdown of amino acids. Pyridoxine exhibits extremely high water-solubility (1 in 5 parts of solute) and is readily absorbed from the GI tract (BP, 2015).

Given the information provided, furosemide and pyridoxine together with the different filler materials will contribute to the drug release profiles of the mini-MUPS-in-capsule drug delivery systems developed in this study as notable by evaluation of various formulation and processing variables (de Kock, 2005:56-57).

3.3 FORMULATION OF BEADS

3.3.1 Factorial design of experiments

In order to study the effects of different formulation factors on the physical and dissolution properties of mini-MUPS-in-capsule drug delivery systems, a full factorial design was employed on the mini-MUPS prepared from the uncoated beads. Factorial design is an important statistical method to determine significant interactions and effects of numerous variables (factors) as well as different levels thereof, on a response. Traditionally, trial-and-error experiments were intended to define the effect of ONE variable on ONE response. However, it has been shown that there are numerous advantages in combining a study of multiple variables in the same factorial experiment. Factorial design can condense the number of experiments that has to be performed by studying multiple factors concurrently. Moreover, it can be utilised to obtain both main effects (from each independent factor) and interaction effects (when both factors must be used to explain the outcome). In formulation, it offers an unbiased, methodical guide to evaluate numerous excipients and their concentrations on dosage form performance, resulting in optimisation of these formulations. It can therefore be stated that the use of this statistical tool eliminates the guesswork from experimentation (de Kock, 2005:120-121).

In this study, four formulation variables were evaluated, namely type of drug (*i.e.* water-soluble or insoluble), type of filler (*i.e.* Avicel® PH 101; MicroceLac® 100 and RetaLac®), binder (*i.e.* Kollidon® VA 64) concentration, and lubricant (*i.e.* magnesium stearate) concentration. Abbreviations were assigned to the factors measured and are presented in Table 3.2. The factors were evaluated at different levels, *i.e.* two types of drug (furosemide and pyridoxine); three types of fillers (*i.e.* Avicel® PH 101; MicroceLac® 100 and RetaLac®), three binder concentrations (0, 3 and 5% w/w); and three lubricant concentrations (0, 0.05 and 0.1% w/w), respectively.

Table 3.2: Formulation factors, variables and levels investigated in this study

Factors	Variables	Levels
Type of drug	Furosemide (F)	2
	Pyridoxine (P)	
Filler material	Avicel® PH 101 (Avi)	3
	MicroceLac® 100 (Mic)	
	RetaLac® (Ret)	
Binder (Kollidon® VA 64)	0% w/w	3
	3% w/w	
	5% w/w	
Lubricant (Magnesium stearate)	0% w/w	3
	0.05% w/w	
	0.1% w/w	

Table 3.3 shows the factorial design with the different compressed uncoated bead formulations (mini-MUPS) that were prepared; for example, Avi0.05F3 represents uncoated beads containing Avicel® PH 101 as filler with furosemide as active ingredient, 0.05% w/w magnesium stearate, and 3% w/w Kollidon® VA 64 were added after uncoated bead manufacture to produce mini-MUPS. Similarly, Ret0.1P5 signifies uncoated beads containing RetaLac® as filler with pyridoxine as active ingredient, with the addition of 0.1% w/w magnesium stearate, and 5% w/w Kollidon® VA 64 in order to produce mini-MUPS.

Table 3.3: Factorial design

				Active ingredients					
				Furosemide			Pyridoxine		
				Binder: Kollidon® VA 64					
				0%	3%	5%	0%	3%	5%
Filler type	Avicel® PH 101	Lubricant: Magnesium stearate	0%	Avi0F0	Avi0F3	Avi0F5	Avi0P0	Avi0P3	Avi0P5
			0.05%	Avi0.05F0	Avi0.05F3	Avi0.05F5	Avi0.05P0	Avi0.05P3	Avi0.05P5
			0.1%	Avi0.1F0	Avi0.1F3	Avi0.1F5	Avi0.1P0	Avi0.1P3	Avi0.1P5
	MicroceLac® 100		0%	Mic0F0	Mic0F3	Mic0F5	Mic0P0	Mic0P3	Mic0P5
			0.05%	Mic0.05F0	Mic0.05F3	Mic0.05F5	Mic0.05P0	Mic0.05P3	Mic0.05P5
			0.1%	Mic0.1F0	Mic0.1F3	Mic0.1F5	Mic0.1P0	Mic0.1P3	Mic0.1P5
	RetaLac®		0%	Ret0F0	Ret0F3	Ret0F5	Ret0P0	Ret0P3	Ret0P5
			0.05%	Ret0.05F0	Ret0.05F3	Ret0.05F5	Ret0.05P0	Ret0.05P3	Ret0.05P5
			0.1%	Ret0.1F0	Ret0.1F3	Ret0.1F5	Ret0.1P0	Ret0.1P3	Ret0.1P5

3.3.2 Bead manufacture by extrusion-spheronisation

Each powder mixture consisting of filler and a model drug (as indicated in the factorial design) was wetted with a mixture of distilled water and ethanol that was slowly added in a controlled manner by means of titration with a burette. The powder was mixed using a mortar and pestle, while wetted. Each wet mass was extruded through a 1 mm sieve (Extruder 20, Caleva®, England) followed by spheronisation with a multi-bowl spheroniser (Caleva®, England) set at predetermined times and speeds as depicted in Table 3.4 (Bashaiwoldu *et al.*, 2011:341). The spheronised beads were air dried in an EcoTherm Labotec oven at 40ºC and weighed every 60 min until there was no variation in the bead mass. The dried beads were sieved using an aperture size of 1.68 mm to establish a more uniform particle size distribution. During the extrusion-spheronisation process it was not possible to prepare the different bead formulations under exactly the same settings, thus small changes were made for each formulation to ensure successful bead preparation as shown in Table 3.4.

Table 3.4: Preparation settings for bead production by means of extrusion-spheronisation

Filler	**Avi***		**Mic***		**Ret***	
Active ingredient	**F***	**P***	**F***	**P***	**F***	**P***
Filler/Drug combination (%)	90/10	90/10	90/10	90/10	90/10	90/10
Binder liquid (Distilled H_2O/Ethanol ratio)	80/20	80/20	74/26	74/26	17/83	17/83
Binder liquid/Powder ratio	1:1	1:1	1:2	1:2	2:1	2:1
Extrusion speed (rpm)	29	29	35	35	32	32
Spheronisation speed (rpm)	1829	2531	1862	1565	2909	2060
Spheronisation time (min)	10	10	8	8	10	0.5

**P = pyridoxine, F = furosemide, Avi =Avicel® PH 101, Mic = MicroceLac® 100, Ret = RetaLac®*

3.3.3 Film coating of beads

3.3.3.1 Introduction

Half of each batch of beads prepared according to the factorial design of experiments were spray coated in a rotating pan coater (Associated Electrical Industries (PTY) LTD, South Africa) using a spray gun with the enteric coating solution for which the composition is shown in Table 3.5.

Table 3.5: Film coating composition

Ingredient	Function	Mass (g)
Eudragit® L 100	Polymer	6.25 g
Eudragit® S 100	Polymer	6.25 g
Triethyl citrate	Plasticiser	1.25 g
Talc	Anti-tacking agent	6.25 g
Acetone	Diluent	68.58 g
Isopropyl alcohol	Diluent	102.84 g
Water	Diluent	8.58 g
	Total	**200.00 g**

In order to compile the coating solution, acetone, isopropyl alcohol and water were mixed to form the solvent. A suspension was prepared from Eudragit® L 100 and Eudragit® S 100 dispersed in half the solvent mixture and mixed for 30 min. Another suspension consisted of talc and triethyl citrate dispersed in the other half of the solvent, which was mixed for 10 min. These two separate suspensions were combined and mixed for an additional 10 min. The beads were film coated using a rotating pan and spray gun. The air heater was turned on 10 min prior to coating in order to heat up the coating pan. The spray suspension was continuously stirred with a gas lift attached to the spray gun in order to prevent caking of the suspension (Evonik, 2012). After coating the beads, it was left for approximately 5 min in the coating pan and rotated to ensure further drying. The coated beads were air dried in an EcoTherm Labotec oven at 40ºC for approximately 24 h (Evonik, 2012).

3.3.4 Preparation of bead mixtures for compression of mini-MUPS

Uncoated beads and coated beads prepared from each formulation (150 g) as indicated in the factorial design (Table 3.3) were placed in separate tightly sealed glass containers. Prior to compression of each formulation, the binder and/or lubricant were added, according to formula composition (Table 3.2) and mixed in a Turbula® mixer (Turbula® T2C, W.A. Bachofen AG Maschinenfabrik, Basel, Switzerland) for 7 min at 69 rpm. After mixing, the various mixtures were stored in tightly sealed glass containers in a dark cupboard at a temperature below 20ºC until compression.

3.4 EVALUATION OF POWDER AND BEAD FLOWABILITY

3.4.1 Introduction

The flow characteristics of a specific powder are directly dependent on the physical properties of the particles within the powder bed. It is not realistic to describe the flow of a

powder with a single flow test as this may not provide sufficient information of its flow properties. For these reasons, multiple testing methods/techniques should be used to analyse the way a powder flows in a given processing environment (Prescott & Barnum, 2000:60). This section describes the different methods utilised to determine the flow properties of the different filler powders as well as the prepared uncoated and coated beads. All methods were performed according to the British Pharmacopoeia (2015) and were done in triplicate.

3.4.2 Morphology of powders and beads

The surface morphology and internal structure of the filler powder particles and the formulated uncoated and coated beads were evaluated by means of scanning electron microscopy (SEM). The powder and bead samples were each mounted on a metal disc with a silicon adhesive and coated in an Eiko® ion coater (model IB-2, Eiko engineering, Japan) using a gold and palladium (66:34) mixture under a vacuum of 1.5 torr. After coating, the internal structures and surfaces of the powders and beads were determined by means of scanning electron microscopy using an Environmental Scanning Electron Microscope with a Field Emission Gun (FEI Quanta® 250, Netherlands). Micrographs of the external surface and internal structure of the powders and beads were taken at several magnifications ranging from 200x to 8000x (Chinyemba, 2012:22).

3.4.3 Particle size and size distributions

Particle size and size distribution analysis of the different filler powders, coated and uncoated beads were determined by means of laser diffraction using a Malvern Mastersizer 2000 instrument fitted with a Hydro 2000 SM small volume sample dispersion unit and a Hydro 2000 MU dispersion unit (Malvern Instruments, Malvern, UK), respectively. The Hydro 2000 SM dispersion unit was engaged during the particle size analysis of the pure powder samples of the fillers, whereas the Hydro 2000 MU was fitted on the instrument for size analysis of the bead formulations at a stirring rate of 2000 rpm. The dispersion medium was chosen for each sample to prevent dissolution of the particles/beads. Absolute ethanol was used as dispersion medium for all the filler powders and uncoated beads of the fillers Avicel® PH 101 and MicroceLac® 100, whereas distilled water was utilised to disperse the coated beads formed from the fillers Avicel® PH 101 and MicroceLac® 100. Cyclohexane was employed as dispersion medium for the uncoated and coated beads prepared from the filler RetaLac®. The small volume dispersion unit was filled with 100 ml dispersion medium, whereas the dispersion unit for the bead formulations was filled with 800 ml dispersion

medium. Prior to particle size analysis, a background measurement was taken to compensate for possible interferences from the dispersion medium as well as potential electrical interferences. In the case of the powders, samples were dispersed in 6 ml absolute ethanol preceding addition to the small volume dispersion unit. A sufficient quantity of the powder sample was added to obtain an obscuration of between 10 and 20%. Analysing the bead size, approximately 1 g of each bead formulation was added to the beaker connected to the Hydro 2000 MU dispersion unit. After a suitable obscuration (≥ 1%) was obtained, the particle sizes of each of the samples were measured. Each measurement consisted of 12 000 snaps (12 sec). The particle size and size distribution of each sample were measured in triplicate and the average size was calculated with Malvern Software (Malvern Instruments, Malvern, UK).

3.4.4 Density

Flow of a powder is significantly affected by the density of that powder. Powders with a higher density are inclined to have a poor flowability, whereas a less dense powder flows better. Density is defined as the weight-volume ratio of a material expressed in $g.cm^{-3}$. Different parameters can be used to obtain information of powder density and frequently used descriptors of density include true density, bulk density, tapped density and porosity (de Kock, 2005:65; Jallo *et al.*, 2012:213-214).

Amidon *et al.* (2009:168) defined the bulk density of a powder as the mass per unit volume including all the inter-particular and intra-particular spaces between the powder particles, whereas tapped density was described as the ratio of the mass to the volume powder occupied in a container after it has been tapped for a predetermined time period, thus eliminating some of the inter-particular spaces between the powder particles.

The bulk and tapped densities of the selected filler powders and the uncoated and coated beads were determined with an Erweka® Tapped Density Tester (SVM 121/221, Germany). A sample of 100 g of each filler powder, as well as the mixtures of the uncoated and coated beads was gently poured into a graduated cylinder and the initial volume it occupied was noted as the bulk volume for each powder or bead formulation. The cylinder containing each powder, uncoated and/or coated bead sample was placed in an Erweka® Tapped Density Tester and set at an amplitude of 5 A. The cylinder was vibrated until a constant volume was obtained which was noted as the tapped volume for each powder or bead formulation. The different densities were calculated from these measurements with the equations given below.

3.4.4.1 Bulk density

Equation 3.1 can be used to determine the amount of powder that can fit in a space including all the inter-particular and intra-particular spaces between the powder particles, which are referred to as the bulk density.

$$\rho_b = \frac{m}{V_b} \quad \textbf{[3.1]}$$

Where P_b is the bulk density, m the mass and V_b the initial volume of the powder/beads in the container (BP, 2015).

3.4.4.2 Tapped density

Amidon *et al.* (2009:168) defined "tapped density" as the ratio of the mass to the volume powder occupied in the container after it has been tapped for a predetermined time period, thus eliminating some of the inter-particular spaces between the powder particles. Equation 3.2 can be used to calculate the tapped density:

$$\rho_t = \frac{m}{V_t} \quad \textbf{[3.2]}$$

Where P_t is the tapped density calculated as the ratio of the mass (m) to the final tapped volume (V_t) of the powder/beads in the container (BP, 2015).

3.4.4.3 Carr's index and Hausner ratio

The bulk and tapped densities were used in calculating the percentage compressibility (or Carr's index) as well as the Hausner ratio (BP, 2015). The following equations were used:

$$\text{Hausner ratio} = \frac{\rho_t}{\rho_B} \quad \textbf{[3.3]}$$

$$\% \text{ Compressibility} = \frac{\rho_t - \rho_B}{\rho_t} \times 100 \quad \textbf{[3.4]}$$

Where P_t represents the tapped density and P_b the bulk density.

3.4.5 Critical orifice diameter

Critical orifice diameter (COD) is defined as the smallest diameter through which a powder can freely flow. The apparatus and method developed by Buys and co-workers (2006:40) were used to determine the COD of the selected filler powders and uncoated and coated bead formulations. The apparatus consisted of copper disks with altered orifice sizes through their centres, ranging from 1.5 to 32 mm. These copper disks are placed on top of each other to form a smooth tunnel.

A cylinder containing 100 g of each filler powder or a formulation of uncoated or coated beads was placed on top of the stacked copper disks, with the widest opening at the top. The assembly were equipped with a shutter and lifted 10 cm above the parallel surface. The shutter was opened to start the powder flow into the smooth tunnel, orifice disks were removed from the bottom till the powder started to flow freely through. The disk that allowed free flow was noted as the critical orifice diameter of the powder or bead formulation (BP, 2015; Buys, 2006:40-41).

3.4.6 Flow rate

Flow rate describes the quantity of powder or beads that is discharged through a funnel per time period. Using a powder flow meter (Apollo Scientific, Erweka, Johannesburg, South Africa), the flow rate was measured by placing 100 g of powder or bead formulation in the funnel. The flow rate was determined when the shutter placed over the funnel outlet was opened, allowing the mixtures to freely flow through the orifice, as determined by the COD flow test. In this study the flow rate was calculated according to weight with the following equation:

$$F = \frac{M}{t} \qquad \textbf{[3.5]}$$

Where F is the flow rate in $g.s^{-1}$; M is the mass (g) and t is the time (s) (BP, 2015).

3.4.7 Angle of repose

A total amount of 100 g of filler powder, uncoated or coated bead formulation was poured into a stainless steel funnel fitted with a shutter (10 mm diameter orifice) and placed at a fixed-height above a glass surface (Lavoie *et al.*, 2002:887). The shutter was opened and the powder or bead mass was discharged from a height of 10 cm onto a horizontal glass surface. The height and diameter of the powder or bead stack (cone) was measured with a graduated ruler. The angle of repose was calculated using the following equation:

$$\tan\theta = \frac{h}{r} \qquad \textbf{[3.6]}$$

Where θ represents the angle of repose, h is the height and r the powder or bead heap radius.

3.4.8 Friability

The conventional friabilator could not be used to determine the friability of the beads due to excessive static forces created on the surface of the beads by the plastic material of this

apparatus. Therefore, an adapted method was used that involved glass containers to avoid this problem. For each bead measurement, 10 g of beads was sieved for a duration of 170 taps, done by hand, in order to remove excess powder particles prior to each measurement using a 350 µm sieve and the weight noted. The beads were placed in a glass container and rotated for a duration of 2 min and 20 sec in order to ensure 100 rotations, using a Turbula® mixer (T2C, W.A. Bachofen AG Maschinenfabrik, Bastle, Switzerland). The Turbula® was used in the place of the friabilator as there was a significant loss on beads due to beads sticking onto the surface of the friabilator. While rotating, static electricity was generated enabling the beads to stick to the surface. After rotation, the bead sample was again sieved and weighed. The following equation was used in calculating the percentage friability:

$$F = \frac{W_1 - W_2}{W_1} \times 100 \qquad \textbf{[3.7]}$$

Where F is the calculated percentage friability; W_1 is the total weight of dusted beads before rotation; and W_2 is the total weight of dusted beads after completion of the rotation (BP, 2015; Viljoen *et al.*, 2013:3).

3.5 PREPARATION OF MINI-MUPS

3.5.1 Introduction

This section focuses on the compression of the uncoated and coated beads into mini-MUPS. Evaluation of mini-MUPS during this study was divided into evaluation of the morphology of the mini-MUPS, evaluation of the physical properties and drug release studies. All physical property test methods were conducted according to the British Pharmacopoeia (2015) and were done in triplicate.

3.5.2 Mixture composition and tablet preparation

Table 3.6 displays the different active ingredient-filler bead combinations with the separate addition of binder and/or lubricant components for the preparation of the mini-MUPS mixture. Each component was accurately weighed in a weighing boat on a Mettler Toledo® analytical balance (Model PB 303-S, Switzerland) in sufficient quantities to produce at least 120 mini-MUPS for each formulation. The different components were mixed in closed glass containers in a Turbula® mixer (T2C, W.A. Bachofen AG Maschinenfabrik, Bastle, Switzerland) for 7 min at 69 rpm. The various mini-MUPS mixtures were stored in glass

containers; Parafilm® was used to cover the container openings and sealed with a lid for at least 24 h until compression.

Table 3.6: Mixture composition for mini-MUPS production

Component	%w/w
Furosemide/Pyridoxine Avicel® PH 101 uncoated/coated beads	qs to 100%
Furosemide/Pyridoxine MicroceLac® uncoated/coated beads	qs to 100%
Furosemide/Pyridoxine RetaLac® uncoated/coated beads	qs to 100%
Kollidon® VA 64	0, 3 or 5 %
Magnesium stearate	0, 0.05 or 0.1%

The mini-MUPS were compressed with a Korsch® XP 1 (K0010281, Korsch AG, Berlin, Germany) single-punch tablet press. Convex faced punches with a diameter of 6 mm were used. Each mini-MUPS weighed approximately 100 mg in order to contain approximately 10 mg of the active compound. Subsequently, three mini-MUPS prepared from uncoated beads and three mini-MUPS prepared from coated beads containing the same active ingredient and filler-system were inserted into a size 0 hard gelatine capsule in order to create a mini-MUPS-in-capsule dosage form.

3.5.3 Evaluation of mini-MUPS

The mini-MUPS prepared from uncoated and coated beads, respectively were evaluated in terms of surface and internal structure morphology, mass variation, hardness, tensile strength, friability, swelling, erosion, disintegration, drug content and drug release.

3.5.3.1 Morphology

Both the surface and internal structure morphology of the different formulated mini-MUPS were evaluated. A mini-MUPS sample was mounted on a metal disc with a silicon adhesive. Each mini-MUPS was coated in an Eiko® ion coater (model IB-2, Eiko engineering, Japan) using a gold and palladium (66:34) mixture under a vacuum of 1.5 torr. After coating, the morphology of the internal structures and surfaces of the mini-MUPS were determined by means of scanning electron microscopy (SEM) using a FEI Quanta® 250 Environmental Scanning Electron Microscope with a Field Emission Gun (FEI, Netherlands). Micrographs of the external surfaces and internal structures of the different mini-MUPS were taken at several magnifications ranging from 200x to 8000x (Chinyemba, 2012:22).

3.5.3.2 Mass variation

From each mini-MUPS formulation, 20 randomly selected units were individually dusted with an art brush to eliminate any excess powder. Each unit was weighed on a Precisa® analytical balance (model 240 A, PAG OERLIKON AG, Zurich, Switzerland). The average weight, standard deviation and the percentage relative standard deviation were calculated and evaluated (Viljoen *et al.*, 2013:3).

3.5.3.3 Crushing strength, diameter and thickness

Crushing strength, diameter and thickness were determined with a Pharma Test® (model PTB-311, Switzerland) tablet test unit at a rate of 0.1 cm.min^{-1}. Randomly selected samples (10 mini-MUPS) from each formulation were compressed diametrically between the platens of the Pharma Test® unit. The tensile strength for each mini-MUPS formulation was calculated using the following equation:

$$\sigma_x = \left(10F/\pi D^2\right) \times \left[(2.84H/D)-(0.126H/W)+(3.15W/D)+0.01\right]^{-1} \quad \textbf{[3.8]}$$

Where σ_x is the tensile strength (N.mm^{-2}); F is the crushing strength (or hardness, measured in Newton); D is the tablet diameter (mm), H is the tablet thickness (mm) and W is the centre cylinder thickness (mm) (BP, 2015; Viljoen *et al.*, 2013:3-4; USP, 2015).

3.5.3.4 Friability

The friability of each mini-MUPS formulation was determined by dusting and weighing 10 mini-MUPS before and after rotation. The mini-MUPS were placed in a friabilator (ERWEKA® GmbH, Heusenstamm, Germany) and operated for the duration of 4 min at 25 rpm. Equation 3.7 was used to calculate the friability of the mini-MUPS.

$$F = \frac{W_1 - W_2}{W_1} \times 100 \quad \textbf{[3.7]}$$

Where F is the calculated percentage friability; W_1 is the total weight of dusted mini-MUPS before rotation; and W_2 is the total weight of dusted mini-MUPS after completion of the rotation (BP, 2015; Viljoen *et al.*, 2013:3).

3.5.3.5 Percentage swelling and erosion

Swelling of mini-MUPS prepared from uncoated and coated beads, respectively were evaluated according to a previously published method (Singh *et al.*, 2009:1123). A sample of 6 mini-MUPS prepared from uncoated beads and 6 mini-MUPS prepared from coated beads were respectively placed in a type II Distek 2500 dissolution system basket (2501049,

North Brunswick, New Jersey, USA) under standard British Pharmacopoeia (2015) conditions (37 ± 0.5°C). The baskets containing the mini-MUPS were weighed and the initial mass recorded. The weight was measured at predetermined time intervals of 1, 2, 3, 5, 13, 21, 30, 60, 90, 120, and 150 min; or until the tablets were fully disintegrated and the weight recorded. At each time point the dissolution apparatus was stopped and the basket was blotted with filter paper and weighed (swollen weight). After the swelling experiments were completed, the swollen mini-MUPS were dried in an EcoTherm Labotec oven at 40ºC until no loss in mass was measured. This weight was noted as the final mass to determine if any erosion occurred (BP, 2015). The percentage swelling and percentage erosion of the samples were calculated according to the following equations:

$$\%\text{Swelling} = \frac{S}{R} \times 100 \quad \textbf{[3.9]}$$

$$\%\text{Erosion} = \frac{T-R}{T} \times 100 \quad \textbf{[3.10]}$$

Where S is the weight of the swollen mini-MUPS, T is the initial weight of the mini-MUPS and R represent the eroded weight of the mini-MUPS (Singh *et al.*, 2009:1123).

3.5.3.6 Disintegration

A basket-rack assembly was used to conduct the disintegration tests on the mini-MUPS prepared from uncoated beads and coated beads according to the British Pharmacopoeia (2015) standards. Using a type II apparatus (Erweka® model D-63150, Heusenstamm, Germany), the disintegration times of six mini-MUPS were performed in distilled water (37 ± 0.5ºC). The disintegration time of each mini-MUPS was noted and the mean disintegration time was calculated (BP, 2015).

3.5.3.7 Assay

One mini-MUPS or 100 mg of beads from each formulation was crushed in a mortar and pestle; dispersed in a mixture of 100 ml ethanol and 200 ml distilled water; and stirred for 4 h. The solution was filtered through a 0.45 µm membrane filter. The containing filtrate were spectrophotometrically analysed at a wavelength of 278 nm (for furosemide) and 324 nm (for pyridoxine), respectively in distilled water using a UV-spectrophotometer (Shimadzu® model UV-1700, Shimadzu Corporation©, Japan) (BP, 2015).

3.5.3.8 Dissolution studies

The dissolution profiles of each mini-MUPS formulation prepared from uncoated beads, each mini-MUPS formulation prepared from coated beads, as well as the formulations of the mini-MUPS-in-capsule systems were obtained. Hydrochloric acid (0.1 M) was used as the

dissolution media (pH 1.2) for the first 120 min. This was followed by a 0.2 M phosphate buffer (pH 6.8) up to approximately 480 min, with the infinity sample having a paddle speed of 150 rpm for 30 min (BP, 2015). In total 900 ml dissolution media was utilised at 37 ± 0.5ºC and at a paddle speed of 100 rpm with a type II apparatus Distek 2500 dissolution system (2501049, North Brunswick, New Jersey, USA) (Bashaiwoldu *et al.*, 2011:343) connected to an Evolution 4300 dissolution auto sampler (4301920) together with a Distek syringe pump (SP02716). All dissolution profiles were obtained in six fold. Samples were drawn at predetermined time intervals of 5, 13, 21, 30, 60, 90, 120, 150, 180, 240, 300, 360, 420, 480 and 540 min and the volume of the sample was replaced with fresh dissolution media (BP, 2015).

In order to compile the acid phase for the first 120 min of dissolution, 600 ml of 0.1 M HCl (pH 1.2) was placed in a dissolution chamber to achieve the predetermined temperature of 37 ± 0.5ºC. Sampling followed the time schedule of 5, 13, 21, 30, 60, 90 and 120 min for the first 120 min in the acidic phase. After 120 min, 300 ml of the 0.2 M trisodium phosphate dodecahydrate buffer (37 ± 0.5°C) were added to each chamber to change the pH from 1.2 to 6.8. After adding the buffer 5 min were allocated for adjusting the pH to 6.8. All pH adjustments were done using either a 2 M HCl solution or a 2 M sodium hydroxide solution. Sampling in the buffer phase followed the time schedule of 150, 180, 240, 300, 360, 420, 480, and 540 min, after which the paddle speed was adjusted to 150 rpm (infinity sample) and stirred for an additional 30 min to ensure maximum drug release. Approximately 5 ml samples were withdrawn through a 10 µm porous membrane filter (053112-SSK, PINFIL10S-DK, Cannula filters, Telford, Pennsylvania, USA) and transferred to clean test tubes, after which 5 ml of fresh dissolution media were replaced depending on the phase at the specific time point. All samples were subsequently analysed with the UV-spectrophotometer.

3.6 UV SPECTROPHOTOMETRIC ANALYSIS OF MODEL DRUGS

3.6.1 Absorbance wavelength

Concentration of the model drugs obtained from the dissolution studies were determined using a Shimadzu ultra violet (UV) spectrophotometer (model UV-1700, Shimadzu Corporation, Kyoto, Japan). The absorbance maxima for furosemide was determined at 276 nm and pyridoxine hydrochloride at 292 nm for the acid stage; and 278 nm and 324 nm for the buffer stage, respectively (BP, 2015). According to the British Pharmacopoeia (2015) the absorbance maxima for furosemide varies from 228, 270 and 333 nm, respectively. The

absorbance maxima for pyridoxine in an acidic media are 288-296 nm and 320-327 nm in an alkaline media, respectively (BP, 2015).

3.6.2 Solution preparation of the active ingredients

In order to compile the furosemide and pyridoxine solutions for the standard curves in the acidic and buffer phases, approximately 50 mg of furosemide or pyridoxine was accurately weighed and transferred to a 500 ml volumetric flask. A volume of 50 ml ethanol was used to aid in dissolving the furosemide. Either 0.1 M hydrochloride solution (pH 1.2) or 0.2 M trisodium phosphate dodecahydrate buffer solution (pH 6.8) were added to make up the stock solution to volume in order to provide a concentration of 100 µg/ml for each of the active ingredients in each phase. The following dilutions were made from the stock solution:

- 5 ml of the stock solution was diluted to 250 ml with HCl/buffer solution to deliver a concentration of 2 µg/ml,
- 10 ml of the stock solution was diluted to 100 ml with HCl/buffer solution to deliver a concentration of 10 µg/ml,
- 20 ml of the stock solution was diluted to 100 ml with HCl/buffer solution to deliver a concentration of 20 µg/ml,
- 15 ml of the stock solution was diluted to 50 ml with HCl/buffer solution to deliver a concentration of 30 µg/ml,
- 20 ml of the stock solution was diluted to 50 ml with HCl/buffer solution to deliver a concentration of 40 µg/ml,
- 25 ml of the stock solution was diluted to 50 ml with HCl/buffer solution to deliver a concentration of 50 µg/ml.

3.6.2.1 Construction of standard curves

Standard curves were constructed prior to each drug release study for both model drugs in both of the dissolution media. The stock solution was prepared as described (section 3.6.2), together with the standard dilutions ranging from 2 to 50 µg/ml. For each dilution containing furosemide, the UV-absorbance was measured at 276 nm and 278 nm in the acidic and buffer dissolution media, respectively. UV-absorbance for pyridoxine containing solutions was measured at 292 nm and 324 nm in the acidic and buffer dissolution media, respectively (de Kock, 2005:73-74). A linear regression analysis was conducted on the UV-absorbance values plotted against the concentrations for each model drug in each phase. The analysis of this data produced the most possible straight line through the plotted coordinates. Complying with the principles of Beer's law, the correlation coefficients of each

standard curve for each model drug-dissolution media was calculated and should be as close as possible to unity ($r^2 \geq 0.9999$ was considered acceptable). In order to calculate the active ingredient concentration in each formulation, the slope (m) and y-intercept (c) were obtained by linear regression (as additional parameters).

3.7 DATA ANALYSIS

The parameters described below were calculated using Microsoft® Excel ™ 2010 for Windows ™ (Microsoft ® Corporation, Seattle, Washington, USA) from the dissolution data obtained.

3.7.1 Mean dissolution time

The Mean Dissolution Time (MDT) was calculated according to the following equation:

$$MDT = \frac{\sum_{j=1}^{n} t_{mid} \Delta x_d}{\sum_{j=1}^{n} \Delta x_d} \quad \textbf{[3.11]}$$

Where j is the sample number, n is the total number of sample times, t_{mid} is the midpoint time between j and j-1, and Δx_d is the additional mass of drug dissolved between j and j-1 (Costa & Lobo, 2001:129).

3.7.2 Difference and similarity factors

The fit factors compare the difference between the percentage drug dissolved per time unit for a test and a reference formulation (Moore & Flanner, 1996:66). The difference factor (f_1) measures the percentage error between two curves over all time points (f_1 = 1-15), where the similarity factor f_2 is a logarithmic transformation of the sum-squared error of differences between the test, T_j and the reference products, R_j over all time points ($f_2 \geq 50$). A f_2-value of 100% demonstrates that the dissolution profiles tested are identical. Thus, the higher the f_1- and the lower the f_2-value, the greater the dissimilarity between the dissolution profiles tested (Uzunović & Vranić, 2007:282). The equations to calculate the fit factors f_1 and f_2 are shown in equations 3.12 and 3.13.

$$\boldsymbol{f_1} = \frac{\sum_{j=1}^{n} |R_j - T_j|}{\sum_{j=1}^{n} (R_j + T_j)/2} \times 100 \quad \textbf{[3.12]}$$

$$\boldsymbol{f_2} = 50 \times \log \left\{ \left[1 + (1/n) \sum_{j=1}^{n} \left| R_j - T_j \right|^2 \right]^{-0.5} \times 100 \right\} \quad \textbf{[3.13]}$$

Where R_j is the reference assay at time point t. T_j is the test assay at time point t, n is the number of time points.

3.8 ACCELERATED STABILITY TESTING

3.8.1 Preparation of stability test samples

Approximately 50 mini-MUPS of each optimised mini-MUPS formulation were placed in clean glass test sample containers and sealed with Parafilm® prior to entering the different stability chambers.

3.8.2 Storage conditions

The physical stability of the optimised mini-MUPS formulations was followed over a period of six months by storing these formulations in stability chambers at the following conditions: 25ºC and 60% relative humidity (RH), 30ºC and 70% RH and 40ºC and 75% RH. Mini-MUPS samples from each set of conditions were evaluated as described below on month 1, 2, 3 and 6.

3.8.3 Evaluation of the physical stability of mini-MUPS

The physical tablet tests (*i.e.* mass variation, tensile strength, friability, disintegration) and assay (described in section 3.5.3) were performed on month 1, 2, 3 and 6 after exposure to the conditions as stated above. All calculations were done using Microsoft® Excel ™ 2010 for Windows ™ (Microsoft ® Corporation, Seattle, Washington, USA).

CHAPTER 4
EVALUATION OF DIFFERENT UNITS IN THE MINI-MUPS-IN-CAPSULE SYSTEM

4.1 INTRODUCTION

The selected fillers and mini-MUPS-in-capsule systems were evaluated in terms of filler powder particle morphology, uncoated and coated bead morphology, flow properties (including density, Carr's index, Hausner ratio, angle of repose, flow rate and critical orifice diameter), as well as the mini-MUPS morphology and physical tablet properties (including mass variation, disintegration, swelling, erosion, hardness, diameter, thickness, tensile strength, friability, drug content and dissolution studies) of the mini-MUPS compressed form the uncoated and coated beads, respectively. Dissolution studies were also done on the mini-MUPS-in-capsule dosage form systems. All tests results conducted in this study were compared to results obtained for Avicel® PH 101 powder and mini-MUPS prepared from Avicel® PH 101 with active ingredients furosemide and pyridoxine respectively.

4.2 EVALUATION OF POWDER AND BEAD FLOWABILITY

Avicel® PH 101, MicroceLac® 100 and RetaLac® filler powders and the uncoated and coated beads produced from these fillers where evaluated according to previously described methods (section 3.4). Morphology, particle size distribution and flow properties were investigated according to British Pharmacopoeia (2015) standards.

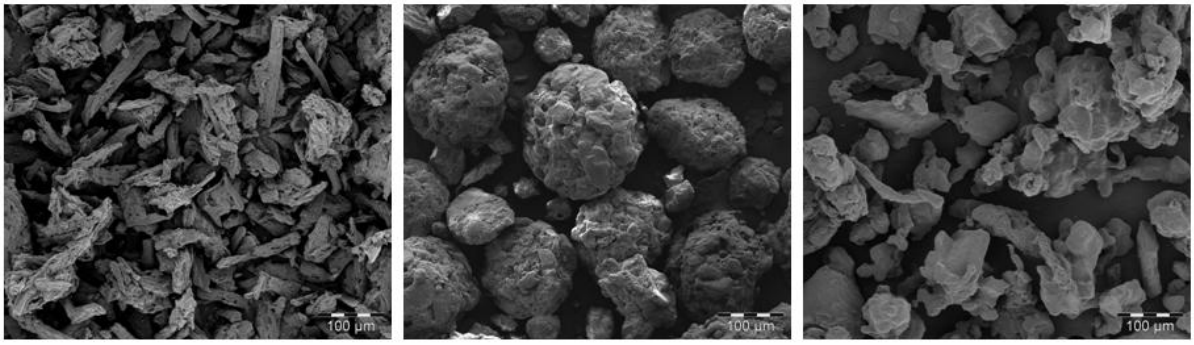
4.2.1 Morphology of powders and beads

As microcrystalline cellulose (Avicel® PH 101) remains the most popular choice in the manufacturing of pellets utilising extrusion-spheronisation, all results obtained for MicroceLac® 100 and RetaLac® in this study will be compared to Avicel® PH 101 as reference standard (Bölcskei *et al.*, 2014:762).

4.2.1.1 Morphology of the filler powders

Scanning electron microscopy (SEM) images of the plain filler powders were taken at a magnification of 200x to 8000x (Figure 4.1) as the different beads were not of similar size.

Studying the separate images, it was evident that MicroceLac® 100 (Figure 4.1.b) would probably depict better flow properties as its particles are not only larger, but also possess spherical shapes when compared to the needle shaped particles of Avicel® PH 101 (Figure 4.1.a) and the porous, irregular structured particles of RetaLac® (Figure 4.1.c) (Meggle, 2014a:2,5; Meggle, 2014b:6).



(a) **(b)** **(c)**

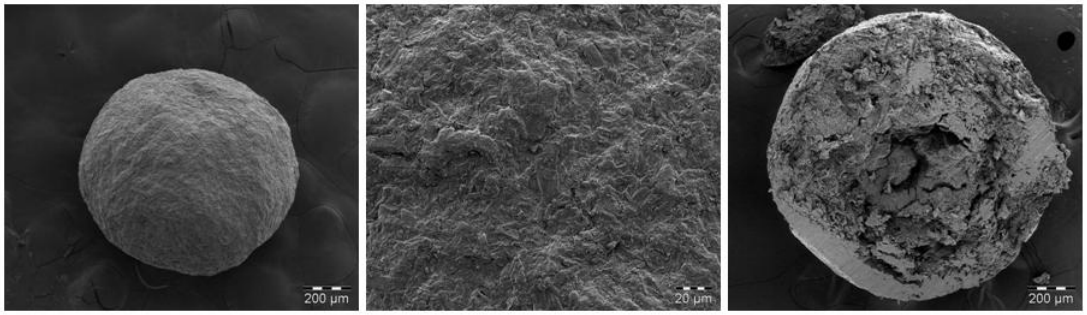
Figure 4.1: SEM images of the pure filler powder particles used in this study: **(a)** Avicel® PH 101; **(b)** MicroceLac® 100; **(c)** RetaLac®

Meggle (2014a:2,5) concluded that the spherical particle shape of MicroceLac® 100 is accomplished due to the co-spray-drying production process utilising 75% α-lactose monohydrate and 25% microcrystalline cellulose . RetaLac® on the other hand, is a co-processed excipient consisting of a 1:1 ratio hypromellose and milled α-lactose monohydrate which created porous, irregular structured powder particles (Meggle, 2014b:6).

4.2.1.2 Morphology of the uncoated beads

Figures 4.2 - 4.4 illustrate the morphology of the uncoated beads produced from Avicel® PH 101, MicroceLac® 100 and RetaLac® on SEM micrographs taken at a magnification of 200x to 8000x as the different beads were not of similar size.

Bashaiwoldu *et al.* (2011:347) explained that using a water/ethanol mixture as wetting agent during extrusion-spheronisation results in more brittle Avicel®/active ingredient beads, with the bead surface showing visible cracks. However, in this study, manufacture of Avicel®/active ingredient beads, of utilising a water/ethanol mixture as wetting agent, did not result in visible cracks on the smooth and continuous bead surface area as seen in Figure 4.2.

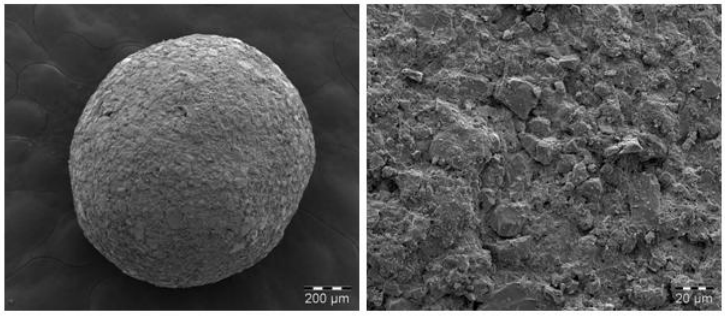


(a) **(b)** **(c)**

Figure 4.2: SEM images of uncoated Avicel® PH 101 beads: **(a)** Single uncoated bead; **(b)** Surface of uncoated bead; **(c)** Uncoated bead cut in half

Furthermore, Bashaiwoldu *et al.* (2011:351) explained that using such a wetting agent during production of beads, larger pores are formed inside the bead, resulting in weaker mini-MUPS as they found that the tensile strength of the beads decreased. Figure 4.2.c portrays a single uncoated Avicel® PH 101 bead cut in half, with a somewhat porous centre clearly visible. As pores were present inside the beads, it can be concluded that the tensile strength of the mini-MUPS will probably be lower as explained by Bashaiwoldu *et al.* (2011:351).

Figure 4.3 depicts an uncoated MicroceLac®/active ingredient bead. A significantly smooth outer surface area is shown in Figure 4.3.a, whereas a larger magnification showed that these beads portrayed a somewhat uneven surface area, compared to uncoated Avicel® beads at the same scale (Figure 4.2.b).

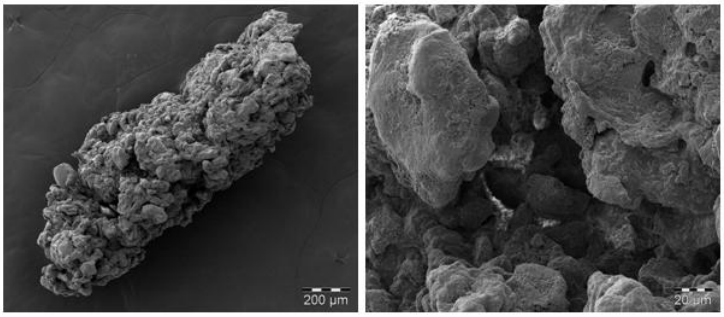


(a) **(b)**

Figure 4.3: SEM images of uncoated MicroceLac® 100 beads: **(a)** Single MicroceLac® 100 bead; **(b)** Surface of uncoated bead

Figure 4.4 illustrates the oblong shaped beads produced by RetaLac®. As with the RetaLac® powder particles, the beads prepared from RetaLac® also depicted porous, irregular and uneven surfaces. RetaLac® beads were manufactured utilising the same extrusion-spheronisation process as with the beads produced from the other filler powders. Flowability was clearly affected by their non-spherical shapes and these beads were more fragile and broke easily when touched compared to the beads prepared from the fillers Avicel® and

MicroceLac®. The RetaLac® beads were possibly more fragile because they are very porous with loose structures when compared to the beads manufactured from Avicel® and MicroceLac® that appear as solid, hard, compact beads.

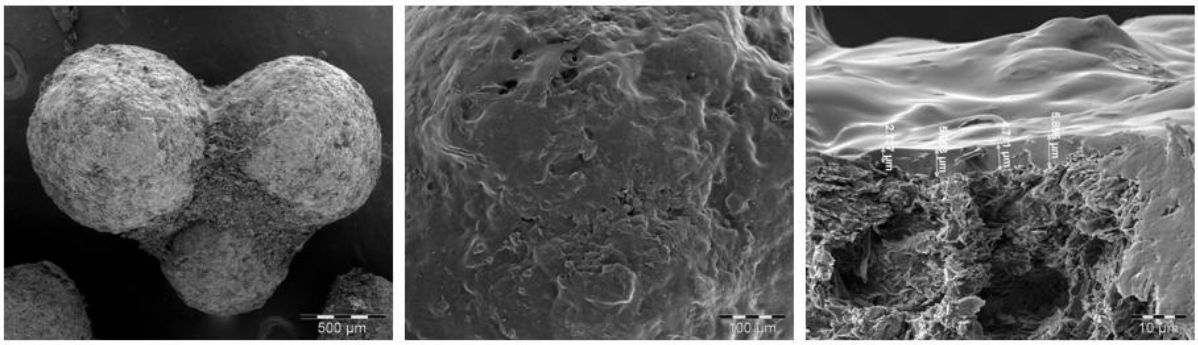


(a) **(b)**

Figure 4.4: SEM images of uncoated RetaLac® beads: **(a)** Single RetaLac® bead; **(b)** Surface of uncoated bead

4.2.1.3 Morphology of the coated beads

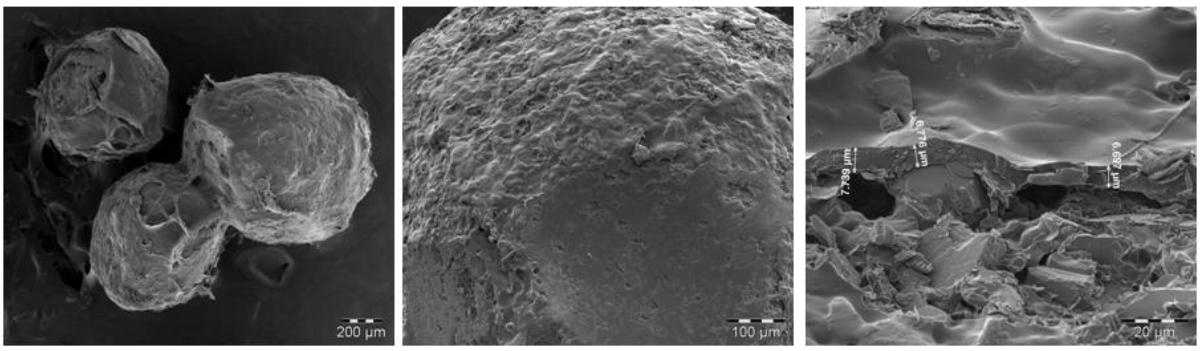
Figures 4.5 - 4.7 depict SEM images taken at different magnifications from beads manufactured by means of extrusion-spheronisation with the different fillers. The beads were coated with a mixture of Eudragit® L 100 and Eudragit® S 100. During preparation of the coated beads for SEM imaging, beads were cut in half. By closely observing the SEM images it could be concluded that the coating was indeed soft and flexible as the blade smeared the film coating when the various beads were cut in half (Figure 4.5.c) (Bashaiwoldu *et al.*, 2011:345). In general, the coating thickness on the RetaLac® beads ranged between 2.66 µm and 7.74 µm as measured on the SEM images.



(a) **(b)** **(c)**

Figure 4.5: SEM images of Avicel® PH 101 coated beads: **(a)** Beads attached to one another due to the coating material; **(b)** Smooth coated surface of the bead with no cracks visible in the coating; **(c)** Coating thickness measured on the bead surface

Figures 4.5.a and 4.6.a depict beads attached to one another due to the stickiness of the coating material during the rotation movement of the pan during the film coating process.

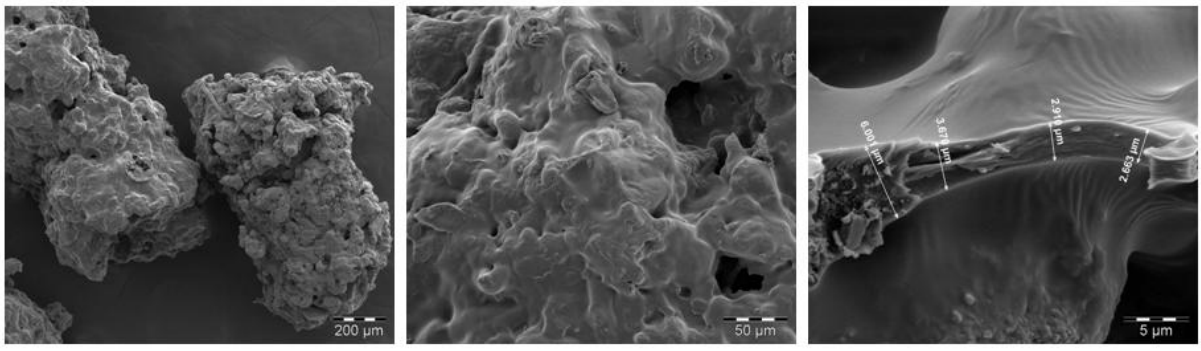


(a) **(b)** **(c)**

Figure 4.6: SEM images of MicroceLac® 100 coated beads: **(a)** Beads attached to each other due to the coating material; **(b)** Smooth coated bead surface with visible indents caused by other beads; **(c)** Coating thickness measured on the bead surface

Figure 4.6.b clearly shows indents in the coated bead surface, which was possibly caused by detachment of beads from each other during rotation during the coating process. The thickness of the film coating on the Avicel® PH 101 beads ranged from 6.697 µm to 7.739 µm as shown on Figure 4.6.c.

Figure 4.7 depicts some indents on the uncoated surface of the RetaLac® coated beads. As these oblong shaped beads portray a rough surface with various shaped indents, it is understandable that the coating material did not cover the complete surface area. The coating of the RetaLac® beads was visibly thinner compared to the other bead coatings. This problem of variable film coating thickness can possibly be overcome through increasing the coating time.



(a) **(b)** **(c)**

Figure 4.7: SEM images of RetaLac® coated beads: **(a)** Complete coated beads; **(b)** Coated bead surface; **(c)** Coating thickness measured on the bead surface

4.2.2 Particle size and size distribution

In this study particle size and size distribution of the filler powders and the uncoated and coated beads (Annexure A) were evaluated with a Malvern Mastersizer (section 3.4.3). Particle size analysis conducted on the powders for Avicel® PH 101 and MicroceLac® 100, both depicted negative skew particle size distributions with a wider span (1.994; 1.830) compared to the RetaLac® powder. RetaLac® powder depicted a normal particle size distribution with a span of 1.716; a mean volume particle size of 163.60 ± 4.84 µm and the average size of particles smaller than 50% ($d_{0.5}$) being 139.19 µm. These findings support the particle size distribution done by Meggle (2014b:6) on RetaLac® powder particles where they concluded that RetaLac® has a $d_{0.5}$ ranging from 100 µm to 200 µm and is homogenous in nature.

In a study conducted by Bashaiwoldu *et al.* (2011:341), a mean volume particle diameter of 54.80 ± 0.54 µm for Avicel® PH 101 powder was obtained. However, in this study a mean volume particle diameter of 77.68 ± 1.11 µm was measured for the Avicel® PH 101 powder.

Meggle (2014a:4) found that MicroceLac® 100 powder exhibited a narrow particle size distribution, which aids the preparation of homogenous powder mixtures prior to compression to ensure tablets of excellent quality. The particle size distribution of the plain MicroceLac® 100 powder exhibited an average volume particle size of 149.56 ± 1.69 µm, which correlate with the findings of Meggle (2014a:4).

According to the particle size and size distribution data obtained from the filler, the following can be concluded: the smaller particles of Avicel® PH 101 will probably cause poorer flowability, due to more contact surface areas and increased bonding forces between the particles, whereas RetaLac® will have better flowability due to larger particles (although the irregular oblong shape may oppose this). MicroceLac® 100 will probably have the best flowability due to larger and more spherical particles compared to the other filler materials investigated in this study.

All the beads produced from the selected fillers containing either furosemide or pyridoxine, exhibited a relatively similar normal particle size distribution with a relatively narrow span (Table 4.1).

Table 4.1: Particle size and size distribution of the different uncoated and coated beads

Furosemide			Pyridoxine	
Span (µm)	**Mean particle size (µm)**		**Span (µm)**	**Mean particle size (µm)**
0.70 ± 0.025	**927.34** ± 64.960	**Avicel® PH 101 UB***	**0.62** ± 0.020	**1096.62** ± 39.561
0.66 ± 0.010	**1003.46** ± 10.473	**Avicel® PH 101 CB***	**0.68** ± 0.009	**958.28** ± 20.500
0.62 ± 0.018	**1092.81** ± 20.494	**MicroceLac® 100 UB***	**0.60** ± 0.012	**1108.72** ± 62.712
0.63 ± 0.001	**1048.62** ± 9.519	**MicroceLac® 100 CB***	**0.63** ± 0.021	**1042.04** ± 65.631
0.84 ± 0.038	**952.80** ± 80.065	**RetaLac® UB***	**0.70** ± 0.069	**934.70** ± 13.166
0.78 ± 0.016	**914.66** ± 29.262	**RetaLac® CB***	**0.77** ± 0.061	**942.01** ± 32.647

**CB= Coated Beads; UB= Uncoated Beads*

4.2.3 Powder and bead flow properties

Flowability of the fillers, uncoated and coated beads containing either furosemide or pyridoxine (active ingredients) was investigated utilising the following parameters: density, Carr's index, Hausner ratio, critical orifice diameter, flow rate and angle of repose. As seen in Table 4.2 adapted from the British Pharmacopoeia (2015), outstanding powder flow properties will be achieved if a material has an angle of repose of less than 30º, whereas the flow properties will be considered less than adequate if an angle of repose higher than 66º is measured. Obtaining a Carr's index of 1 to 10% and a Hausner ratio smaller than 1.11, will again predict excellent flowability (BP, 2015).

Table 4.2: Flowability scale prerequisites

Flow property	Carr's index (%)	Hausner ratio	Angle of repose (º)
Excellent	1-10	1.00-1.11	25-30
Good	11-15	1.12-1.18	31-35
Fair	16-20	1.19-1.25	36-40
Passable	21-25	1.26-1.34	41-45
Poor	26-31	1.35-1.45	46-55
Very poor	32-37	1.46-1.59	56-65
Extremely poor	> 38	> 1.60	> 66

4.2.3.1 Powder flow properties

The flow properties tested in this study for the plain filler powders are shown in Table 4.3. Flowability tests conducted by Meggle (2014a:6) showed that plain MicroceLac® powder attained an angle of repose of 34º; a Hausner ratio of 1.26 and a Carr's index of 20.69%, overall indicating that this filler powder exhibits good to passable flowability. Furthermore, Meggle (2014b:7) also found that RetaLac® powder depicts fair to poor flow properties, having an angle of repose of 36º; a Hausner ratio of 1.35 and a Carr's index of 26.09%. Thus, the results obtained as shown in Table 4.3 for the MicroceLac® and RetaLac® powders depicted better flowability when compared to results Meggle (2014a:6; 2014b:7) obtained. Saha and Shahiwala (2009:203), on the other hand, explained that powder flow properties of microcrystalline cellulose are comparatively poor due to small particle size, as also seen in this study. Taking all flow properties into consideration, MicroceLac® 100 depicted superior flow. Even though the flow rate of MicroceLac® 100 was significantly lower than that of Avicel® PH 101, it must be kept in mind that the orifice diameter through which the powders flowed differed as Avicel® depicted no flow through any of the orifices and MicroceLac® 100 freely flowed through an orifice with 1.5 mm diameter. RetaLac® also displayed enhanced flow properties compared to the reference standard, Avicel® PH 101.

Table 4.3: Flow properties of the selected plain filler powders

	Fillers		
	Avicel® PH 101	**MicroceLac® 100**	**RetaLac®**
Bulk density (g/cm^3)	**0.59** ± 0.011	**0.01** ± 0.005	**0.02** ± 0.008
Tapped density (g/cm^3)	**0.88** ± 0.024	**0.01** ± 0.006	**0.02** ± 0.010
Hausner ratio	**1.49** ± 0.019	**1.19** ± 0.004	**1.34** ± 0.007
Carr's Index (%)	**33.81** ± 0.847	**15.83** ± 0.407	**25.50** ± 0.953
COD (mm)	**N/F**	**1.5**	**3**
Flow rate (g/s^{-1})	**8.48** ± 1.227	**1.45** ± 0.038	**0.91** ± 0.009
Angle of repose (º)	**23.79** ± 1.160	**19.94** ± 0.105	**26.96** ± 0.384

**COD= Critical Orifice Diameter, N/F= No Flow*

4.2.3.2 Uncoated and coated bead flow properties

The flow properties of the uncoated and coated beads containing either furosemide or pyridoxine are shown in Tables 4.4 and 4.5, respectively.

Considering the flow properties (Table 4.4) for uncoated beads produced from MicroceLac® 100 and Avicel® PH 101 containing furosemide, these bead formulations depicted excellent flow compared to the formulations that contained RetaLac®. In conclusion, Avicel® PH 101 and MicroceLac® 100 coated beads containing furosemide portrayed excellent and good flow, respectively, whereas RetaLac® depicted only fair flow.

The overall flow properties of the uncoated bead filler/pyridoxine formulations (Table 4.5) showed that Avicel® PH 101, MicroceLac® 100 and RetaLac® beads depicted excellent, good and fair flow, respectively. Examining the coated bead filler/pyridoxine flow properties Avicel® PH 101 and MicroceLac® 100 both depicted excellent flow, whereas RetaLac® portrayed poor flowability. Examination of these bead flowability tests it can be concluded that the active ingredients furosemide and pyridoxine played a part in the flowability of the beads. Furosemide has fine particles that are cohesive and agglomerate easily exhibiting strong bonding forces to the powder particles in creating beads (de Kock, 2005:56; de Villiers *et al.*, 1993:159,161).

Table 4.4: Flow properties of uncoated and coated beads for the active ingredient furosemide

	Filler - furosemide					
	Avicel® PH 101		**MicroceLac® 100**		**RetaLac®**	
	UB	**CB**	**UB**	**CB**	**UB**	**CB**
Bulk density (g/cm^3)	**0.91** ± 0.133	**0.79** ± 0.040	**0.79** ± 0.021	**0.74** ± 0.007	**0.58** ± 0.011	**0.56** ± 0.008
Tapped density(g/cm^3)	**0.96** ± 0.099	**0.83** ± 0.054	**0.82** ± 0.031	**0.85** ± 0.015	**0.71** ± 0.021	**0.71** ± 0.023
Hausner ratio	**1.05** ± 0.034	**1.06** ± 0.025	**1.05** ± 0.002	**1.15** ± 0.014	**1.23** ± 0.054	**1.26** ± 0.020
Carr's index (%)	**5.07** ± 4.297	**5.78** ± 2.153	**4.71** ± 0.163	**12.81** ± 0.620	**18.89** ± 3.600	**20.30** ± 1.423
COD (mm)	**4**	**6**	**6**	**6**	**6**	**6**
Flow rate (g/s^{-1})	**6.12** ± 0.050	**6.20** ± 0.051	**6.20** ± 0.051	**5.52** ± 0.081	**3.41** ± 0	**3.97** ± 0.031
Angle of repose (º)	**12.04** ± 1.413	**10.81** ± 2.161	**14.73** ± 5.863	**15.69** ± 1.653	**18.99** ± 0.643	**19.55** ± 1.154

**CB= Coated Beads, COD= Critical Orifice Diameter, UB= Uncoated Beads*

Table 4.5: Flow properties of uncoated and coated beads for the active ingredient pyridoxine

	Filler - pyridoxine					
	Avicel® PH 101		MicroceLac® 100		RetaLac®	
	UB*	CB*	UB*	CB*	UB*	CB*
Bulk density (g/cm^3)	**0.99** ± 0.033	**0.75** ± 0.011	**0.74** ± 0.018	**0.77** ± 0.012	**0.56** ± 0.013	**0.55** ± 0.008
Tapped density (g/cm^3)	**1.01** ± 0.027	**0.80** ± 0.035	**0.86** ± 0.010	**0.83** ± 0.014	**0.70** ± 0.016	**0.78** ± 0.054
Hausner ratio	**1.01** ± 0.013	**1.07** ± 0.030	**1.15** ± 0.035	**1.08** ± 0.018	**1.24** ± 0.020	**1.42** ± 0.109
Carr's index (%)	**1.31** ± 0.690	**6.48** ± 2.597	**13.33** ± 2.720	**7.69** ± 1.553	**19.46** ± 0.987	**29.52** ± 5.080
COD* (mm)	**5**	**7**	**6**	**6**	**6**	**6**
Flow rate (g/s^{-1})	**4.55** ± 0.028	**5.55** ± 1.800	**5.28** ± 0.037	**5.91** ± 0.092	**3.47** ± 0.024	**3.72** ± 0.037
Angle of repose (º)	**24.46** ± 0.638	**15.78** ± 1.958	**12.89** ± 2.762	**10.03** ± 2.024	**19.86** ± 1.184	**18.83** ± 1.700

**CB= Coated Beads, COD= Critical Orifice Diameter, UB= Uncoated Beads*

Uncoated and coated beads for each filler/active ingredient formulation depicted enhanced flow properties compared to the filler powders. Beads formulated with Avicel® PH 101 depicted excellent to good flowability compared to the plain filler powder having significantly poor flow characteristics. Beads produced from either furosemide or pyridoxine and MicroceLac® 100 formulations showed excellent to good flow properties, whereas the plain filler depicted only fair powder flow. Flowability tests conducted on RetaLac® powder showed that this powder exhibited passable flow. Both uncoated and coated beads for the RetaLac®/furosemide formulation depicted fair flow, whereas uncoated RetaLac®/pyridoxine beads also showed fair flow with the coated RetaLac®/pyridoxine beads depicting poor flow.

In general, it can be concluded that the uncoated beads for both active ingredients portrayed increased flowability compared to the coated beads. In each test, beads produced from furosemide depicted enhanced flowability compared to the pyridoxine beads, showing that the properties of the active ingredient do contribute to the flowability of beads.

4.2.4 Uncoated and coated bead friability

The friability results obtained from the uncoated and coated beads containing either furosemide or pyridoxine are displayed in Table 4.6. All of the uncoated and coated beads, except the coated beads produced from the RetaLac®/pyridoxine formulation, adhered to the official criteria of the British Pharmacopoeia (2015), *i.e.* a maximum weight loss of only 1%.

However, due to the fact that the beads were destined to be compressed into mini-MUPS; as well as that these mini-tablets were encapsulated; friability of the beads was not considered to impact directly on the final dosage form. Thus, for the purpose of this study the friability of RetaLac® coated beads was not considered problematic.

Table 4.6: Percentage friability of uncoated and coated beads

Active ingredient: furosemide	Average friability (%)	Active ingredient: pyridoxine	Average friability (%)
Avicel® PH 101 UB*	0.08	**Avicel® PH 101 UB***	0.62
Avicel® PH 101 CB*	0.16	**Avicel® PH 101 CB***	0.02
MicroceLac® 100 UB*	0.01	**MicroceLac® 100 UB***	0.43
MicroceLac® 100 CB*	0.04	**MicroceLac® 100 CB***	0.02
RetaLac® UB*	0.25	**RetaLac® UB***	0.02
RetaLac® CB*	0.25	**RetaLac® CB***	1.59

**CB= Coated beads; UB= Uncoated beads*

The friability test conducted on the uncoated and coated beads complied with the British Pharmacopoeia (2015) as the results obtained showed that the loss on mass did not exceed 1%. However, the coated RetaLac®/pyridoxine beads depicted a mass loss of 1.59%. As the beads were intended to be compressed in order to produce uncoated and coated mini-MUPS, respectively, this result was not considered as important.

4.3 EVALUATION OF MINI-MUPS

In this study, mini-MUPS-in-capsule drug delivery systems were designed to contain compressed mini-tablets of the uncoated and coated beads (uncoated mini-MUPS and coated mini-MUPS). Uncoated and coated mini-MUPS were evaluated in terms of morphology, physical tablet properties and dissolution profiles. All tests conducted were done according to British Pharmacopoeia (2015) standards and as described in section 3.5.3.

4.3.1 Morphology of mini-MUPS

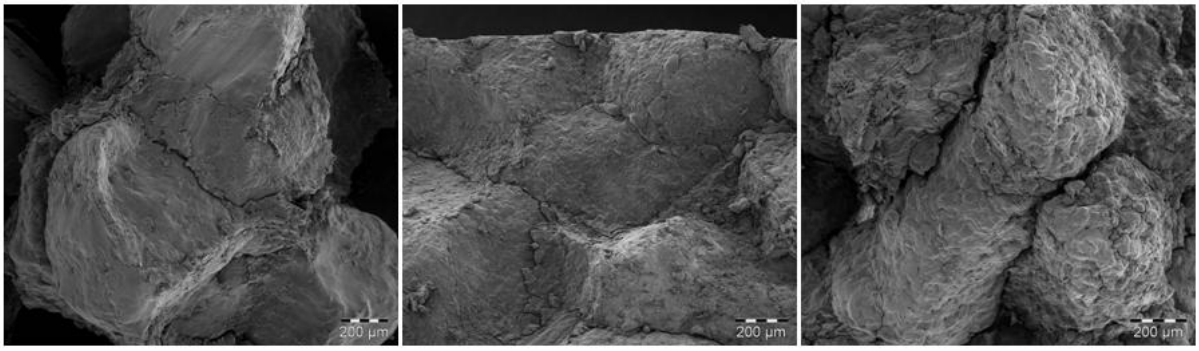
SEM images were taken at several magnifications from mini-MUPS comprising compressed uncoated beads and compressed coated beads, respectively. The individual uncoated and coated mini-MUPS for the fillers utilised were cut in half to clarify what effect the compression forces had on the uncoated and coated beads during compression. Figures 4.8 and 4.9 illustrate how the beads were deformed during compression of the uncoated and coated beads in order to create mini-MUPS.

Abdul *et al.* (2010:4) explained that during compaction, the deformation and bead fragmentation of Avicel® beads are low to non-existent; similar to what is seen in Figure 4.8.a. Other research scientists also reached the same conclusion, namely that during extrusion-spheronisation very hard beads are formed. The use of water during wet granulation of Avicel® proved to decrease this filler's compressibility and thus beads made from Avicel® are characterised as hard and do not deform during compression (Saha & Shahiwala, 2009:203; Torrado & Augsburger, 2008:514).

Bashaiwoldu *et al.* (2011:345) conducted a study where they produced MUPS compressed from uncoated and coated beads prepared from Avicel® PH 101. They stated that compression of coated beads with a film thickness of between 9 - 34 µm produced weak, inconsistent tablets. As seen in Figures 4.8.a and 4.9.a, the same results were obtained in this study with a coating thickness ranging between 2.66-7.74 µm. Not only can little to none deformation and fragmentation of the beads be observed, but relatively large openings between beads can be seen (especially for RetaLac®), which possibly indicated that binding of the beads during compression was insufficient. Saha and Shahiwala (2009:203), as well as Torrado and Augsburger (2008:514) classified Avicel® beads as hard; we however can state that the coated surface of these beads were soft (section 4.3.1.3).

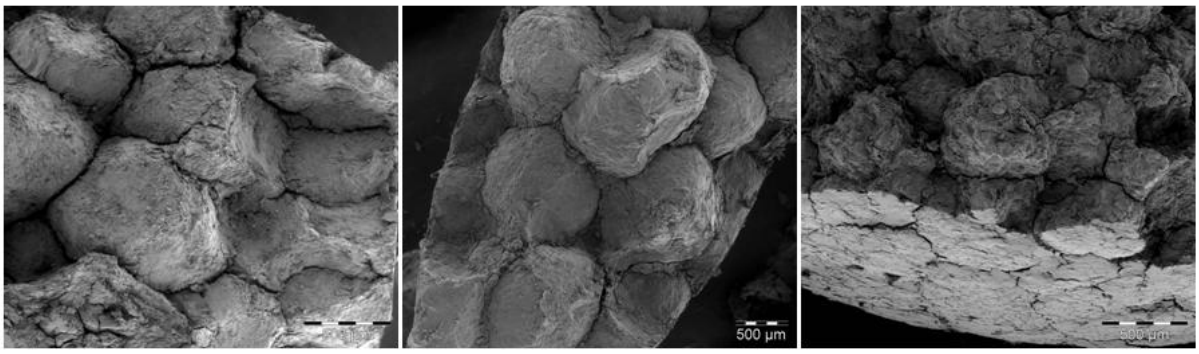
As seen in Figure 4.5.c it could be concluded that the coating was indeed soft and flexible as the blade smeared the film coating when the various beads were cut in half. Although the film coating covering the beads were soft, the coating did not merge during compression. Furthermore, the addition of Kollidon VA 64 (binder) had no significant effect on the binding forces between the individually coated beads of any of the fillers used.

The surface areas of both uncoated and coated Avicel® PH 101 mini-MUPS were relatively uneven. Comparing the uncoated and coated mini-MUPS it is clearly visible that the coated mini-MUPS produced larger gaps in the tablet itself, which might possibly serve as an indication that poor binding occurred.



(a) **(b)** **(c)**

Figure 4.8: SEM images of uncoated beads compressed into mini-MUPS: **(a)** Avicel® PH 101; **(b)** MicroceLac® 100; **(c)** RetaLac®



(a) **(b)** **(c)**

Figure 4.9: SEM images of coated beads compressed into mini-MUPS: **(a)** Avicel® PH 101; **(b)** MicroceLac® 100; **(c)** RetaLac®

Figures 4.8.b and 4.9.b illustrate the uncoated and coated mini-MUPS for the filler MicroceLac® 100. In each uncoated and coated mini-MUPS the surface area were presented as smooth and shiny. As seen in these images these beads also appear to be hard as deformation occurred to a small extent as previously seen with the Avicel® mini-MUPS. A possible reason could be that the 75% α-lactose monohydrate contributed towards the deformation, however being spray-dried with microcrystalline cellulose helped against total deformation.

Meggle (2014b:3, 6) found that RetaLac® powder exhibits plastic and brittle fracture deformation properties resulting in enhanced direct compression properties. From the images in Figures 4.8.c and 4.9.c, it can be concluded that the RetaLac® beads in this case also exhibited plastic deformation. The beads itself was brittle allowing mini-MUPS with smooth surfaces to be formed during compression. The mini-MUPS produced from RetaLac® uncoated as well as RetaLac® coated beads had an increased hardness compared to the other mini-MUPS filler formulations.

Film coatings on all the bead formulations seemed soft as the coating deformed during compression. Comparison of the mini-MUPS prepared from the uncoated and coated beads, revealed that the uncoated beads exhibited more deformation during compression than the coated beads irrespective of the filler used.

4.3.2 Evaluation of the physical properties of mini-MUPS

The physical properties of mini-MUPS prepared from both uncoated and coated beads were evaluated in terms of mass variation; crushing strength; diameter; thickness; tensile strength; friability; disintegration; drug content; swelling; erosion and dissolution profiles. Annexure B contains the entire data set generated from the physical tests on the mini-MUPS.

4.3.2.1 Selection of optimised formulations

A factorial design was utilised to establish the optimum formulations for each active ingredient (furosemide, pyridoxine) and filler (Avicel® PH 101, MicroceLac® 100, RetaLac®) combination. Mini-MUPS were eliminated from the initial formulations as indicated in the factorial design according to the official criteria set for physical tablet tests (including mass variation, tensile strength, disintegration, friability) by the British Pharmacopoeia (2015) and dissolution profile evaluations in order to obtain the optimised formulations that are indicated in bold in the white blocks in Table 4.7.

In total 9 formulations of each filler/active ingredient combination were used to manufacture uncoated mini-MUPS. In each case only 3 to 4 formulations of a specific filler/active ingredient combination were manufactured as the different combinations resulted in various problems (being too sticky or breaking to the touch) that caused certain formulations not being able to be manufactured and undergo testing, for example:

Pyridoxine/Avicel®/MicroceLac® combinations once mixed with 3 or 5 % w/w binder stuck to the glass container and/or fill shoe of the tablet press even though the formulations also contained lubricant. In turn this resulted in beads being too sticky to compress or once compressed stuck to the dies making further manufacture impossible. Pyridoxine/RetaLac® formulations that were not manufactured for testing as it resulted in capping once the mini-MUPS were ejected from the die.

Furosemide/MicroceLac® combinations resulted in capping with the increase in binder concentration. The Avicel® and RetaLac® formulations that were not manufactured for testing were the mini-MUPS formulations that broke directly after ejection from the die or once touched. Thus only 21 formulations were successfully manufactured and tested.

Table 4.7: Optimised mini-MUPS formulations (in the white blocks and in bold) determined by a full factorial design

				Active ingredients					
				Furosemide			Pyridoxine		
				Binder: Kollidon® VA 64					
				0%	3%	5%	0%	3%	5%
Filler type	Avicel® PH 101	Lubricant: Magnesium stearate	0%	Avi0F0	**Avi0F3**	Avi0F5	**Avi0P0**	Avi0P3	Avi0P5
			0.05%	Avi0.05F0	Avi0.05F3	Avi0.05F5	Avi0.05P0	Avi0.05P3	Avi0.05P5
			0.1%	Avi0.1F0	Avi0.1F3	Avi0.1F5	Avi0.1P0	Avi0.1P3	Avi0.1P5
	MicroceLac® 100		0%	Mic0F0	Mic0F3	Mic0F5	Mic0P0	Mic0P3	Mic0P5
			0.05%	Mic0.05F0	**Mic0.05F3**	Mic0.05F5	**Mic0.05P0**	Mic0.05P3	Mic0.05P5
			0.1%	Mic0.1F0	Mic0.1F3	Mic0.1F5	Mic0.1P0	Mic0.1P3	Mic0.1P5
	RetaLac®		0%	Ret0F0	Ret0F3	Ret0F5	Ret0P0	Ret0P3	Ret0P5
			0.05%	Ret0.05F0	Ret0.05F3	Ret0.05F5	Ret0.05P0	Ret0.05P3	Ret0.05P5
			0.1%	Ret0.1F0	**Ret0.1F3**	Ret0.1F5	Ret0.1P0	**Ret0.1P3**	Ret0.1P5

4.3.2.2 Uncoated beads

Physical tests (as described in section 3.5.3) were conducted on all of the mini-MUPS formulations prepared from uncoated beads as outlined in the factorial design (Table 3.3). After careful evaluation of the results obtained from the physical tests of the different mini-MUPS formulations prepared from uncoated beads, the optimised formulations were selected (Table 4.7), beads prepared, film coated and the physical properties of the mini-MUPS compressed from these beads were then determined.

From Table 4.8 it is clear that the Avicel® formulations containing no magnesium stearate (lubricant) or Kollidon® VA 64 (binder) failed to comply with the British Pharmacopoeia (2015) requirement for mass variation as the mass of these mini-MUPS deviated by more than 15% from the average mass. It seemed as though an increase in binder concentration played a more prominent role compared to the lubricant concentration in terms of increasing the tablet properties (tensile strength) because the %RSD for mass variation decreased, regardless the type of active ingredient included.

Table 4.8 Physical test data for mini-MUPS prepared from uncoated beads containing Avicel® PH 101 and either furosemide or pyridoxine *(%RSD is indicated in parentheses)*

Formulation	Average mass (mg)	Tensile strength ($N.mm^{-2}$)	Friability (%)	Disintegration (min)
Avi0F0	87.86 (26.06)	0.70 (72.87)	82.85 (35.84)	***ND**
Avi0F3	96.62 (2.05)	2.50 (13.40)	3.69 (0.78)	13.33 (19.37)
Avi0F5	99.14 (2.53)	2.92 (15.28)	1.43 (63.43)	10.00 (24.49)
Avi0P0	93.19 (18.52)	1.12 (22.92)	72.46 (65.83)	11.00 (39.83)
Avi0P5	92.90 (5.56)	****NV**	88.45 (22.62)	2.17 (26.38)
Avi0.05P3	88.74 (4.39)	1.17 (31.16)	72.04 (67.22)	8.33 (39.19)
Avi0.05P0	96.41 (4.13)	0.27 (93.14)	100.00 (0.00)	5.00 (48.99)

**ND= Considered non-disintegrating; **NV= No value*

Furthermore, an increase in Kollidon® VA 64 concentration in the Avicel®/furosemide formulations resulted in a decrease in the percentage friability and an increase in tensile

strength as the binding forces between the beads increased (Bühler, 1992:217). Interestingly for these formulations, the disintegration times also decreased with an increase in binder concentration. Kollidon® VA 64 is soluble in aqueous media, therefore, the higher the concentration in a formulation, the faster disintegration will occur (Bühler, 1992:217).

On the other hand, Avicel® PH 101/pyridoxine formulations did not depict the same trends concerning friability and tensile strength. In these formulations, it seemed as though the addition of a lubricant played a more important role compared to that of the binder concentration. As the magnesium stearate concentration increased, the percentage friability increased and the tensile strength decreased. However, addition of the lubricant and/or binder to the Avicel® PH 101/pyridoxine formulations decreased the disintegration times.

The faster disintegration times might be due to the overall poorer binding between the Avicel® PH 101/pyridoxine beads and not directly due to the increase in the magnesium stearate concentration. Normally, magnesium stearate will form a hydrophobic layer around particles or tablets which will then repel water molecules from entering the tablet pores during disintegration, thus, extending disintegration of the tablets (Uzunović & Vranić, 2007:280). In this study, conversely, the magnesium stearate decreased the binding properties by rather forming a layer around the beads, shielding each bead from binding interactions with other beads.

Results obtained for the mini-MUPS prepared from uncoated MicroceLac® 100/active ingredient formulations are presented in Table 4.9. Contrary to results attained for the Avicel® formulations, the MicroceLac® 100 formulations showed relatively longer disintegration times as the binder and/or lubricant concentrations increased.

The MicroceLac® 100/pyridoxine formulations practically showed no disintegration which was preferred for this study as the aim was to obtain modified release profiles. Additionally, the percentage friability decreased and the tensile strength increased for all MicroceLac® 100 formulations as the binder and lubricant concentrations increased. It was therefore clear that magnesium stearate did not affect binding and disintegration to the same extent in these formulations. Mass variation was not considerably affected by the type of active ingredient or excipients added, though a slight decline in mass was seen for the MicroceLac® 100/pyridoxine formulations as the Kollidon® VA 64 and magnesium stearate concentrations increased. All of the mini-MUPS of the MicroceLac® 100 formulations did, however, comply with the British Pharmacopoeia (2015) prerequisites. The decrease in tablet mass might have been due to an increase in bulk density of the bead formulations. The same volume in the tablet die would have been filled, however, due to the bulk density

of the added excipients; fewer particles would have flowed into the tablet die, which in turn would produce tablets of a lower mass after compression.

Table 44.9: Physical test data for mini-MUPS prepared from uncoated beads containing MicroceLac® 100 and either furosemide or pyridoxine *(%RSD is indicated in parentheses)*

Formulation	Average mass (mg)	Tensile strength ($N.mm^{-2}$)	Friability (%)	Disintegration (min)
Mic0.05F0	100.52 (9.21)	0.86 (32.60)	68.92 (39.56)	7.16 (7.24)
Mic0.05F3	98.02 (1.52)	1.40 (16.48)	0.12 (20.09)	12.37 (3.92)
Mic0.05F5	97.68 (6.75)	2.08 (16.09)	0.18 (15.86)	13.22 (10.02)
Mic0.1F5	100.10 (2.83)	1.26 (30.19)	0.19 (10.30)	12.15 (7.66)
Mic0P0	106.97 (2.47)	3.66 (11.23)	0.37 (37.16)	***ND**
Mic0.05P0	96.37 (6.29)	3.47 (12.24)	0.06 (61.19)	14.72 (4.72)
Mic0.05P3	90.36 (4.93)	6.35 (6.44)	0.11 (48.02)	***ND**

**ND= Considered non-disintegrating*

All uncoated formulations (Table 4.10) containing RetaLac® as filler were considered non-disintegrating (disintegration times exceeded 15 min). As mentioned before, RetaLac® consists of α-lactose monohydrate and hypromellose in a 1:1 ratio. The α-lactose monohydrate is soluble in water and will therefore not affect disintegration negatively.

However, hypromellose forms a gel-like matrix system when in solution and will therefore not disintegrate. The drug particles are dispersed as solid particles within the formed matrix and the particles at the surface will dissolve first and thus release the drug rapidly. Thereafter, drug particles at successively increasing distance from the surface will dissolve and will be released by diffusion in liquid filled pores in the gel to the exterior of the system. Disintegration of this type of system will therefore be extended (Alderborn, 2013:525). Moreover, no differences in mass variation or percentage friability could be found. All the RetaLac® formulations, irrespective of the active ingredient included, adhered to the British Pharmacopoeia (2015) criteria.

Table.4.10: Physical test data for mini-MUPS prepared from uncoated beads containing RetaLac® and either furosemide or pyridoxine *(%RSD is indicated in parentheses)*

Formulation	Average mass (mg)	Tensile strength ($N.mm^{-2}$)	Friability (%)	Disintegration (min)
Ret0.05F0	96.25 (2.41)	2.72 (7.98)	0.03 (88.54)	***ND**
Ret0.1F0	98.41 (1.12)	2.34 (21.74)	0.03 (75.07)	***ND**
Ret0.1F3	98.34 (2.30)	2.38 (23.55)	0.12 (30.24)	***ND**
Ret0P0	95.15 (2.96)	4.30 (7.97)	0.12 (69.48)	***ND**
Ret0.05P0	101.06 (1.36)	3.96 (12.27)	0.06 (55.22)	***ND**
Ret0.1P0	98.32 (2.04)	3.07 (5.33)	0.66 (19.30)	***ND**
Ret0.1P3	104.42 (1.34)	3.73 (13.15)	0.06 (90.71)	***ND**

**ND= Considered non-disintegrating*

In fact, these mini-MUPS were overall less friable and depicted higher overall tensile strengths compared to the other filler mini-MUPS formulations. These findings are in concurrence with the observations of Meggle (2014b:14) who stated that all tablets produced from RetaLac® containing 10% active ingredient depicted friability values of less than 1%. A small decrease in tensile strength with an increase in excipient (*i.e.* binder and lubricant) concentrations was observed and overall the RetaLac®/pyridoxine formulations depicted slightly higher tensile strengths.

4.3.2.3 Coated beads

As discussed in section 4.3.2.2 a full factorial design was conducted to determine the optimum formulae for each mini-MUPS formulation prepared from uncoated beads utilising physical tablet tests and dissolution profiles. In order to choose the optimum formulations, the overall tablet test results; dissolution profiles (Annexure C) as well as trying to maintain similar binder and lubricant concentrations between the formulations, were considered important factors for this study. The optimised formulations are presented in Table 4.11.

All of the formulations depicted an average tablet mass close to 100 mg. Tensile strength and percentage friability were measured, although these mini-MUPS were intended for

encapsulation into hard gelatine capsules to form mini-MUPS-in-capsule-systems, where the mini-MUPS will be protected and not be exposed to abrasion by extreme handling and transport (Alderborn, 2013:529-530). The optimised RetaLac® formulations depicted a notably higher tensile strength compared to the other optimised formulations, whereas the optimised Avicel® formulations were more brittle compared to the other formulations. Interestingly, the optimised RetaLac® and MicroceLac® formulations containing pyridoxine portrayed increased tensile strength and decreased percentage friability values compared to the formulations containing furosemide.

Table 4.11: Physical test data for the optimised formulations of mini-MUPS prepared from uncoated beads containing either furosemide or pyridoxine *(%RSD is indicated in parentheses)*

Formulation	Average mass (mg)	Tensile strength ($N.mm^{-2}$)	Friability (%)	Disintegration (min)
Avi0F3	96.62 (2.05)	2.50 (13.40)	3.69 (0.78)	13.33 (19.37)
Avi0P0	93.19 (18.52)	1.12 (22.92)	72.46 (65.83)	11.00 (39.83)
Mic0.05F3	98.02 (1.52)	1.40 (16.48)	0.12 (20.09)	12.37 (3.92)
Mic0.05P0	96.75 (6.29)	3.47 (12.24)	0.06 (61.19)	14.72 (4.72)
Ret0.1F3	98.34 (1.12)	2.38 (23.55)	0.12 (30.24)	***ND**
Ret0.1P3	104.42 (2.04)	3.73 (13.15)	0.06 (90.71)	***ND**

**ND= Considered non-disintegrating*

The Avicel® formulations, however, did not follow the same trend. Additionally, one of the objectives of this study was to be able to prepare a modified release system. Disintegration results of tablets are a good indication in determining whether modified release was obtained from the specific formulations. According the British Pharmacopoeia specifications, if 6 tablets of a specific formulation disintegrate within 15 min, the tablets comply with the criteria and are considered conventional immediate release tablets (BP, 2015). However, in order to obtain modified release tablets, one would consider a disintegration time of more than 15 min as non-disintegrating and thus satisfactory. For the mini-MUPS prepared from uncoated beads forming part of the immediate release component of the mini-MUPS-in-capsule system in this study, a disintegration time of less than 15 min was considered

satisfactory. As all of the optimised formulations portrayed disintegration times exceeding 10 min this contributed towards modifying drug release. The optimised bead formulations were manufactured, then coated and compressed and the physical properties of the mini-MUPS were determined as shown in Table 4.12.

Equating the mini-MUPS prepared from uncoated and coated bead formulations, it could be observed that on average the weight of the mini-MUPS increased slightly after the beads were coated. The %RSD for mass variation indicated that all the mini-MUPS of the individual formulations were of almost the same weight as these values were very low. The British Pharmacopoeia (2015) states that for a formulation with an average tablet weight of between 80 - 250 mg, to adhere to the criteria, the weight of two or more tablets may not deviate with more than 7.5% from the average weight.

Table 4.12: Physical test data for the mini-MUPS prepared from coated beads containing either furosemide or pyridoxine *(%RSD is indicated in parentheses)*

Formulation	Average mass (mg)	Tensile strength ($N.mm^{-2}$)	Friability (%)	Disintegration (min)
Avi0F3	102.66 (4.86)	1.01 (28.71)	28.81 (10.70)	3.55 (20.71)
Avi0P0	103.73 (5.89)	0.00 (0.00)	100.00 (0.00)	2.53 (43.38)
Mic0.05F3	102.46 (3.87)	1.31 (14.98)	0.08 (20.70)	14.4 (5.01)
Mic0.05P0	100.35 (3.89)	0.85 (31.32)	63.45 (53.02)	10.00 (13.29)
Ret0.1F3	99.79 (1.53)	1.52 (11.94)	0.11 (40.96)	***ND**
Ret0.1P3	99.48 (2.41)	2.30 (18.63)	0.23 (31.24)	***ND**

**ND= Considered non-disintegrating*

All MicroceLac® and RetaLac® coated formulations adhered to the requirement for mass variation, but the optimised Avicel® formulations containing coated beads failed as more than two mini-MUPS deviated more than 7.5% of the mini-MUPS average mass (one formulation deviating by more than 15%) in tablet weight (Annexure B.2). Also, tensile strength values decreased more prominently and the coated mini-MUPS showed overall higher friability values. It seems as though the coating did not melt or merged during compression in order to form stronger bonding mechanisms between individual beads, but rather fractured and formed a barrier which hindered the beads in the different formulations to be able to properly

bind during compaction (*i.e.* it reduced particle contact). In fact, overall the physical properties of the coated Avicel® formulations were more affected than the other formulations, whereas the coated RetaLac® formulations were affected to a lesser extent.

The disintegration times of the coated Avicel® formulations decreased by approximately 9 min, regardless the active ingredient incorporated. Disintegration times may not have been extended due to the fact that the coating was fractured during compression and/or that the individual beads were not coated sufficiently. Another reason could be that the film coating layer reduced the inter-particular forces between the compressed individual beads, resulting in lower tensile strength, which in turn led to decreased disintegration times as water could have penetrated more rapidly through the pores of the mini-MUPS; and the beads fractured more easily once in contact with the disintegration medium. The Avicel® and MicroceLac® formulations were still considered disintegrating as all the tablets disintegrated within 15 min regardless if they were coated or not. The RetaLac® formulations on the other hand, were considered non-disintegrating. Mini-MUPS prepared from coated and uncoated beads produced from these formulations swelled once in contact with water as RetaLac® consists of 50% hypromellose. These mini-MUPS formed a matrix in the water and rather eroded than disintegrated.

4.3.3 Assay

The assay for content of active ingredient was performed on all optimised mini-MUPS prepared from the uncoated and coated beads (Annexure 4.13 and 4.14). The different mini-MUPS prepared from uncoated and coated beads were formulated to each contain 10 mg active ingredient (either furosemide or pyridoxine). Theoretically, every mini-MUPS-in-capsule-system therefore contained 60 mg active ingredient (three uncoated mini-MUPS and three coated mini-MUPS). No noticeable differences could be observed between the individual uncoated or coated tablet formulations, regardless the active ingredient added.

Table 4.13: Assay for the optimised mini-MUPS prepared from uncoated beads and coated beads containing furosemide *(%RSD is indicated in parentheses)*

	Uncoated beads			**Coated beads**		
	A0F3	**M0.05F3**	**R0.1F3**	**A0F3**	**M0.05F3**	**R0.1F3**
Average mg/100mg tablet	7.81 (5.78)	10.33 (0.76)	9.97 (1.64)	6.11 (7.72)	9.39 (0.76)	9.34 (4.89)

Table 4.14: Assay for the optimised mini-MUPS prepared from uncoated beads and coated beads containing pyridoxine *(%RSD is indicated in parentheses)*

	Uncoated beads			Coated beads		
	A0P3	**M0.05P3**	**R0.1P3**	**A0P0**	**M0.05P0**	**R0.1P3**
Average mg/100mg tablet	7.99 (9.08)	8.95 (8.89)	8.22 (4.20)	9.39 (1.06)	8.43 (0.75)	7.90 (6.78)

4.3.4 Swelling and erosion

The swelling and erosion properties of the optimised mini-MUPS prepared from uncoated and coated beads were evaluated as described in section 3.5.3.5. Tables 4.15 - 4.18 portray the swelling and erosion values obtained for Avicel® PH 101 ® and MicroceLac® 100 mini-MUPS prepared from uncoated and coated beads. Swelling starts right from the beginning as the weight and water uptake of a mini-MUPS increase. Once the mini-MUPS disintegrated into the individual beads, the test was concluded (Sriamornsak *et al.*, 2007:213-215). Note that a mini-MUPS were considered disintegrated when 100% eroded into single beads. The Avicel® PH 101 formulations (regardless the active ingredient incorporated) depicted none to minor swelling for less than 1 min. Erosion rapidly occurred thereafter; and the samples were totally (100%) eroded before 2 min passed.

Table 4.15: Swelling and erosion for Avicel®/furosemide formulations *(%RSD is indicated in parentheses)*

		A0F3			
		Uncoated beads		Coated beads	
		mini-MUPS mass (mg)	**% Swelling**	**mini-MUPS mass (mg)**	**% Swelling**
Time (min)	**0**	102.63 (6.73)	0.00	103.82 (3.77)	0.00
	1	100.77 (0.57)	-1.81*	100.77 (0.22)	-2.94*

** A negative value is an indication of erosion*

Table 4.16: Swelling and erosion for Avicel®/pyridoxine formulations *(%RSD is indicated in parentheses)*

		A0P0			
		Uncoated beads		Coated beads	
		mini-MUPS mass (mg)	% Swelling	mini-MUPS mass (mg)	% Swelling
Time (min)	0	104.63 (2.44)	0.00	101.58 (5.75)	0.00
	1	101.18 (0.65)	-3.29*	99.35 (3.26)	-2.20*

** A negative value is an indication of erosion*

The MicroceLac® 100 formulations tested remained longer intact. Negligible to minor swelling could be observed; though the samples rather eroded more slowly compared to the Avicel® PH 101 formulations. MicroceLac® 100 formulations containing furosemide eroded more rapidly, *i.e.* within 2 - 3 min the samples were considered totally eroded, whereas the uncoated MicroceLac® 100 formulation containing pyridoxine depicted total erosion after 5 min which was also considered relatively fast.

Table 4.17: Swelling and erosion for MicroceLac®/furosemide formulations *(%RSD is indicated in parentheses)*

		M0.05F3			
		Uncoated beads		Coated beads	
		mini-MUPS mass (mg)	% Swelling	mini-MUPS mass (mg)	% Swelling
Time (min)	0	105.48 (6.83)	0.00	100.38 (4.76)	0.00
	1	100.33 (0.09)	-4.88**	100.48 (0.09)	0.09
	2	100.38 (0.10)	-4.83**	100.45 (0.04)	0.07
	3	*	*	100.42 (0.09)	0.04
	5	*	*	*	*

** The dark background is an indication where no further swelling occurred; ** Negative values are an indication of erosion*

Table 4.18: Swelling and erosion for MicroceLac®/pyridoxine formulations *(%RSD is indicated in parentheses)*

		M0.05P0			
		Uncoated beads		Coated beads	
		mini-MUPS mass (mg)	% Swelling	mini-MUPS mass (mg)	% Swelling
Time (min)	0	105.68 (4.87)	0.00	100.67 (2.44)	0.00
	1	100.65 (0.16)	-4.76**	99.14 (3.15)	-1.52**
	2	100.6 (0.12)	-4.81**	99.26 (3.15)	-1.40**
	3	100.72 (0.25)	-4.69**	*	*
	5	100.64 (0.14)	-4.77**	*	*

** The dark background is an indication where no further swelling occurred; **Negative values are an indication of erosion*

RetaLac® formulations, on the other hand, portrayed prominent swelling properties as can be observed Tables 4.19 and 4.20 As previously discussed, the filler RetaLac® consists of hypromellose and α-lactose monohydrate (1:1), forming a hydrophobic matrix when in solution. Levina and Rajabi-Siahboomi (2004:2746), as well as Sriamornsak *et al.* (2007:211) stated that hypromellose (hydroxypropyl methylcellulose, HPMC) are frequently used as an oral drug delivery system, mainly to enable sustained drug release. Furthermore, Sriamornsak *et al.* (2007:211, 218-219) stated that HPMC is compatible with most excipients and are formulated utilising an easy, inexpensive and economical process for prolonged drug release with no need for special equipment. Drug release from a hydrophobic matrix usually occurs through the formation of a hydrated viscous layer around the tablet, acting as a barrier to yield drug release through water penetration, as well as the movement of drug solutes out of the matrix tablet. These hydration properties of the polymer and the physical characteristics or the hydrated gel layer may influence drug release (Alderborn, 2013:525-526; Qiu, 2009a:472,490).

Kavanagh and Corrigan (2004:141) explained the process of swelling and erosion of a matrix system, as once the tablet or in this case, mini-MUPS is exposed to water or dissolution medium, the dry polymer becomes hydrated, swells and forms a barrier gel layer, decreasing diffusion of the drug out of the matrix. The more the polymer chain becomes

hydrated the more the gel is diluted, moving closer to the point of "disentanglement concentration", this being the critical polymer concentration below which the polymer chains disentangle and detach from a gelled matrix.

Table 4.19: Swelling and erosion for RetaLac®/furosemide formulations *(%RSD is indicated in parentheses)*

		R0.1F3			
		Uncoated beads		Coated beads	
		mini-MUPS mass (mg)	% Swelling	mini-MUPS mass (mg)	% Swelling
Time (min)	0	98.52 (2.89)	0.00	100.63 (1.21)	0.00
	1	102.39 (0.05)	3.93	103.32 (0.60)	2.67
	2	103.41 (0.05)	4.96	104.35 (0.60)	3.70
	3	104.44 (0.05)	6.01	105.38 (0.60)	4.72
	5	105.46 (0.05)	7.04	106.42 (0.59)	5.75
	13	106.49 (0.05)	8.09	107.46 (0.60)	6.79
	21	107.52 (0.05)	9.14	108.50 (0.61)	7.82
	30	*	*	109.55 (0.61)	8.86
	60	*	*	110.60 (0.62)	9.91
	90	*	*	111.39 (40.83)	10.69
	120	*	*	*	*
	150	*	*	*	*
Erosion (%)		2.98 (99.56)		2.66 (22.68)	

**The dark background is an indication where no further swelling occurred*

Table 4.20: Swelling and erosion for RetaLac®/pyridoxine formulations *(%RSD is indicated in parentheses)*

		R0.1P3			
		Uncoated beads		Coated beads	
		mini-MUPS mass (mg)	% Swelling	mini-MUPS mass (mg)	% Swelling
Time (min)	0	95.23 (3.53)	0.00	100.37 (1.6)	0.00
	1	102.91 (0.13)	8.06	103.36 (0.16)	2.98
	2	103.94 (0.13)	9.15	104.4 (0.16)	4.02
	3	104.97 (0.13)	10.23	105.44 (0.16)	5.05
	5	106.00 (0.13)	11.31	106.47 (0.16)	6.08
	13	107.04 (0.13)	12.40	107.51 (0.16)	7.11
	21	108.07 (0.13)	13.48	108.56 (0.16)	8.16
	30	109.11 (0.13)	14.58	109.60 (0.16)	9.20
	60	*	*	110.65 (0.16)	10.24
	90	*	*	111.70 (0.16)	11.29
	120	*	*	112.75 (0.16)	12.33
	150	*	*	113.80 (0.16)	13.38
Erosion (%)		4.51 (85.33)		41.17 (9.10)	

**The dark background is an indication where no further swelling occurred*

Once this transpired, the polymer will undergo simultaneous swelling, dissolution and diffuse into the bulk medium, resulting in erosion of the polymer. Erosion is known as the rate-controlling step for drug release of poorly soluble or water-insoluble drugs (*i.e.* furosemide) from a water swellable matrix system. Water penetration or diffusion, however, is

considered the rate-controlling step, for highly water-soluble drugs (*i.e.* pyridoxine) (Kavanagh & Corrigan, 2004:141; Yin *et al.*, 2013:1025). Swelling behaviour of the matrix is therefore an indication of the rate at which the dissolution media is absorbed (Sriamornsak *et al.*, 2007:213-215).

From Figure 4.10 it is clear that the coated RetaLac® formulations, regardless the active ingredient incorporated, swelled more and for a longer time period than the uncoated RetaLac® formulations. This might have been due to the coating inhibiting proper liquid penetration into the tablets as well as dissolution and diffusion of the polymer (hypromellose) into the bulk medium, thus decreasing the degree of swelling. This observation was more evident for the RetaLac® formulations containing the highly water-soluble pyridoxine, where, as stated the rate-controlling step for this type of matrix-system is considered water penetration or diffusion.

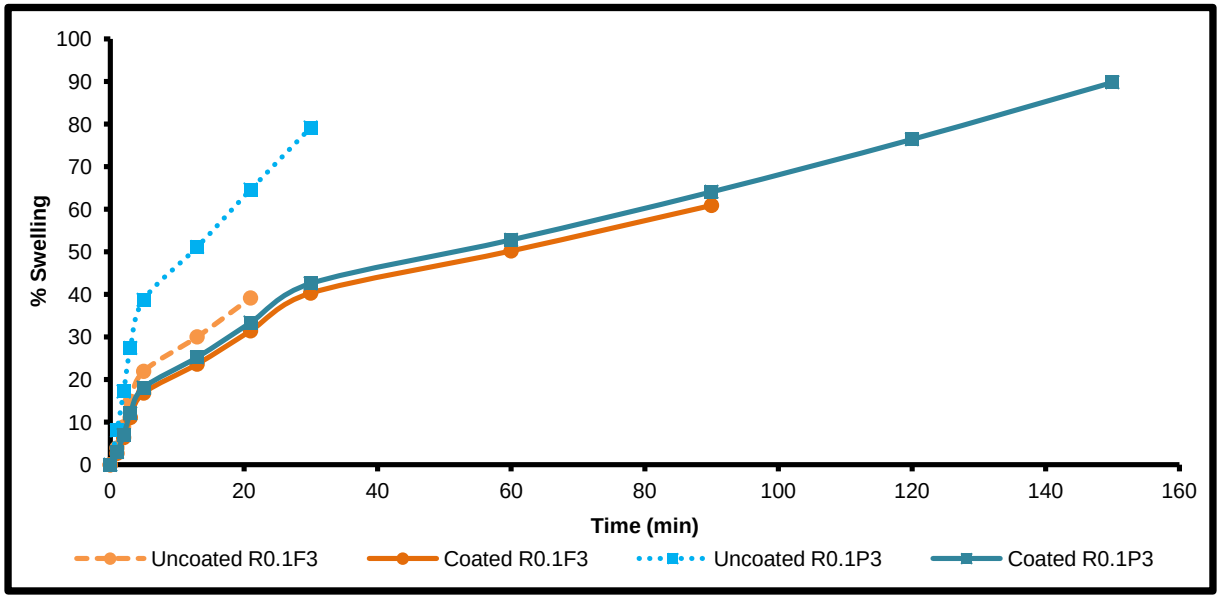


Figure 4.10: Furosemide or pyridoxine average percentage swelling of uncoated and coated RetaLac® formulations

Furthermore, the RetaLac® formulations containing furosemide, a poorly water-soluble drug, showed a lesser degree of swelling compared to the formulations containing pyridoxine. For example the uncoated RetaLac®/furosemide (R0.1F3) formulation depicted a total percentage swelling of 39.17% compared to the coated RetaLac®/pyridoxine (R0.1P3) formulation that showed a total percentage swelling of 89.81%. These RetaLac® formulations containing furosemide also depicted swelling times shorter than the formulations containing pyridoxine. For example the uncoated RetaLac®/furosemide

(R0.1F3) formulation depicted the fastest maximum swelling time of 21 min compared to the coated RetaLac®/pyridoxine (R0.1P3) formulation that swelled for approximately 150 min.

Erosion of the RetaLac®/furosemide formulations occurred more rapidly. The uncoated and coated RetaLac®/furosemide formulations started eroding after 21 and 90 min, respectively; whereas the RetaLac®/pyridoxine formulations only started eroding after 30 and 150 min, respectively. However, the RetaLac®/furosemide formulations depicted a maximum erosion percentage of less than 3%, regardless if it was coated or not; whereas the uncoated RetaLac®/pyridoxine formulation presented a maximum erosion percentage of 4.51% and the coated RetaLac®/pyridoxine formulation depicted a maximum erosion percentage of 41.17%. The dissolution of the film coating material may have contributed towards the final loss in mass of the coated mini-MUPS. As stated before, erosion is known as the rate-controlling step for drug release of poorly soluble drugs (*i.e.* furosemide) from a water swellable matrix system. Therefore, the fact that the RetaLac®/furosemide formulations showed less erosion of the mini-MUPS may be an indication that dissolution of the active ingredient from these formulations could be to a lesser extent and/or delayed. Levina and Rajabi-Siahboomi (2004:2753) concluded that water absorption was increased from tablets containing HPMC with added microcrystalline cellulose or lactose. These findings were in concurrence with the findings of this study.

4.3.5 Dissolution studies

A total of 21 mini-MUPS formulations prepared from uncoated beads (to establish an immediate release component); 6 mini-MUPS formulations prepared from coated beads (establish a modified release component) and 6 mini-MUPS-in-capsule formulations (containing three mini-MUPS prepared from uncoated beads and three mini-MUPS prepared from coated beads) were evaluated to determine the effects of each factor in the dissolution profiles of the different formulations. The similarity and difference factors as well as the mean dissolution times were calculated. All data regarding average percentage drug release of mini-MUPS prepared from optimised uncoated beads, coated beads and mini-MUPS-in-capsule systems; mean dissolution times (MDT); difference as well as similarity factors are presented in Annexure C.

4.3.5.1 Dissolution of mini-MUPS prepared from uncoated beads

Dissolution results obtained for all of the mini-MUPS formulations prepared from uncoated beads (as determined by the factorial design) are presented in Figures 4.11– 4.16 and Tables 4.21 – 4.23.

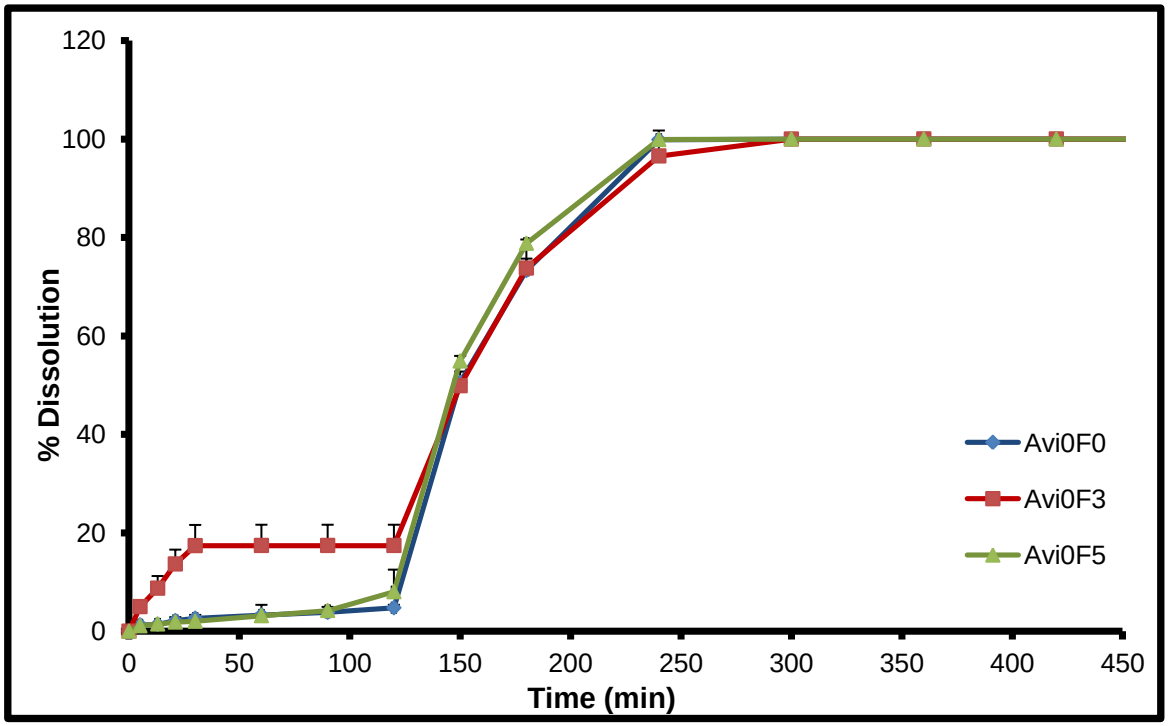


Figure 4.11: Percentage drug dissolution for the different Avicel® PH 101/furosemide formulations prepared from uncoated beads as a function of time

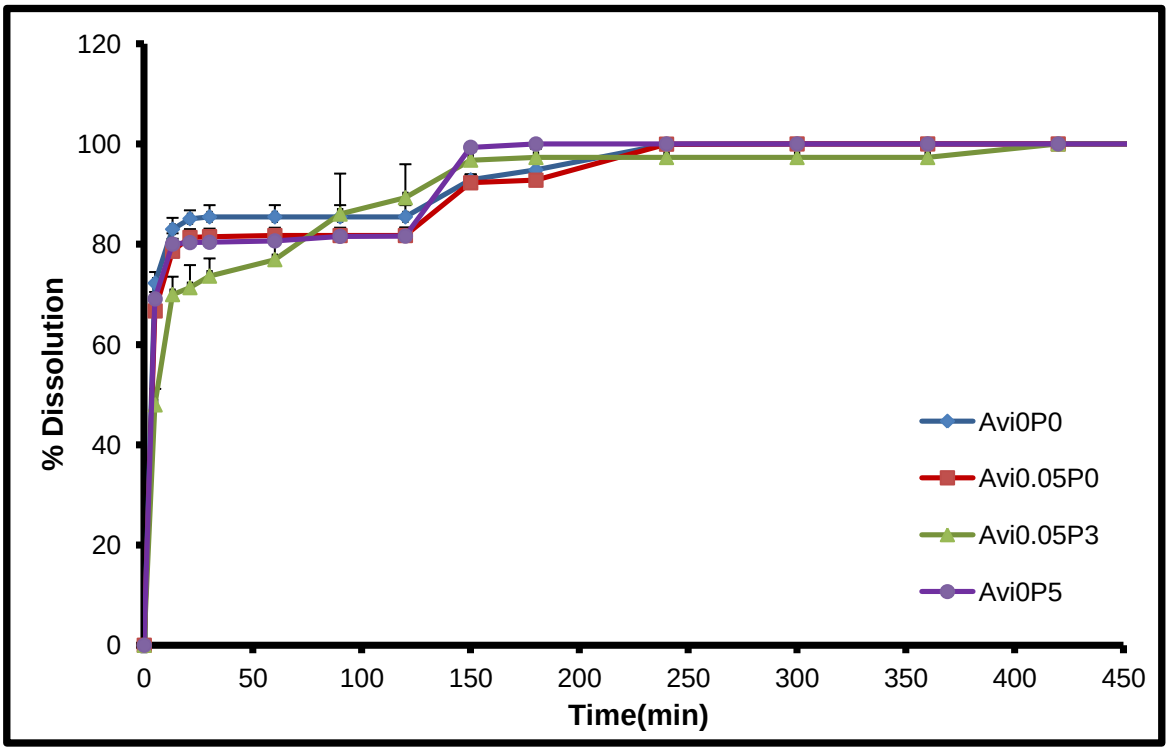


Figure 4.12: Percentage drug dissolution for the different Avicel® PH 101/pyridoxine formulations prepared from uncoated beads as a function of time

Table 4.21: Percentage drug dissolution for the different Avicel® PH 101 formulations prepared from uncoated beads for the active ingredients furosemide and pyridoxine

Average % in solution							
Time (min)	**Avi0F3**	**Avi0F0**	**Avi0F5**	**Avi0P0**	**Avi0P5**	**Avi0.05P3**	**Avi0.05P0**
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	4.99	1.29	1.10	72.25	69.09	47.96	66.69
13	8.74	1.40	1.47	82.99	80.03	69.95	78.59
21	13.66	2.16	1.85	85.09	80.32	71.34	81.35
30	17.38	2.63	2.01	85.46	80.39	73.64	81.51
60	17.41	3.29	3.15	85.46	80.66	76.92	81.73
90	17.41	3.84	4.21	85.46	81.57	86.02	81.73
120	17.41	4.76	8.02	85.46	81.62	89.27	81.73
150	49.87	50.54	54.89	92.83	99.33	96.78	92.25
180	73.77	73.29	78.80	94.82	100.00	97.30	92.77
240	96.55	99.85	99.88	99.97	100.00**	97.30	99.96
300	99.98	100.00	100.00	100.00	100.00**	97.30	100.00
360	100.00**	100.00**	100.00**	100.00**	100.00**	97.30	100.00**
420	100.00**	100.00**	100.00**	100.00**	100.00**	99.99	100.00**
480	100.00**	100.00**	100.00**	100.00**	100.00**	100.00	100.00**
MDT (min)*	16.99	18.71	17.94	5.30	5.13	5.91	6.13
f_1*	-	13.31	14.35	-	3.76	8.44	2.94
f_2*	-	53.00	53.02	-	69.4	49.72	73.49

** f_1 = Difference factor; f_2 = Similarity factor; MDT = Mean dissolution time. ** The dark background = no further dissolution*

No clear differences in dissolution profiles could be observed between mini-MUPS formulations manufactured from a certain filler, *i.e.* an increase in either magnesium stearate and/or Kollidon® VA 64 concentrations in the mini-MUPS formulations manufactured from Avicel®; MicroceLac® or RetaLac® did not influence drug release from these mini-MUPS as the dissolution profiles were almost similar and followed the same trend. However, visible differences between the different mini-MUPS formulations were noticed from the start of the dissolution experiments. The mini-MUPS from the Avicel®- and MicroceLac® formulations rapidly disintegrated into beads on the bottom of the glass dissolution vessels, whereas the mini-MUPS manufactured from RetaLac® rather floated in and on the surface of the dissolution media; and formed a gel-matrix from where the active ingredient first had to diffuse from prior to dissolution.

Similarly, clear difference could be observed between the mini-MUPS formulations containing furosemide and pyridoxine, regardless the type of filler employed. Mini-MUPS formulations that contained furosemide as an active ingredient depicted dissolution profiles showing delayed drug release in the acidic phase (pH = 1.2) from the start of the dissolution experiments until 120 min. As indicated in section 3.2.1. furosemide is a poorly water-soluble active ingredient, but it is freely soluble in diluted alkali and drug release occurs

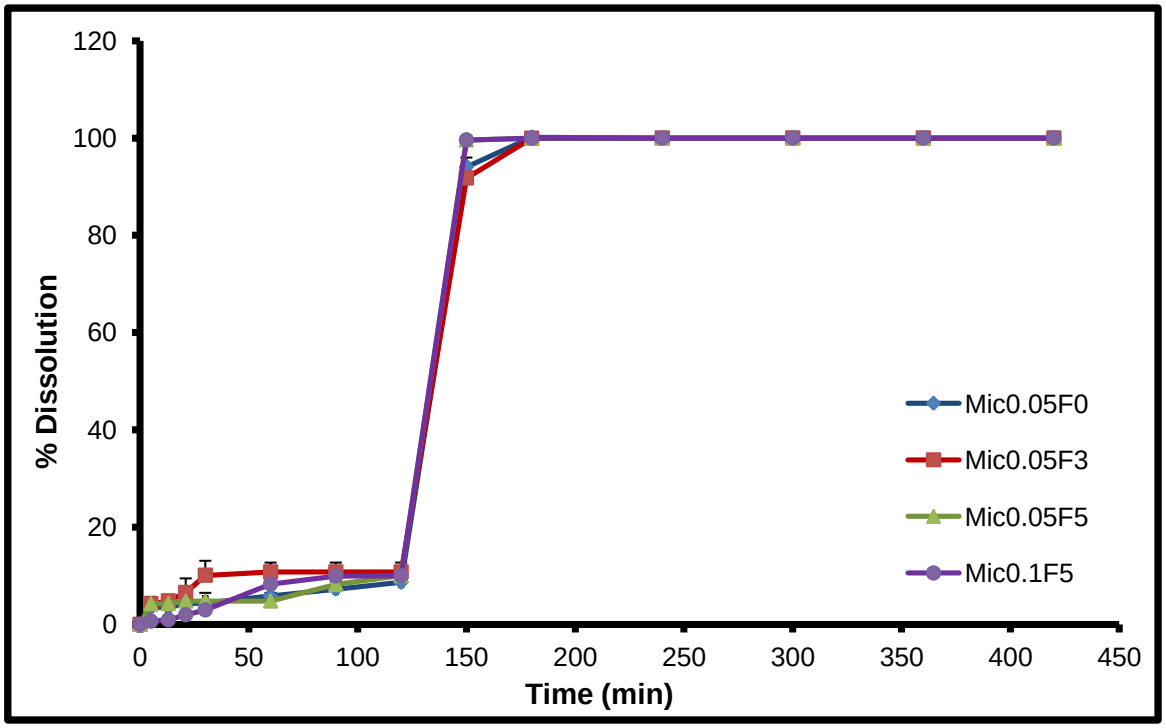


Figure 4.13: Percentage drug dissolution for the different MicroceLac® 100/furosemide formulations prepared from uncoated beads as a function of time

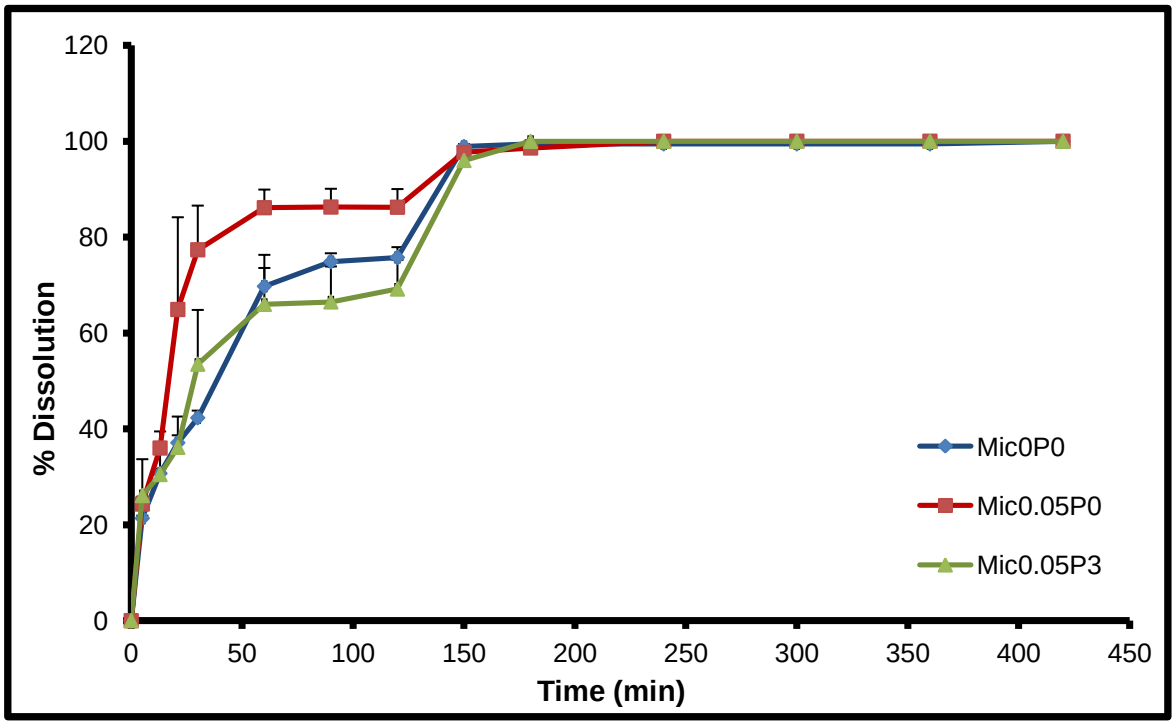


Figure 4.14: Percentage drug dissolution for the different MicroceLac® 100/pyridoxine formulations prepared from uncoated beads as a function of time

Table 4.22: Percentage drug dissolution for the different MicroceLac® 100 formulations prepared from uncoated beads for the active ingredients furosemide and pyridoxine

Average % in solution							
Time (min)	**Mic0.05F3**	**Mic0.05F0**	**Mic0.05F5**	**Mic0.1F5**	**Mic0.05P0**	**Mic0P0**	**Mic0.05P3**
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	4.21	3.53	4.27	0.64	24.33	21.36	26.09
13	4.83	3.51	4.28	0.92	36.01	30.72	30.49
21	6.55	4.26	4.79	1.98	64.90	37.11	36.16
30	10.10	4.39	4.79	2.97	77.36	42.31	53.49
60	10.78	5.83	4.79	8.29	86.13	69.74	65.98
90	10.79	7.27	8.22	9.96	86.27	74.88	66.48
120	10.79	8.66	10.04	9.97	86.23	75.76	69.17
150	91.78	94.07	99.58	99.61	97.68	98.90	95.99
180	99.95	100.00	100.00	100.00	98.59	99.44	99.98
240	100.00	100.00**	100.00**	100.00**	99.99	99.44	100.00
300	100.00**	100.00**	100.00**	100.00**	100.00	99.44	100.00**
360	100.00**	100.00**	100.00**	100.00**	100.00**	99.44	100.00**
420	100.00**	100.00**	100.00**	100.00**	100.00**	100.00	100.00**
MDT (min)*	13.85	14.44	14.41	14.67	6.43	10.03	8.81
f_1*	17.91	21.65	21.91	22.96	12.53	22.68	22.68
f_2*	41.99	39.97	38.53	38.34	34.45	26.97	27.7
f_1***	-	3.56	3.82	4.8	-	11.81	12.53
f_2***	-	77.87	73.56	71.36	-	41.77	41.88

** f_1 = Difference factor; f_2 = Similarity factor; MDT = Mean dissolution time. ** Dark background = of no further dissolution; *** f_1 = Difference factors for the various MicroceLac® formulations compared with the optimised MicroceLac® formulations; *** f_2 = Similarity factors for the various MicroceLac® formulations compared with the optimised MicroceLac® formulations*

through erosion as the rate-controlling step (Kavanagh & Corrigan, 2004:141; Martindale, 2015; Yin *et al.*, 2013:1025). When the pH was adjusted to 6.8, drug release increased in the following 30 min from approximately 5 – 55% for all of the uncoated Avicel® mini-MUPS/furosemide formulations; from nearly 9 – 100% for all of the uncoated MicroceLac® mini-MUPS/furosemide formulations; and from roughly 13 – 98% for all of the uncoated RetaLac® mini-MUPS/furosemide formulations. The following rank order could be established for the percentage drug released from furosemide mini-MUPS formulations within the first 120 min (considering the fillers utilised): RetaLac® > MicroceLac® > Avicel®. In contrast with these results, mini-MUPS/pyridoxine formulations portrayed dissolution profiles displaying rapid drug release within the first 120 min as water penetration or diffusion is

considered the rate-controlling step for water-soluble drugs (i.e. pyridoxine) (Kavanagh & Corrigan, 2004:141; Yin *et al.*, 2013:1025).

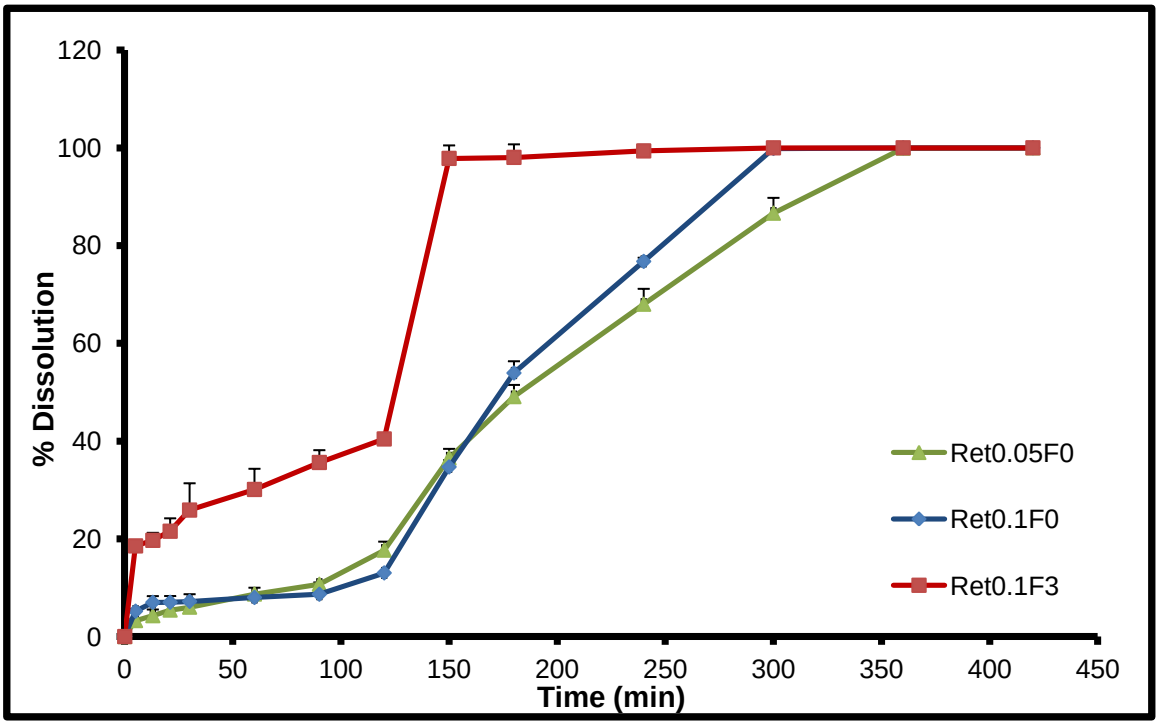


Figure 4.15: Percentage drug dissolution for the different RetaLac®/furosemide formulations prepared from uncoated beads as a function of time

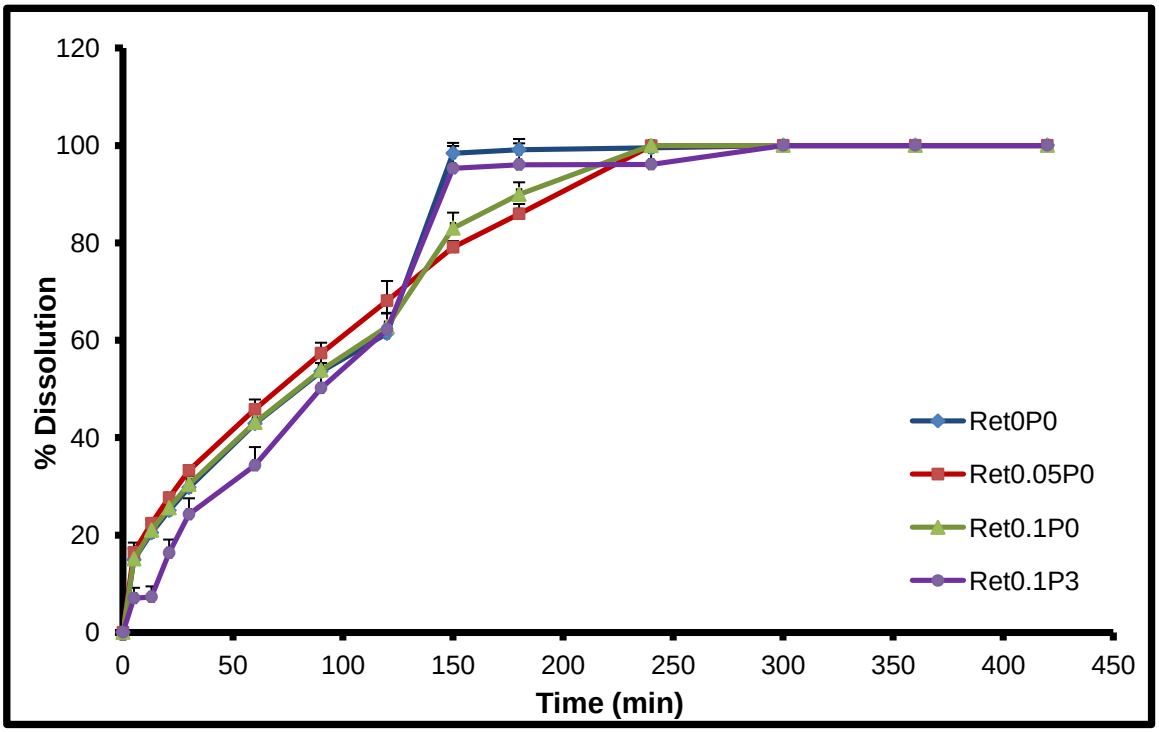


Figure 4.16: Percentage drug dissolution for the different RetaLac®/pyridoxine formulations prepared from uncoated beads as a function of time

Table 4.23: Percentage drug dissolution for the different RetaLac® formulations prepared from uncoated beads for the active ingredients furosemide and pyridoxine

Average % in solution							
Time (min)	**Ret0.1F3**	**Ret0.05F0**	**Ret0.1F0**	**Ret0.1P3**	**Ret0P0**	**Ret0.05P0**	**Ret0.1P0**
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	18.55	3.27	5.19	7.02	14.89	16.42	15.07
13	19.69	4.27	7.00	7.28	20.44	22.39	20.96
21	21.55	5.42	7.02	16.33	25.03	27.66	25.57
30	25.88	6.02	7.20	24.24	29.73	33.23	30.39
60	30.10	8.70	8.00	34.29	42.85	45.78	43.08
90	35.62	10.70	8.66	50.16	53.55	57.33	53.86
120	40.43	17.68	13.02	62.21	61.38	68.10	62.76
150	97.84	36.55	34.71	95.29	98.37	79.07	82.99
180	98.04	49.09	53.92	96.05	99.11	85.93	89.93
240	99.36	68.00	76.76	96.12	99.48	99.92	99.94
300	100.00	86.62	99.87	99.98	100.00	100.00	100.00
360	100.00**	99.93	100.00	100.00	100.00**	100.00**	100.00**
420	100.00**	100.00	100.00**	100.00**	100.00**	100.00**	100.00**
MDT (min)*	12.22	21.93	21.04	12.90	11.68	13.23	12.97
f_1*	27.52	19.68	15.59	36.29	32.21	31.21	32.27
f_2*	36.83	44.62	49.48	17.98	20.97	22.04	21.13
f_1***	-	36.95	33.76	-	8.35	14.45	10.51
f_2***	-	27.79	28.61	-	60.21	50.59	56.14

** f_1 = Difference factor; f_2 = Similarity factor; MDT = Mean dissolution time. ** Dark background = of no further dissolution; *** f_1 = Difference factors for the various RetaLac® formulations compared with the optimised RetaLac® formulations; *** f_2 = Similarity factors for the various RetaLac® formulations compared with the optimised RetaLac® formulations*

The Avicel® mini-MUPS/pyridoxine formulations released approximately 80% active ingredient within the first 120 min, irrespective of the excipients included in the formulations or the fact that microcrystalline cellulose is practically insoluble in diluted acids (Martindale, 2015); and in the following 30 min (after pH adjustment to 6.8) approximately 92% pyridoxine have already been released. The MicroceLac® mini-MUPS/pyridoxine formulations released roughly 70% pyridoxine within the first 120 min and approximately 95% within 150 min also irrespective of the formulation compositions. Lastly, mini-MUPS/pyridoxine formulations manufactured from RetaLac® depicted an active ingredient release of nearly 60% after the first 120 min and approximately 80% after 150 min. Thus, the following rank order could be established for the initial percentage drug released from pyridoxine mini-MUPS formulations within the first 120 min (considering the fillers utilised): Avicel® > MicroceLac® > RetaLac®. Levina and Rajabi-Siahboomi (2004:2753) found that water absorption was increased from tablets containing HPMC with added microcrystalline cellulose or lactose resulting in faster drug release. In this study, however, this was not the case. Drug release depended

predominantly on the type of active ingredient incorporated as well as on the discriminating media in which dissolution occurred.

Mean dissolution time (MDT) is defined as the average dissolution time of the percentage drug released for all the points tested during the dissolution and the higher the MDT the slower the drug release (Tables 4.21 - 4.23) (Qiu, 2009b:384). In general formulations containing pyridoxine exhibited lower MDT-values compared to formulations containing furosemide, indicating that dissolution of the formulations containing pyridoxine was overall faster. This was expected as pyridoxine is a water-soluble active ingredient. Furthermore, RetaLac® mini-MUPS formulations depicted the highest MDT-values, regardless the active ingredient incorporated. This indicated that dissolution from these formulations was more delayed compared to the other mini-MUPS formulations, which was probably due to the gel-matrix formation as explained earlier. Differences in MDT-values for the Avicel®- and MicroceLac® mini-MUPS formulations were less prominent; however, the MDT-values for the MicroceLac®/furosemide mini-MUPS formulations were slightly lower compared to the Avicel®/furosemide mini-MUPS formulations, indicating that dissolution occurred more rapidly from the MicroceLac®/furosemide mini-MUPS formulations. As seen in Tables 4.21 and 4.22 differences in the pyridoxine formulations were not as clear.

The f_1- and f_2-values were calculated (Tables 4.21 - 4.23; Tables C.1 – C.3; Annexure C) for each filler/active ingredient mini-MUPS prepared from uncoated beads combination in order to establish if any significant differences could be obtained between these formulations. No statistical significant differences ($f_1 < 15\%$; $f_2 > 50\%$) in the dissolutions profiles of the Avicel®/furosemide or Avicel®/pyridoxine mini-MUPS formulations prepared from uncoated beads were observed, which indicated that the addition of magnesium stearate and/or Kollidon® VA 64, irrespective of the concentrations added, did not alter the dissolution behaviour of these formulations. The same conclusion could be drawn for the MicroceLac®/active ingredient formulations (regardless the active ingredient) as well as for the RetaLac®/pyridoxine formulations ($f_1 < 15\%$; $f_2 > 50\%$). However, the f_2-values of the MicroceLac®/pyridoxine formulations showed slight dissimilarity ($f_2 = 41.77\%$ and 41.88%, respectively) between these formulations. The addition of excipients to these formulations might therefore have had a slight influence on the dissolution behaviour; this was however not evident in this study. Even though the MDT-values of the RetaLac®/furosemide formulations did not show substantial differences, the f_1- and f_2-values did identify statistical significant differences ($f_1 > 15\%$; $f_2 < 50\%$) between the dissolution profiles of these formulations. The addition of 3% Kollidon® VA 64 decreased the dissolution time, whereas the addition of magnesium stearate in higher concentrations, *i.e*. 0.1%, increased dissolution time. An overall evaluation of the different fillers effect on dissolution profiles, irrespective of

the active ingredients used depicted the following. Avicel® PH 101 or microcrystalline cellulose had a faster drug release even though it is practically insoluble in diluted acids (Figure 4.11 and 4.12) (Martindale, 2015). MicroceLac® 100 portrayed somewhat similar dissolution profiles compared with Avicel®. A possible reason could be that as 75% of this co-compressed excipient exist out of α-lactose monohydrate being freely, but slowly soluble in water, enabled slower drug release (Figure 4.13 and 4.14) (Martindale, 2015; Meggle, 2014a:2,5). As previously stated the filler RetaLac® forms a hydrophilic mini-MUPS matrix and drug release usually occurs through the hydrated viscous layer forming around the tablet acting as a barrier and yielding drug release (Sriamornsak *et al.*, 2007:211). Thus RetaLac® formulations portray more delayed drug release compared to the other fillers used (Figure 4.15 and 4.16).

4.3.5.2 Comparison of the dissolution profiles of the optimised uncoated mini-MUPS

As stated before, after careful evaluation of the results obtained from the various tablet tests and dissolution profiles of the different formulations of mini-MUPS prepared from uncoated beads the optimised bead formulations were selected (Table 4.7), prepared, coated and compressed into mini-MUPS after which the physical properties were tested. The optimised formulations for each filler/active ingredient as indicated in the factorial design are shown in Figures 4.17 and 4.18. The Avicel® formulations were used as the "reference standard", and thus, the f_1- and f_2-values were calculated utilising these formulations as the references. Figure 4.17 depict the average percentage drug in solution of the optimised uncoated formulations containing furosemide.

The f_1- and f_2-values showed definite differences ($f_1 > 15\%$; $f_2 < 50\%$) between all of the optimised uncoated formulations containing furosemide. Observing the MDT-values, it could be seen that the Avicel® and MicroceLac® formulations depicted a more delayed drug release (higher MDT) compared to the RetaLac® formulation (lower MDT). The following rank order concerning the dissolution times of the respective formulations could be established: RetaLac® > MicroceLac® > Avicel®. The formation of a hydrophobic gel-matrix might have aided in the dissolution of furosemide from the RetaLac® formulation as it probably created a more preferable environment where the furosemide particles were possibly in closer contact with the dissolution medium. Likewise, the RetaLac® oblong shaped beads were more porous and thus fragile, enabling faster water penetration and drug release.

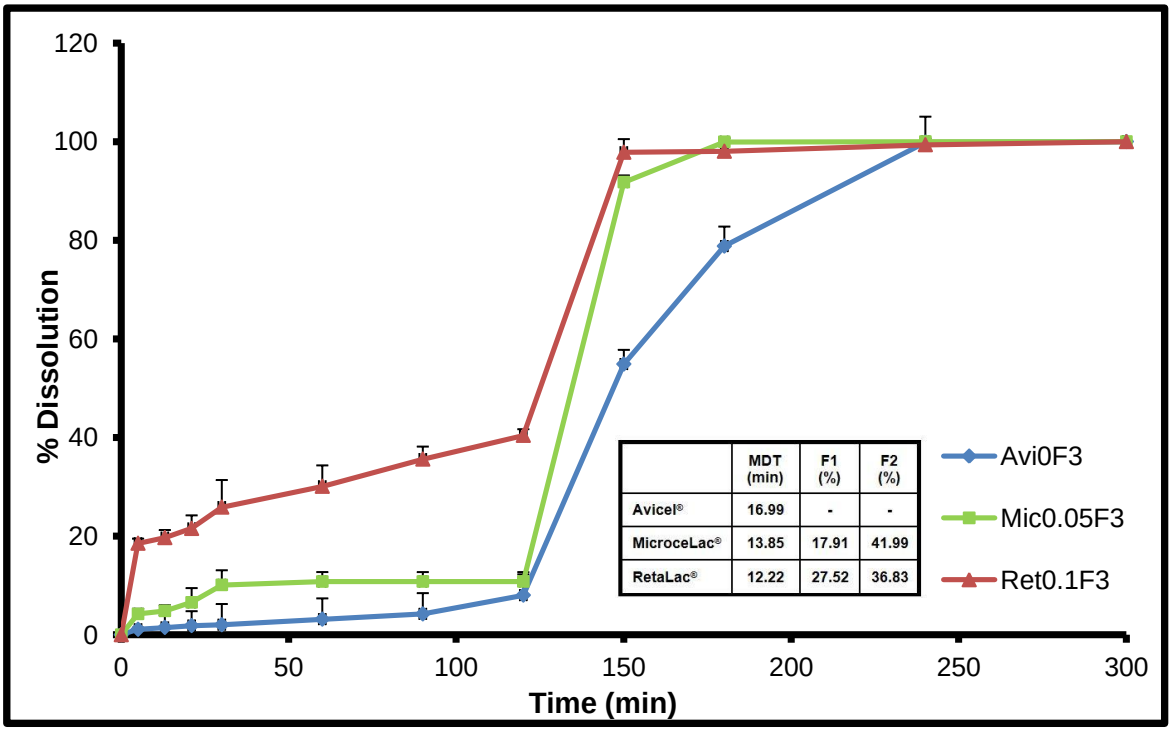


Figure 4.17: Percentage drug dissolution for the optimised furosemide formulations prepared from uncoated beads plotted as a function of time. The mean dissolution time, similarity and difference factors are also shown

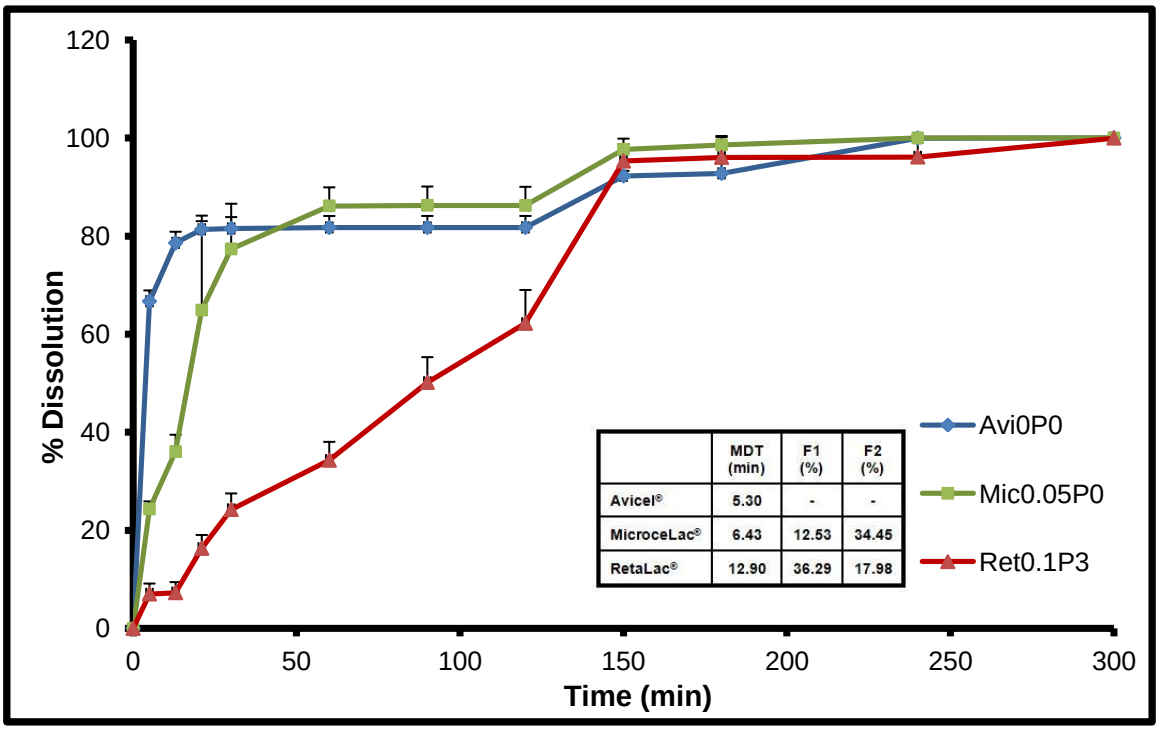


Figure 4.18: Percentage drug dissolution for the optimised pyridoxine formulations prepared from uncoated beads plotted as a function of time. The mean dissolution time, similarity and difference factors are also shown

Furthermore, the beads manufactured from microcrystalline cellulose (i.e. Avicel®) or co-compressed lactose/microcrystalline cellulose (*i.e.* MicroceLac®), were harder, thus delaying water penetration into, and finally drug release from the pores in the various beads. Again, the f_1- and f_2-values displayed significant differences ($f_1 > 15\%$; $f_2 < 50\%$) between all of the optimised uncoated formulations containing pyridoxine (Figure 4.18). However, these formulations containing pyridoxine depicted a rank order for the MDT-values contradictory to that obtained for the furosemide formulations: Avicel® > MicroceLac® > RetaLac®. The MDT-values for the pyridoxine formulations were lower compared to the values of the furosemide formulations, indicating that drug release from these formulations was faster as expected for freely soluble drugs. Moreover, the RetaLac®/pyridoxine formulation depicted the slowest drug release from the formulation which could be attributed to the gel-matrix in which the rate determining step for drug dissolution is water penetration or diffusion.

4.3.5.3 Evaluation of the dissolution profiles of mini-MUPS prepared from coated beads

Bashaiwoldu *et al.* (2011:352) and Nikowitz *et al.* (2014:1007) found that drug release from mini-MUPS prepared from coated beads were mainly dependent on film coating thickness and the amount of damage to the film coating during compression of the coated beads. However, formulations of mini-MUPS prepared from uncoated and coated beads in this study predominately depended on the type of formulation utilised as well as the active ingredient incorporated in order to produce delayed drug release. Furthermore, Bashaiwoldu *et al.* (2011:352) concluded that deformation of coated beads during compaction resulted in film rupture and/or complete disintegration of beads into original powder particles once in contact with dissolution media. They found that they had no control over the merging or fracturing of the film coating during compaction, and thus, also no control over drug release. They could therefore not manufacture coated beads with modified drug release, irrespective of the coating thickness.

Results obtained in this study (Figure 4.19 and 4.20) showed that coating of the different bead formulations, irrespective of the active ingredient integrated, did not meaningfully delay release of the active ingredient from the various formulations. During the compression process of the mini-MUPS prepared from coated beads the individual beads deformed, however no plasticisation of the coating occurred. The coating rather cracked, exposing the active ingredient to the dissolution media, even though the mini-MUPS remained intact. Comparison of the MDT-values of the mini-MUPS formulations prepared from the uncoated beads with the mini-MUPS formulations prepared from the coated beads (Tables C1 and C.2; Annexure C) confirmed that substantial delayed release was not achieved.

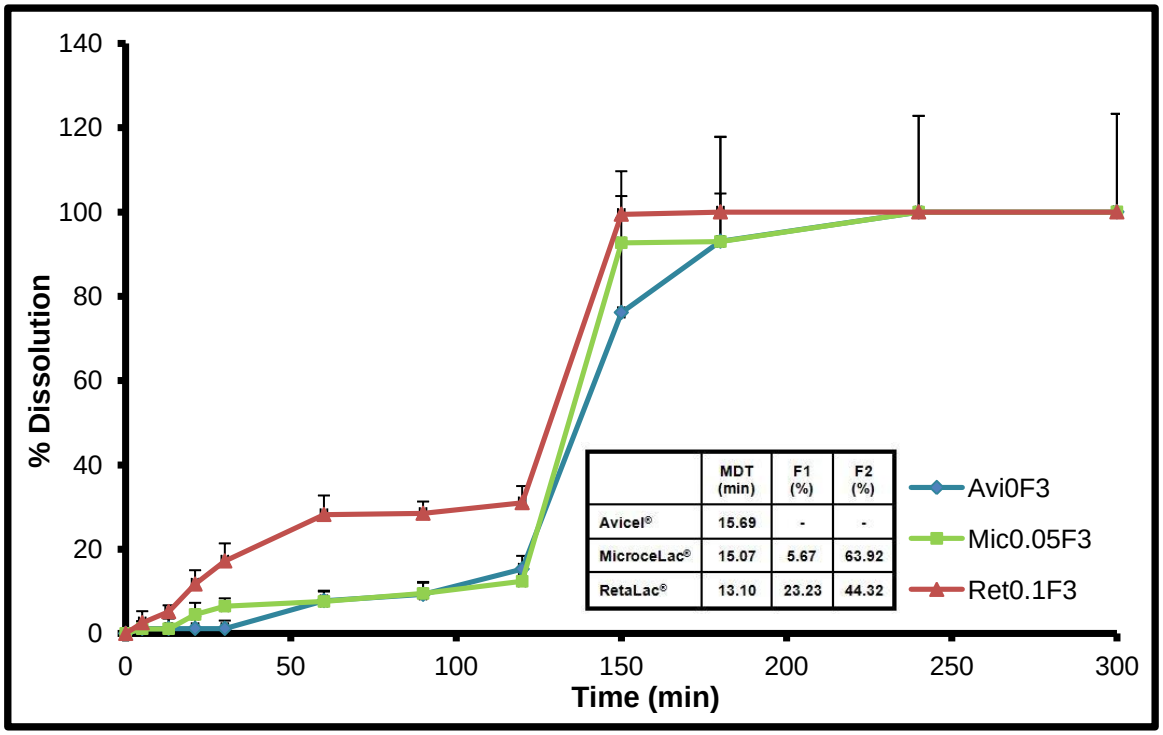


Figure 4.19: Percentage drug dissolution for the optimised furosemide formulations prepared from coated beads plotted as a function of time. The mean dissolution time, similarity and difference factors are also shown

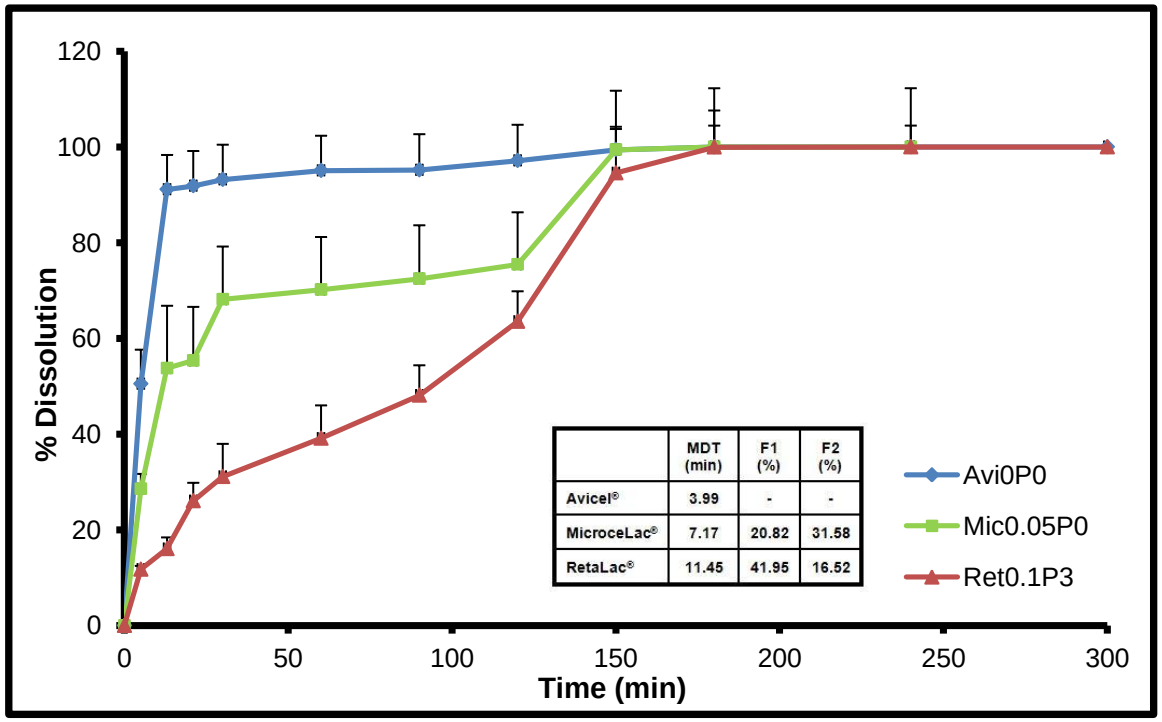


Figure 4.20: Percentage drug dissolution for the optimised pyridoxine formulations prepared from coated beads plotted as a function of time. The mean dissolution time, similarity and difference factors are also shown

Dissolution of the coated MicroceLac® and RetaLac® formulations were marginally slower compared to the dissolution rate of the uncoated mini-MUPS of the optimised formulations (Annexure C). In fact, coating of the optimised Avicel® mini-MUPS formulations actually caused a slightly more rapid dissolution of the active ingredient. The optimised Avicel® formulations were more brittle and disintegrated faster compared to the other formulations as discussed in section 4.3.2, thus indicating that dissolution of the active ingredients of these formulations would be relatively faster compared to the other coated formulations.

4.3.5.4 Evaluation of the dissolution profiles of mini-MUPS-in-capsules

Beads were produced utilising extrusion-spheronisation; half of these beads were coated and compressed to create coated mini-MUPS, whereas the other half were left uncoated and compressed to create uncoated mini-MUPS. Subsequently, three uncoated and three coated mini-MUPS from the same filler and active ingredient were encapsulated in size 0 hard gelatine capsules. Uncoated and coated compressed beads were utilised in order to create an immediate drug release component with delayed drug release properties. Half of the beads were film coated with a Eudragit® L /S 100 mixture in order to delay drug release until a pH of between 5.5 and 7 was reached, thus avoiding drug release in the stomach (Mahato, 2007:155; Nollenberger & Albers, 2013:465-468). The uncoated and coated mini-MUPS were inserted into hard gelatine capsules to create a single, convenient dosage form. The dissolution profiles for the mini-MUPS-in-capsule systems for each filler/active ingredient formulation are displayed in Figures 4.21 and 4.22.

The mini-MUPS-in-capsule systems illustrated overall higher MDT-values (Table 4.24) compared to the optimised coated and uncoated mini-MUPS formulations. All of the mini-MUPS-in-capsule systems exhibited a pulsatile drug release profile, regardless the filler type or active ingredient incorporated.

Pulsatile drug release systems are formulated for drug release to occur in a time-controlled manner where an initial dose is followed by a lag time before the next part of the dose is released (Maroni *et al.*, 2013b:362; Qiu & Zhou, 2011:27). A pulsatile drug release system has a non-monolithic and multi-cargo drug release drug-blood profile as it is compiled using a combination of immediate release and delayed release mechanisms. The delayed release mechanism of pulsatile release systems contain site-specific and/or time-controlled drug delivery mechanisms to ensure that the drug is released at a specific area in the GI-tract; or at a predetermined time (Qiu, 2009a:483).

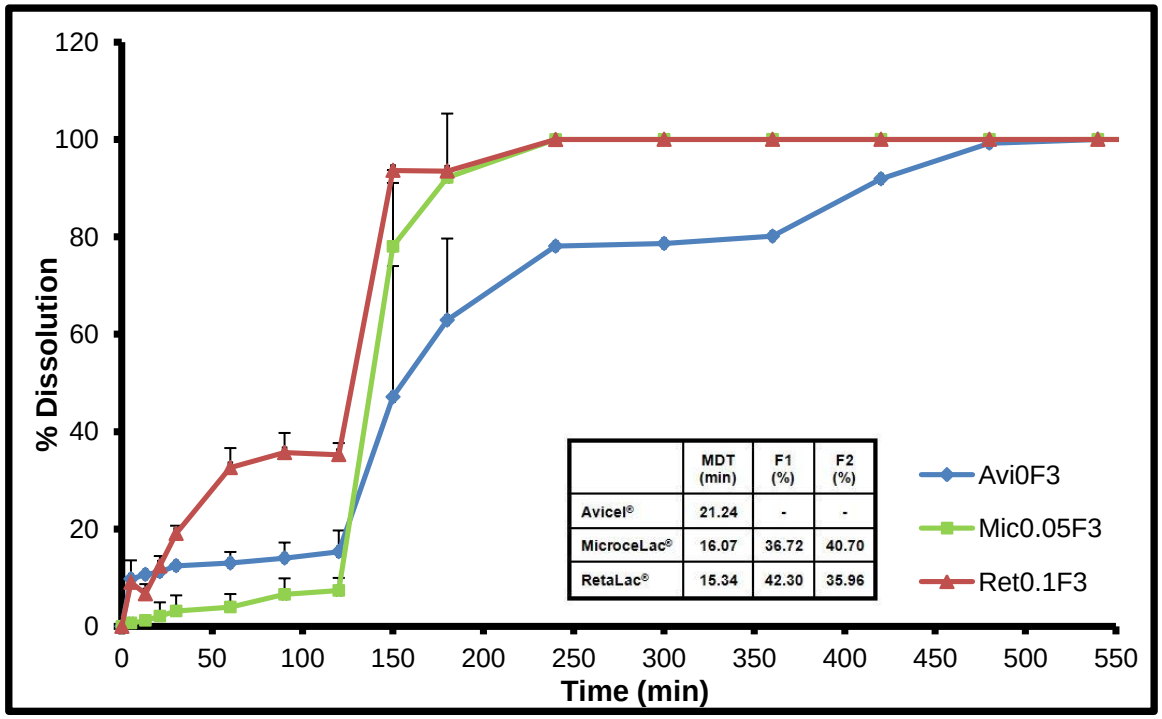


Figure 4.21: Percentage drug dissolution for the optimised furosemide mini-MUPS-in-capsule dosage form formulations plotted as a function of time. The mean dissolution time, similarity and difference factors are also shown

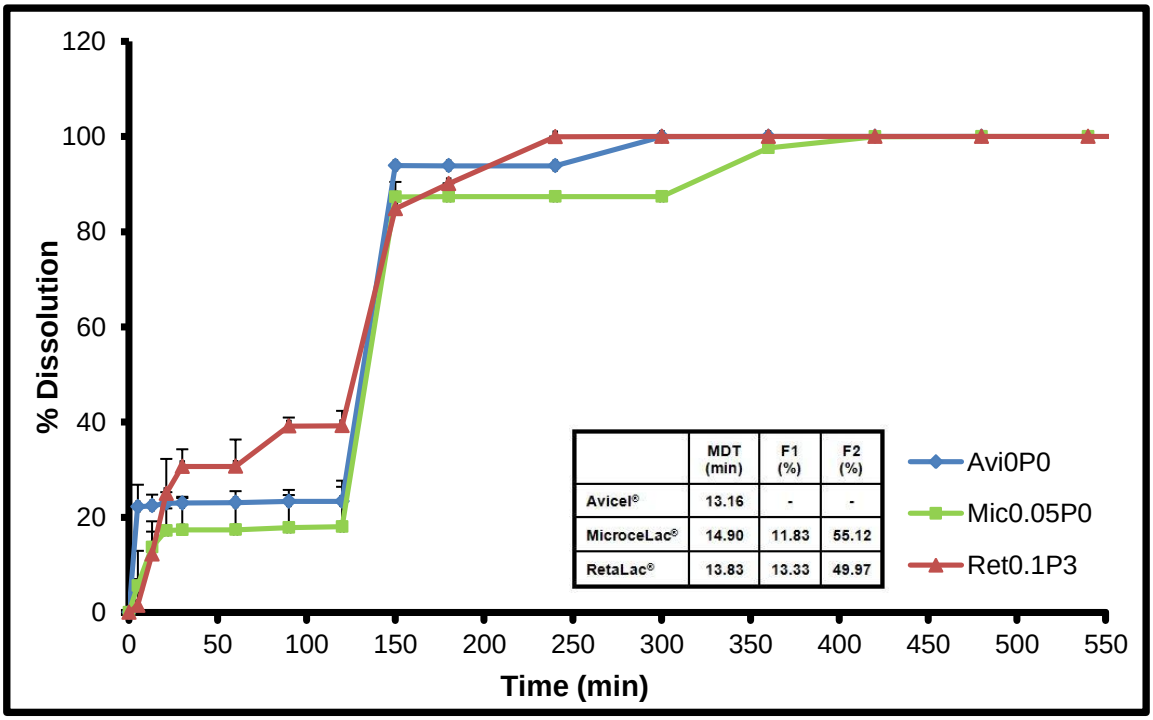


Figure 4.22: Percentage drug dissolution for the optimised pyridoxine mini-MUPS-in-capsule dosage form formulations plotted as a function of time. The mean dissolution time, similarity and difference factors are also shown

Table 4.24: Summary of mean dissolution time and fit factors for optimised formulations

	Avi0F3	Avi0P0	Mic0.05F3	Mic0.05P0	Ret0.1F3	Ret0.1P3
	Optimised uncoated mini-MUPS					
MDT (min)*	16.99	5.30	13.85	6.43	12.22	12.90
f1	-	-	17.91	12.53	27.52	36.29
f2	-	-	41.99	34.45	36.83	17.98
	Optimised coated mini-MUPS					
MDT (min)*	15.69	3.99	15.07	7.17	13.10	13.83
f1	-	-	5.67	20.82	23.23	13.33
f2	-	-	63.92	31.58	44.32	49.97
	Optimised Mini-MUPS-in-capsule dosage forms					
MDT (min)*	21.24	13.16	16.07	14.90	15.34	13.83
f1	-	-	36.72	11.83	42.30	13.33
f2	-	-	40.70	55.12	35.96	49.97

**MDT= Mean dissolution time*

From a comparison of the dissolution profiles of the mini-MUPS-in-capsule systems, irrespective of the active ingredient added (Figures 4.15 and 4.16), it was clear that within the first 120 min, the Avicel®- and MicroceLac® mini-MUPS-in-capsule systems released less drug compared to the RetaLac® mini-MUPS-in-capsule systems. A possible reason might be that once again the RetaLac® beads were more fragile (even though the mini-MUPS depicted a higher tensile strength compared to the other formulations), thus water penetration were faster and the mini-MUPS swelled extensively more allowing faster erosion of the matrix and hence faster drug release. On the other hand, negligible to no swelling occurred with the different Avicel® and MicroceLac® mini-MUPS formulations as discussed in section 4.4.4, thus drug release was delayed as water probably struggled to penetrate into the mini-MUPS. Taking the f_1- and f_2-values into account, significant differences in the dissolution profiles of the mini-MUPS-in-capsule systems containing furosemide could be observed, whereas no significant differences could be obtained for the mini-MUPS-in-capsule systems containing pyridoxine. Therefore, as identified previously, the type (*i.e.* water-soluble or insoluble) of active ingredient definitely plays an important role in the drug release profile.

4.4 ACCELERATED STABILITY TESTING

Accelerated stability was performed on the optimised mini-MUPS formulations (Annexure D). As previously described (section 3.8) these formulations were stored in three different stability chambers with predetermined conditions varying in temperature and relative humidity over a six month period. At months 1, 2, 3 and 6 physical tablet tests as previously described in section 3.5.3 were conducted on the stored mini-MUPS samples for each set of conditions.

4.4.1 Evaluation of the physical stability of mini-MUPS

Accelerated stability testing was done in order to determine if there were any changes in the physical characteristics of the mini-MUPS. Tables D.1 – D.8 in annexure D contain all of the results obtained during the six month period. Note that various human, machine and process errors could have occurred during the manufacturing processes.

The assays tested for each mini-MUPS prepared from uncoated and coated furosemide beads respectively showed no noticeable difference between the various months and stability chamber conditions. Conversely, pyridoxine content of the mini-MUPS prepared from uncoated and coated beads displayed a decrease in mg pyridoxine / 100 mg tablet after two months for each chamber condition (Figure 4.23 and 4.24), regardless the type of filler utilised in the mini-MUPS formulations. The mini-MUPS formulations prepared from uncoated or coated beads depicted the same trend in decrease in content. Note that the lined bars in Figure 4.24 represent coated formulations.

Regarding the variation in mass results obtained for all of the mini-MUPS formulations, no noticeable differences could be observed. Tables D.7 and D.8 (Annexure D) depict these compliance results for all 6 months in the three chamber conditions.

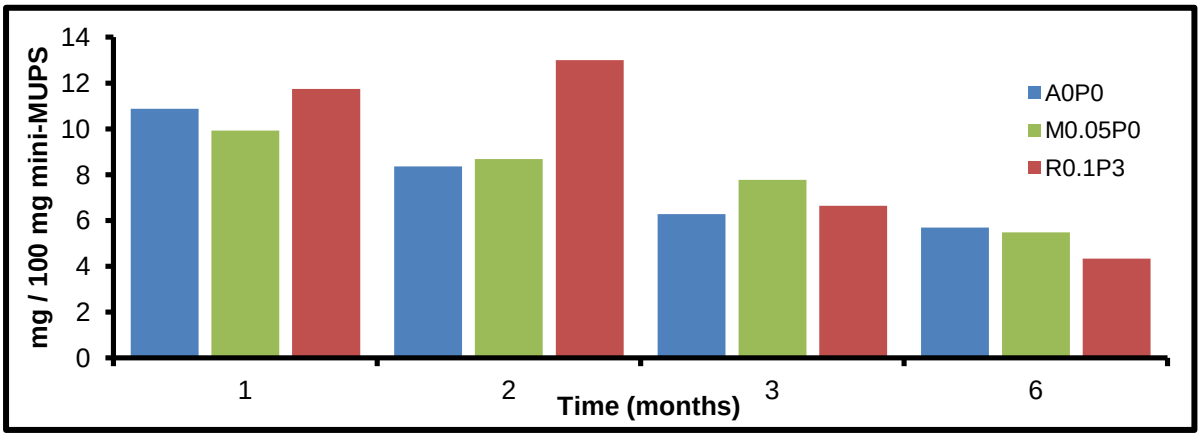


Figure 4.23: Assay tested on mini-MUPS prepared from uncoated beads for duration of 6 months in a stability chamber with 25ºC/60% RH conditions

The Avicel® mini-MUPS formulations depicted the least optimal results concerning mass variation as most of these formulations failed the British Pharmacopoeia (2015) prerequisite. All of the RetaLac® mini-MUPS formulations passed this prerequisite during the six month stability testing in the different chamber conditions, showing that these formulations were stable in terms of mass variation. The MicroceLac® mini-MUPS formulations were less stable; where it was visible that coating of these mini-MUPS formulations produced tablets

that varied less in weight over the six month period. Lastly, it was interesting to observe that the mini-MUPS formulations containing furosemide were more susceptible to variation in mass.

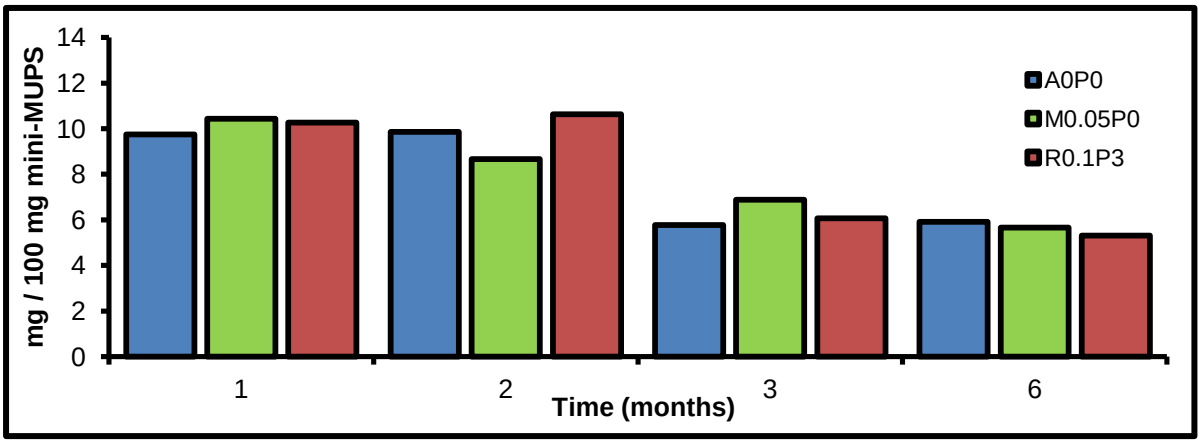


Figure 4.24: Assay tested on mini-MUPS prepared from coated beads for duration of 6 months in a stability chamber with 25ºC/60% RH conditions

Most of the furosemide and pyridoxine formulations depicted no evident variation regarding average tensile strength and %RSD between the various months and chamber conditions. Exceptions included:

- A0P0 coated formulation depicted no tensile strength during the six month period.
- M0.05F3 mini-MUPS prepared from uncoated beads showed higher tensile strength values with a decreased %RSD for each month in chamber 3 (40ºC/75% RH) compared to the other chambers that depicted no substantial differences.
- The R0.1F3 coated formulation showed a marked decrease in tensile strength in chamber 1 (25ºC/60% RH) for all 6 months.
- The M0.05P0 uncoated formula in chamber 3 showed lower tensile strength values compared to the other two chambers for the six month period.
- The coated M0.05P0 formulation depicted increased tensile strength values in chamber 3 after three months.

During friability testing MicroceLac® and RetaLac® mini-MUPS formulations depicted friability values of less than 1%. The only exceptions were the coated R0.1F3 formulation stored in chamber 2 and the coated M0.05P0 formulation stored in chamber 2. At month 1 the M0.05P0 formulation depicted a friability value of 2.53% and at month 3 the R0.1F3 formulation portrayed a friability value of 9.65%, which was probably due to human error. All

of the Avicel® mini-MUPS formulations failed the friability test for all six months. These formulations were extremely brittle and all fractured instantly when handled.

During stability testing the disintegration time for the Avicel® uncoated formulations for both active ingredients depicted a slight increase in the disintegration time over the six month period. The furosemide and pyridoxine Avicel® formulations for the coated mini-MUPS depicted a slight decrease and no variation in disintegration time, respectively. Uncoated and coated MicroceLac®/active ingredient formulations portrayed no noticeable variation in disintegration time during stability testing, except the coated MicroceLac®/pyridoxine formulations that showed a slight decrease in disintegration time in chamber 1 for all six months of testing. Both the uncoated and coated RetaLac® formulations for both active ingredients were considered non-disintegrating during stability testing over the six month period.

Varying ambient conditions played a role in the physical stability of the different mini-MUPS formulations. Comparing the different mini-MUPS formulations it was evident that most of the Avicel® formulations were more negatively affected by all stability chamber conditions. Moreover, it could be seen that in general all stability tests done on both coated and uncoated MicroceLac®- and RetaLac® formulations containing either furosemide or pyridoxine depicted excellent results with some deviations.

CHAPTER 5
SUMMARY AND FUTURE PROSPECTS

5.1 SUMMARY

The aim of this study was to develop and evaluate mini-MUPS-in-capsule drug delivery systems as a novel, solid oral dosage form for sustained drug release.

The following objectives set, were met:

- Bead formulations were successfully prepared by means for extrusion-spheronisation utilising three different fillers namely, Avicel® PH 101; MicroceLac® 100 and RetaLac®,
- A portion of each of the optimised filler/active ingredient formulations were coated with a 50:50 mixture of Eudragit® L 100 and Eudragit® S 100 in a rotating pan coater. The physical properties of the coated mini-MUPS as well as the dissolution studies conducted showed that none of the physical properties were changed by film coating of the beads,
- The morphology of the filler powders, uncoated and coated beads was evaluated by means of scanning electron microscopy and it depicted that beads produced from Avicel® PH 101 and MicroceLac® 100 were more spherical compared to RetaLac® that formed oblong shaped beads,
- The flow properties, particle size and particle size distribution of the filler powders, uncoated and coated beads (all the bead formulations as determined by a factorial design) were successfully evaluated. Formulation of beads for each filler/active ingredient combination depicted enhanced flow properties compared to the filler powders as expected due to enlarged particle sizes. In general, it could be concluded that the uncoated beads for both active ingredients portrayed increased flowability compared to the coated beads and that the beads in general portrayed better flowability than the filler powders,
- The friability of the uncoated and coated beads was successfully evaluated, by utilising an adapted method from the British Pharmacopoeia, which indicated all the bead formulations complied to the requirements of the BP,
- Uncoated beads were compressed, utilising a Korsch® single tablet press according to a factorial design which eliminated uncompressible formulations and delivered formulations that were compressed successfully into mini-MUPS,

- Coated beads were successfully directly compressed, utilising a Korsch® single tablet press, to manufacture mini-MUPS,
- The morphology and physical properties of the mini-MUPS containing uncoated and coated beads were successfully evaluated. It could be concluded that each factor (filler, active ingredient, binder and lubricant) did indeed have a pronounced effect on the formulations of the mini-MUPS containing uncoated and coated beads,
- Swelling and erosion experiments conducted on the optimised mini-MUPS containing uncoated beads and mini-MUPS containing coated formulations showed that the coated pyridoxine formulation portrayed a higher percentage swelling concentration and longer time period of swelling compared to the uncoated formulations and the furosemide formulations,
- Dissolution profiles of the mini-MUPS containing uncoated and coated beads showed that drug release were influenced by the type of filler and active ingredient utilised as well as the binder and lubricant concentration,
- The mini-MUPS-in-capsule drug delivery systems were successfully prepared. Dissolution studies done on these dosage forms depicted pulsatile drug release,
- During accelerated stability testing it could be concluded that the different stability conditions in fact did have an effect on the mini-MUPS containing uncoated and coated beads for each filler/active ingredient combination.

Mini-MUPS-in-capsule systems are novel and exciting dosage forms that need to be explored further in more detail. This study confirmed that mini-MUPS-in-capsule drug delivery systems have potential to be used as modified release dosage forms by the pharmaceutical industry. Coating of the beads did improve tablet properties, as well as sustaining drug release from the different formulations. However, the mini-MUPS-in-capsule systems tested in the study illustrated pulsatile drug and not sustained drug release.

5.2 FUTURE PROSPECTS

After completion of this study, the following recommendations for future investigations can be made:

- Incorporation of multiple, active ingredients, into the mini-MUPS-in-capsule system in order to create a fixed dose combination product for a predetermined disease condition,
- Utilising multiple coating layers to achieve sustained drug release as in this study it showed that the integrity of the film coating played an important role in the drug release profiles,

- Determining the optimised increase in mass of the beads in order to obtain sustained release using film coating, as well as the right coating polymers for the specified coating,
- Analyse and optimise the coating thickness for optimal drug release profiles,
- Dissolution testing on all three stability chambers optimised formulations to establish the effect of stability on drug release profiles,
- Utilising *in vivo* studies to evaluate the effect of sustained drug release on plasma drug levels over an extended period of time,
- Undertaking additional swelling and erosion studies on various filler powders and comparing the data with the data obtained from dissolution studies.
- Further studies are needed in order to establish an improved mini-MUPS-in-capsule dosage form for different active ingredients.

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APSSA/SAAPI CONFERENCE 2015

ABSTRACT

Development of a compressed bead-in-capsule drug delivery system for sustained release

Leandri Esterhuizen-Rudolph, Josias H. Hamman and Joe M. Viljoen

Centre of Excellence for Pharmaceutical Sciences, North-West University, Potchefstroom, 2531, South Africa

Purpose: Formulation of a unique mini-tablet-in-capsule drug delivery system prepared from beads for modified drug release.

Methods: Powder flow properties for selected filler materials (i.e. Avicel® PH 101, MicroceLac® 100; RetaLac®), uncoated and coated beads were conducted. Beads were formulated using extrusion-spheronisation; half of each formulation of beads was coated using a rotating pan coater. Uncoated and coated beads were compressed into tablets referred to as multiple unit pellet systems (MUPS) with a 6 mm diameter. Scanning electron microscopy (SEM) images were taken of the uncoated and coated beads; and the optimum mini-tablet formulations. After a full factorial design, physical test, and dissolution evaluations were conducted on the mini-tablets in order to obtain the optimum formulations for each model compound (i.e. furosemide and pyridoxine) and filler combination used in this study. Accelerated stability testing was performed on the mini-MUPS for a three month period.

Results: Beads were successfully manufactured by means of extrusion spheronisation with all three the selected filler materials and optimised by means of a factorial design. The mini-MUPS-in-capsule systems tested in this study illustrated pulsatile drug release and the systems containing RetaLac® depicted more suitable pulsatile drug release.

Conclusions: A mini-MUPS-in-capsule system is a novel and exciting dosage form that provides modified drug release properties with high potential for use as a modified drug release system in the pharmaceutical industry.

ANNEXURE A

PARTICLE SIZE AND SIZE DISTRIBUTION

A.1: Avicel® PH 101 powder

A.2: MicroceLac® 100 powder

A.3: RetaLac® powder

A.4: Avicel® PH 101 uncoated beads containing active ingredient furosemide

A.5: Avicel® PH 101 coated beads containing active ingredient furosemide

A.6: Avicel® PH 101 uncoated beads containing active ingredient pyridoxine

A.7: Avicel® PH 101 coated beads containing active ingredient pyridoxine

A.8: MicroceLac® 100 uncoated beads containing active ingredient furosemide

A.9: MicroceLac® 100 coated beads containing active ingredient furosemide

A.10: MicroceLac® 100 uncoated beads containing active ingredient pyridoxine

A.11: MicroceLac® 100 coated beads containing active ingredient pyridoxine

A.12: RetaLac® uncoated beads containing active ingredient furosemide

A.13: RetaLac® coated beads containing active ingredient furosemide

A.14: RetaLac® uncoated beads containing active ingredient pyridoxine

A.15: RetaLac® coated beads containing active ingredient pyridoxine

A.1: Avicel® PH 101 powder

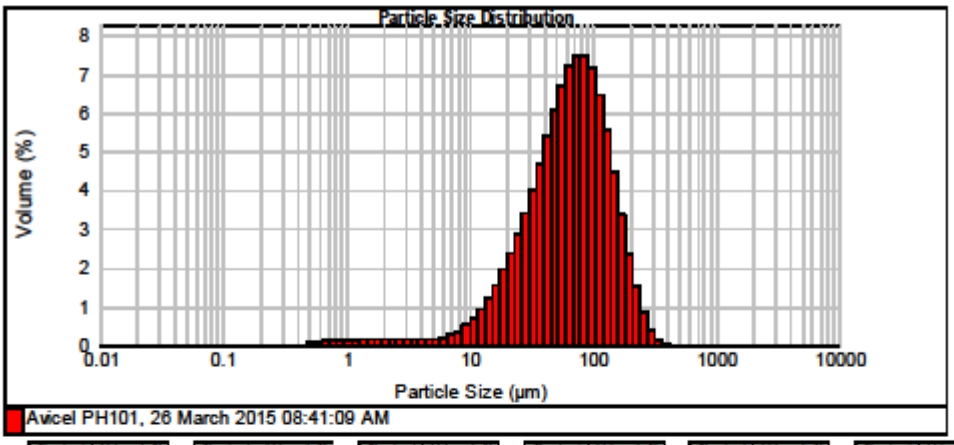
MALVERN INSTRUMENTS

MASTERSIZER 2000

Result Analysis Report

Sample Name: Avicel PH101	**SOP Name:** RIIP (2000SM)	**Measured:** 26 March 2015 08:41:09 AM	
Sample Source & type: Supplier	**Measured by:** Administrator	**Analysed:** 26 March 2015 08:41:10 AM	
Sample bulk lot ref: Sample 1	**Result Source:** Measurement		
Particle Name: Titanium Dioxide	**Accessory Name:** Hydro 2000SM (A)	**Analysis model:** General purpose	**Sensitivity:** Enhanced
Particle RI: 2.741	**Absorption:** 0.1	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 14.89 %
Dispersant Name: Alcohol	**Dispersant RI:** 1.320	**Weighted Residual:** 0.456 %	**Result Emulation:** Off
Concentration: 0.0586 %Vol	**Span :** 1.994	**Uniformity:** 0.622	**Result units:** Volume
Specific Surface Area: 0.212 m²/g	**Surface Weighted Mean D[3,2]:** 28.351 um	**Vol. Weighted Mean D[4,3]:** 77.677 um	

d(0.1): 20.198 um **d(0.5):** 65.792 um **d(0.9):** 151.374 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.12	11.482	0.99	120.226	5.56	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.13	13.183	1.22	138.038	4.47	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.14	15.136	1.56	158.489	3.38	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.14	17.378	1.95	181.970	2.35	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.14	19.953	2.39	208.930	1.51	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.14	22.909	2.88	239.883	0.85	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.14	26.303	3.43	275.423	0.41	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.13	30.200	4.03	316.228	0.12	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.13	34.674	4.69	363.078	0.02	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.13	39.811	5.39	416.869	0.00	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.13	45.709	6.09	478.630	0.00	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.14	52.481	6.73	549.541	0.00	5754.399	0.00
0.052	0.00	0.550	0.05	5.754	0.15	60.256	7.23	630.957	0.00	6606.934	0.00
0.060	0.00	0.631	0.07	6.607	0.19	69.183	7.52	724.436	0.00	7585.776	0.00
0.069	0.00	0.724	0.08	7.586	0.25	79.433	7.50	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.09	8.710	0.36	91.201	7.16	954.993	0.00	10000.000	
0.091	0.00	0.955	0.11	10.000	0.50	104.713	6.48	1096.478	0.00		
0.105	0.00	1.096	0.12	11.482	0.69	120.226		1258.925	0.00		

Operator notes:

Malvern Instruments Ltd.
Malvern, UK

Mastersizer 2000 Ver. 5.60
Serial Number : MAL1007548

File name: 13 April 2015
Record Number: 2

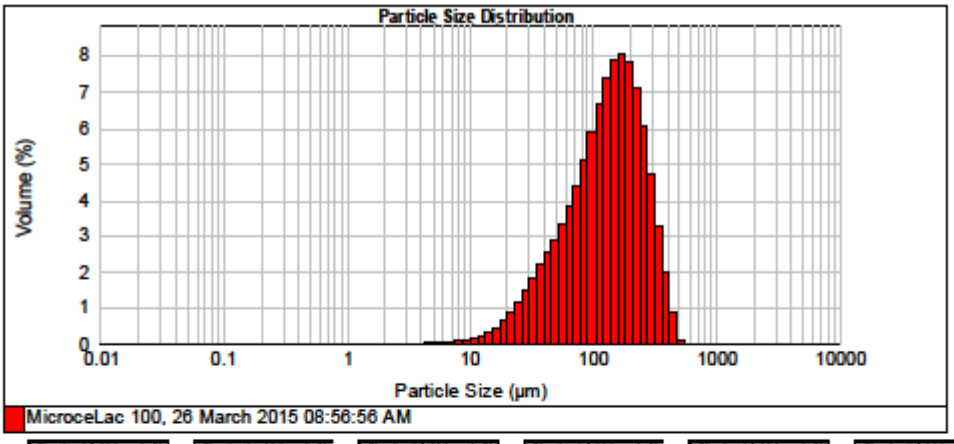
A.2: MicroceLac® 100 powder

MASTERSIZER 2000

Result Analysis Report

Sample Name: MicroceLac 100	**SOP Name:** RIIP (2000SM)	**Measured:** 26 March 2015 08:56:56 AM	
Sample Source & type:	**Measured by:** Administrator	**Analysed:** 26 March 2015 08:56:57 AM	
Sample bulk lot ref: Sample 1	**Result Source:** Edited		
Particle Name: Titanium Dioxide	**Accessory Name:** Hydro 2000SM (A)	**Analysis model:** General purpose	**Sensitivity:** Enhanced
Particle RI: 2.741	**Absorption:** 0.1	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 10.82 %
Dispersant Name: Alcohol	**Dispersant RI:** 1.320	**Weighted Residual:** 0.680 %	**Result Emulation:** Off
Concentration: 0.1297 %Vol	**Span :** 1.830	**Uniformity:** 0.566	**Result units:** Volume
Specific Surface Area: 0.0736 m²/g	**Surface Weighted Mean D[3,2]:** 81.529 um	**Vol. Weighted Mean D[4,3]:** 149.556 um	

d(0.1): 39.931 um d(0.5): 133.037 um d(0.9): 283.391 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.22	120.226	7.38	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.32	138.038	7.89	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.46	158.489	8.06	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.65	181.970	7.80	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.00	19.953	0.89	208.930	7.11	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	1.18	239.883	6.03	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	1.49	275.423	4.72	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.00	30.200	1.83	316.228	3.32	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	2.18	363.078	2.01	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.08	39.811	2.54	416.869	0.92	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.07	45.709	2.92	478.630	0.12	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.07	52.481	3.34	549.541	0.00	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.08	60.256	3.83	630.957	0.00	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.09	69.183	4.41	724.436	0.00	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.10	79.433	5.11	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.12	91.201	5.88	954.993	0.00	10000.000	
0.091	0.00	0.955	0.00	10.000	0.16	104.713	6.68	1096.478	0.00		
0.105	0.00	1.096	0.00	11.482		120.226		1258.925	0.00		

Operator notes:

Malvern Instruments Ltd.
Malvern, UK

Mastersizer 2000 Ver. 5.60
Serial Number : MAL1007548

File name: 13 April 2015
Record Number: 68

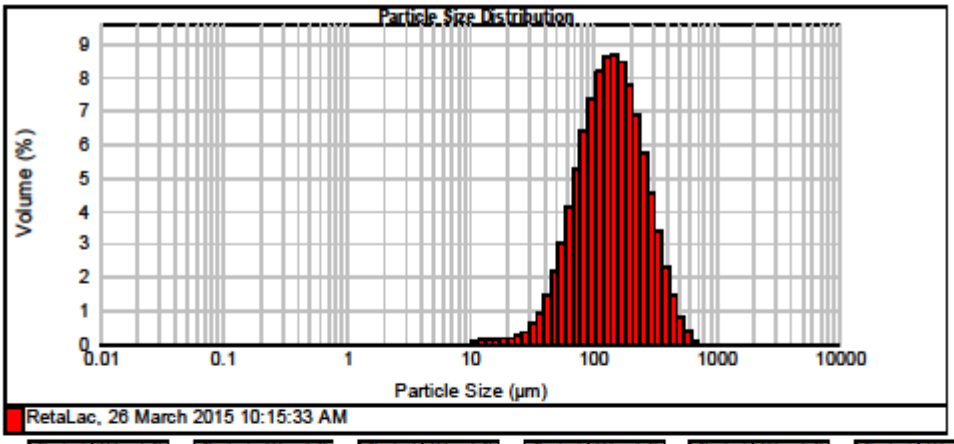
A.3: RetaLac® powder

MASTERSIZER 2000

Result Analysis Report

Sample Name: RetaLac	**SOP Name:** RIIP (2000SM)	**Measured:** 26 March 2015 10:15:33 AM	
Sample Source & type: Supplier	**Measured by:** Administrator	**Analysed:** 26 March 2015 10:15:34 AM	
Sample bulk lot ref: Sample 1	**Result Source:** Measurement		
Particle Name: Titanium Dioxide	**Accessory Name:** Hydro 2000SM (A)	**Analysis model:** General purpose	**Sensitivity:** Enhanced
Particle RI: 2.741	**Absorption:** 0.1	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 13.72 %
Dispersant Name: Alcohol	**Dispersant RI:** 1.320	**Weighted Residual:** 0.608 %	**Result Emulation:** Off
Concentration: 0.2271 %Vol	**Span :** 1.716	**Uniformity:** 0.536	**Result units:** Volume
Specific Surface Area: 0.0547 m²/g	**Surface Weighted Mean D[3,2]:** 109.706 um	**Vol. Weighted Mean D[4,3]:** 163.600 um	

d(0.1): 61.192 um **d(0.5):** 139.192 um **d(0.9):** 300.016 um



Size (µm)	Volume In %
0.010	0.00
0.011	0.00
0.013	0.00
0.015	0.00
0.017	0.00
0.020	0.00
0.023	0.00
0.026	0.00
0.030	0.00
0.035	0.00
0.040	0.00
0.046	0.00
0.052	0.00
0.060	0.00
0.069	0.00
0.079	0.00
0.091	0.00
0.105	0.00

Size (µm)	Volume In %
0.105	0.00
0.120	0.00
0.138	0.00
0.158	0.00
0.182	0.00
0.209	0.00
0.240	0.00
0.275	0.00
0.316	0.00
0.363	0.00
0.417	0.00
0.479	0.00
0.550	0.00
0.631	0.00
0.724	0.00
0.832	0.00
0.955	0.00
1.096	0.00

Size (µm)	Volume In %
1.096	0.00
1.259	0.00
1.445	0.00
1.660	0.00
1.905	0.00
2.188	0.00
2.512	0.00
2.884	0.00
3.311	0.00
3.802	0.00
4.365	0.00
5.012	0.00
5.754	0.00
6.607	0.00
7.586	0.00
8.710	0.00
10.000	0.00
11.482	0.07

Size (µm)	Volume In %
11.482	0.10
13.183	0.12
15.136	0.14
17.378	0.16
19.953	0.19
22.909	0.25
26.303	0.37
30.200	0.58
34.674	0.93
39.811	1.45
45.709	2.16
52.481	3.08
60.256	4.11
69.183	5.25
79.433	6.38
91.201	7.37
104.713	8.15
120.226	8.61

Size (µm)	Volume In %
120.226	8.61
138.038	8.71
158.489	8.44
181.970	7.80
208.930	6.88
239.883	5.74
275.423	4.54
316.228	3.36
363.078	2.31
416.869	1.45
478.630	0.81
549.541	0.40
630.957	0.10
724.436	0.00
831.764	0.00
954.993	0.00
1096.478	0.00
1258.925	0.00

Size (µm)	Volume In %
1258.925	0.00
1445.440	0.00
1659.587	0.00
1905.461	0.00
2187.762	0.00
2511.886	0.00
2884.032	0.00
3311.311	0.00
3801.894	0.00
4365.158	0.00
5011.872	0.00
5754.399	0.00
6606.934	0.00
7585.776	0.00
8709.636	0.00
10000.000	0.00

Operator notes:

Malvern Instruments Ltd.
Malvern, UK

Mastersizer 2000 Ver. 5.60
Serial Number : MAL1007548

File name: 13 April 2015
Record Number: 11

A.4: Avicel® PH 101 uncoated beads containing active ingredient furosemide

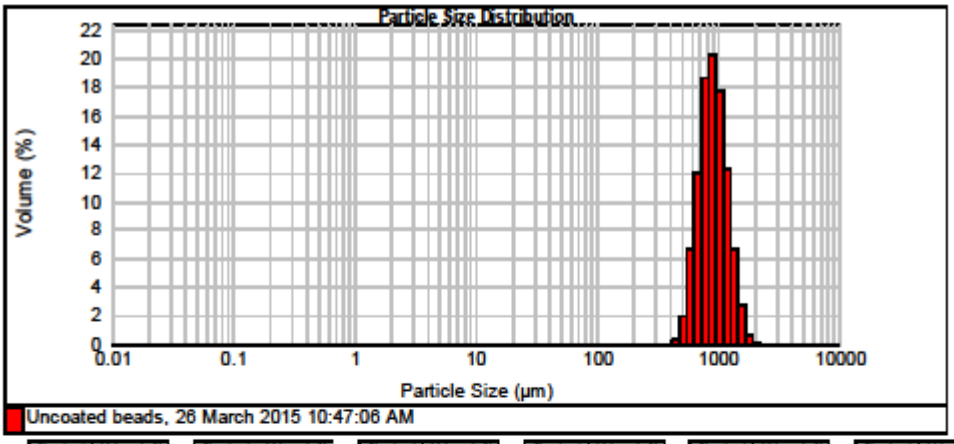
MALVERN INSTRUMENTS

MASTERSIZER 2000

Result Analysis Report

Sample Name: Uncoated beads	**SOP Name:** Leandri (Beads)	**Measured:** 26 March 2015 10:47:06 AM	
Sample Source & type:	**Measured by:** Administrator	**Analysed:** 26 March 2015 10:47:07 AM	
Sample bulk lot ref: AF 1 (Sample 2)	**Result Source:** Measurement		
Particle Name: Titanium Dioxide	**Accessory Name:** Hydro 2000MU (A)	**Analysis model:** General purpose	**Sensitivity:** Normal
Particle RI: 2.741	**Absorption:** 0.1	**Size range:** 0.020 to 2000.0... um	**Obscuration:** 0.93 %
Dispersant Name: Alcohol	**Dispersant RI:** 1.320	**Weighted Residual:** 4.808 %	**Result Emulation:** Off
Concentration: 0.1148 %Vol	**Span :** 0.695	**Uniformity:** 0.216	**Result units:** Volume
Specific Surface Area: 0.00692 m²/g	**Surface Weighted Mean D[3,2]:** 866.798 um	**Vol. Weighted Mean D[4,3]:** 927.342 um	

d(0.1): 639.946 um d(0.5): 891.643 um d(0.9): 1259.683 um



Size (µm)	Volume In %
0.010	0.00
0.011	0.00
0.013	0.00
0.015	0.00
0.017	0.00
0.020	0.00
0.023	0.00
0.026	0.00
0.030	0.00
0.035	0.00
0.040	0.00
0.046	0.00
0.052	0.00
0.060	0.00
0.069	0.00
0.079	0.00
0.091	0.00
0.105	0.00

Size (µm)	Volume In %
0.105	0.00
0.120	0.00
0.138	0.00
0.158	0.00
0.182	0.00
0.209	0.00
0.240	0.00
0.275	0.00
0.316	0.00
0.363	0.00
0.417	0.00
0.479	0.00
0.550	0.00
0.631	0.00
0.724	0.00
0.832	0.00
0.955	0.00
1.096	0.00

Size (µm)	Volume In %
1.096	0.00
1.259	0.00
1.445	0.00
1.660	0.00
1.905	0.00
2.188	0.00
2.512	0.00
2.884	0.00
3.311	0.00
3.802	0.00
4.365	0.00
5.012	0.00
5.754	0.00
6.607	0.00
7.586	0.00
8.710	0.00
10.000	0.00
11.482	0.00

Size (µm)	Volume In %
11.482	0.00
13.183	0.00
15.136	0.00
17.378	0.00
19.953	0.00
22.909	0.00
26.303	0.00
30.200	0.00
34.674	0.00
39.811	0.00
45.709	0.00
52.481	0.00
60.256	0.00
69.183	0.00
79.433	0.00
91.201	0.00
104.713	0.00
120.226	0.00

Size (µm)	Volume In %
120.226	0.00
138.038	0.00
158.489	0.00
181.970	0.00
208.930	0.00
239.883	0.00
275.423	0.00
316.228	0.00
363.078	0.00
416.869	0.32
478.630	1.95
549.541	6.89
630.957	12.07
724.436	18.66
831.764	20.32
954.993	17.75
1096.478	12.21
1258.925	6.85

Size (µm)	Volume In %
1258.925	6.85
1445.440	2.70
1659.587	0.66
1905.461	0.04
2187.762	0.00
2511.886	0.00
2884.032	0.00
3311.311	0.00
3801.894	0.00
4365.158	0.00
5011.872	0.00
5754.399	0.00
6606.934	0.00
7585.776	0.00
8709.636	0.00
10000.000	0.00

Operator notes:

Malvern Instruments Ltd.
Malvern, UK

Mastersizer 2000 Ver. 5.60
Serial Number : MAL1007548

File name: 13 April 2015
Record Number: 17

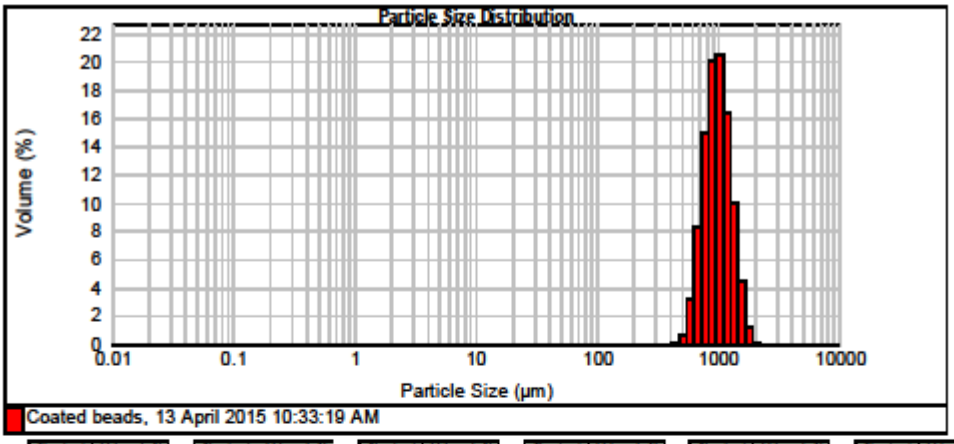
A.5: Avicel® PH 101 coated beads containing active ingredient furosemide

MASTERSIZER 2000

Result Analysis Report

Sample Name: Coated beads	**SOP Name:** Leandri (Beads)	**Measured:** 13 April 2015 10:33:19 AM	
Sample Source & type:	**Measured by:** Administrator	**Analysed:** 13 April 2015 10:33:20 AM	
Sample bulk lot ref: A-CF (Sample 1)	**Result Source:** Measurement		
Particle Name: Titanium Dioxide	**Accessory Name:** Hydro 2000MU (A)	**Analysis model:** General purpose	**Sensitivity:** Normal
Particle RI: 2.741	**Absorption:** 0.1	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 0.74 %
Dispersant Name: Water	**Dispersant RI:** 1.330	**Weighted Residual:** 7.453 %	**Result Emulation:** Off
Concentration: 0.0999 %Vol	**Span :** 0.661	**Uniformity:** 0.208	**Result units:** Volume
Specific Surface Area: 0.00636 m²/g	**Surface Weighted Mean D[3,2]:** 942.703 um	**Vol. Weighted Mean D[4,3]:** 1003.458 um	

d(0.1): 704.403 um **d(0.5):** 971.501 um **d(0.9):** 1346.859 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.00	120.226	0.00	1258.925	10.03
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.00	138.038	0.00	1445.440	4.45
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.00	158.489	0.00	1659.587	1.14
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.00	181.970	0.00	1905.461	0.07
0.017	0.00	0.182	0.00	1.905	0.00	19.953	0.00	208.930	0.00	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	0.00	239.883	0.00	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	0.00	275.423	0.00	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.00	30.200	0.00	316.228	0.00	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	0.00	363.078	0.00	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.00	39.811	0.00	416.869	0.06	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.00	45.709	0.00	478.630	0.60	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.00	52.481	0.00	549.541	3.22	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.00	60.256	0.00	630.957	8.35	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.00	69.183	0.00	724.436	15.05	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.00	79.433	0.00	831.764	20.09	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.00	91.201	0.00	954.993	20.58	10000.000	0.00
0.091	0.00	0.955	0.00	10.000	0.00	104.713	0.00	1096.478	16.36		
0.105	0.00	1.096	0.00	11.482	0.00	120.226	0.00	1258.925			

Operator notes:

Malvern Instruments Ltd.
Malvern, UK

Mastersizer 2000 Ver. 5.60
Serial Number : MAL1007548

File name: 13 April 2015
Record Number: 43

A.6: Avicel® PH 101 uncoated beads containing active ingredient pyridoxine

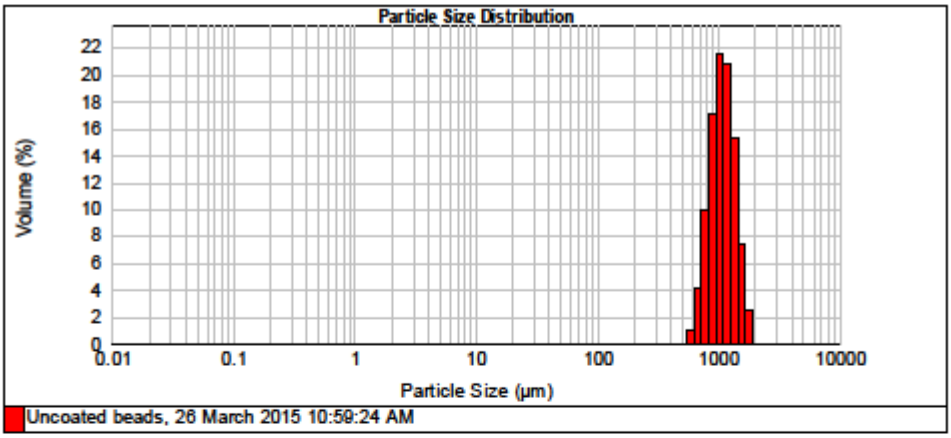
MALVERN INSTRUMENTS

MASTERSIZER 2000

Result Analysis Report

Sample Name: Uncoated beads	**SOP Name:** Leandri (Beads)	**Measured:** 26 March 2015 10:59:24 AM	
Sample Source & type:	**Measured by:** Administrator	**Analysed:** 26 March 2015 10:59:25 AM	
Sample bulk lot ref: AP 1 (Sample 1)	**Result Source:** Measurement		
Particle Name: Titanium Dioxide	**Accessory Name:** Hydro 2000MU (A)	**Analysis model:** General purpose	**Sensitivity:** Normal
Particle RI: 2.741	**Absorption:** 0.1	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 1.17 %
Dispersant Name: Alcohol	**Dispersant RI:** 1.320	**Weighted Residual:** 6.201 %	**Result Emulation:** Off
Concentration: 0.1725 %Vol	**Span :** 0.620	**Uniformity:** 0.198	**Result units:** Volume
Specific Surface Area: 0.00579 m²/g	**Surface Weighted Mean D[3,2]:** 1036.271 um	**Vol. Weighted Mean D[4,3]:** 1096.621 um	

d(0.1): 781.574 um d(0.5): 1069.908 um d(0.9): 1444.939 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.00	120.226	0.00	1258.925	15.37
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.00	138.038	0.00	1445.440	7.36
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.00	158.489	0.00	1659.587	2.57
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.00	181.970	0.00	1905.461	0.04
0.017	0.00	0.182	0.00	1.905	0.00	19.953	0.00	208.930	0.00	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	0.00	239.883	0.00	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	0.00	275.423	0.00	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.00	30.200	0.00	316.228	0.00	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	0.00	363.078	0.00	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.00	39.811	0.00	416.869	0.00	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.00	45.709	0.00	478.630	0.00	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.00	52.481	0.00	549.541	0.00	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.00	60.256	0.00	630.957	1.06	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.00	69.183	0.00	724.436	4.20	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.00	79.433	0.00	831.764	10.05	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.00	91.201	0.00	954.993	17.01	10000.000	0.00
0.091	0.00	0.955	0.00	10.000	0.00	104.713	0.00	1096.478	21.51		
0.105	0.00	1.096	0.00	11.482	0.00	120.226	0.00	1258.925	20.75		

Operator notes:

Malvern Instruments Ltd.
Malvern, UK

Mastersizer 2000 Ver. 5.60
Serial Number : MAL1007548

File name: 13 April 2015
Record Number: 19

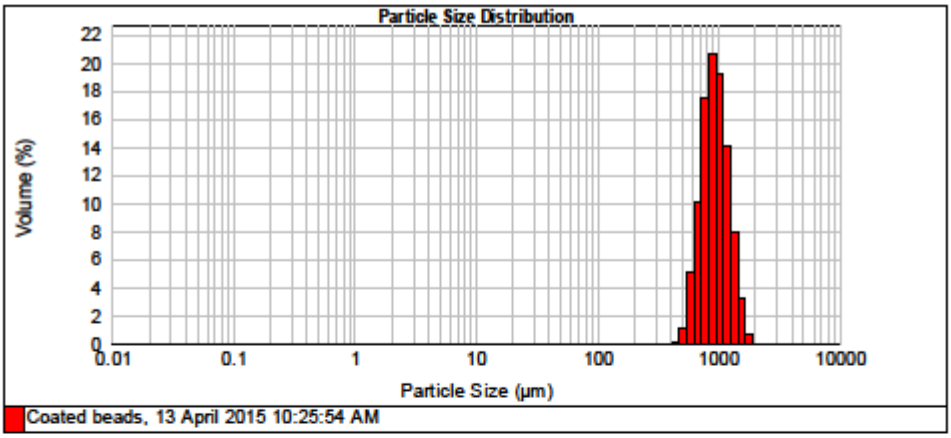
A.7: Avicel® PH 101 coated beads containing active ingredient pyridoxine

MASTERSIZER 2000

Result Analysis Report

Sample Name: Coated beads	**SOP Name:** Leandri (Beads)	**Measured:** 13 April 2015 10:25:54 AM	
Sample Source & type: AP 1	**Measured by:** Administrator	**Analysed:** 13 April 2015 10:25:55 AM	
Sample bulk lot ref:	**Result Source:** Measurement		
Particle Name: Titanium Dioxide	**Accessory Name:** Hydro 2000MU (A)	**Analysis model:** General purpose	**Sensitivity:** Normal
Particle RI: 2.741	**Absorption:** 0.1	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 1.63 %
Dispersant Name: Water	**Dispersant RI:** 1.330	**Weighted Residual:** 9.247 %	**Result Emulation:** Off
Concentration: 0.2096 %Vol	**Span :** 0.676	**Uniformity:** 0.214	**Result units:** Volume
Specific Surface Area: 0.00668 m²/g	**Surface Weighted Mean D[3,2]:** 898.483 um	**Vol. Weighted Mean D[4,3]:** 958.284 um	

d(0.1): 666.530 um d(0.5): 924.897 um d(0.9): 1291.892 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.00	120.226	0.00	1258.925	7.86
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.00	138.038	0.00	1445.440	3.23
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.00	158.489	0.00	1659.587	0.78
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.00	181.970	0.00	1905.461	0.04
0.017	0.00	0.182	0.00	1.905	0.00	19.953	0.00	208.930	0.00	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	0.00	239.883	0.00	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	0.00	275.423	0.00	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.00	30.200	0.00	316.228	0.00	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	0.00	363.078	0.00	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.00	39.811	0.00	416.869	0.15	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.00	45.709	0.00	478.630	1.18	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.00	52.481	0.00	549.541	5.18	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.00	60.256	0.00	630.957	10.16	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.00	69.183	0.00	724.436	17.52	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.00	79.433	0.00	831.764	20.64	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.00	91.201	0.00	954.993	19.29	10000.000	
0.091	0.00	0.955	0.00	10.000	0.00	104.713	0.00	1096.478	13.97		
0.105	0.00	1.096	0.00	11.482	0.00	120.226	0.00	1258.925			

Operator notes:

Malvern Instruments Ltd.
Malvern, UK

Mastersizer 2000 Ver. 5.60
Serial Number : MAL1007548

File name: 13 April 2015
Record Number: 39

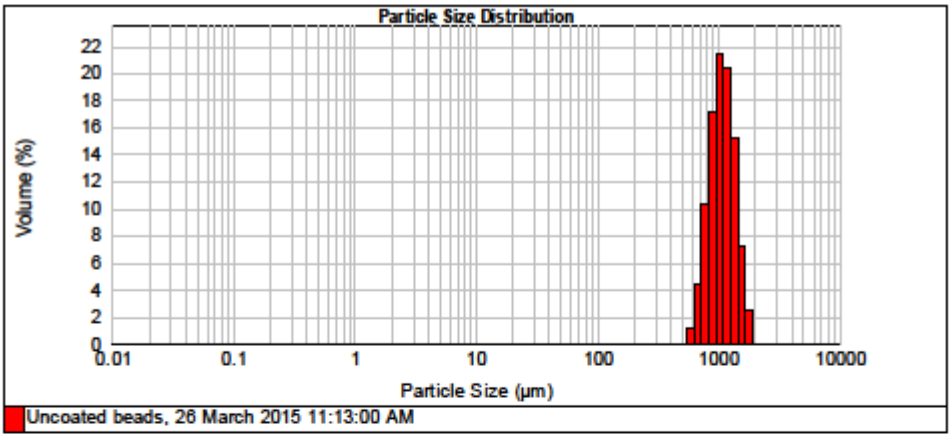
A.8: MicroceLac® 100 uncoated beads containing active ingredient furosemide

MASTERSIZER 2000

Result Analysis Report

Sample Name: Uncoated beads	**SOP Name:** Leandri (Beads)	**Measured:** 26 March 2015 11:13:00 AM	
Sample Source & type:	**Measured by:** Administrator	**Analysed:** 26 March 2015 11:13:01 AM	
Sample bulk lot ref: MF 1 (Sample 1)	**Result Source:** Measurement		
Particle Name: Titanium Dioxide	**Accessory Name:** Hydro 2000MU (A)	**Analysis model:** General purpose	**Sensitivity:** Normal
Particle RI: 2.741	**Absorption:** 0.1	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 1.24 %
Dispersant Name: Alcohol	**Dispersant RI:** 1.320	**Weighted Residual:** 3.499 %	**Result Emulation:** Off
Concentration: 0.1822 %Vol	**Span :** 0.624	**Uniformity:** 0.199	**Result units:** Volume
Specific Surface Area: 0.00581 m²/g	**Surface Weighted Mean D[3,2]:** 1032.072 um	**Vol. Weighted Mean D[4,3]:** 1092.808 um	

d(0.1): 777.408 um d(0.5): 1065.350 um d(0.9): 1442.223 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.00	120.226	0.00	1258.925	15.11
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.00	138.038	0.00	1445.440	7.25
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.00	158.489	0.00	1659.587	2.55
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.00	181.970	0.00	1905.461	0.05
0.017	0.00	0.182	0.00	1.905	0.00	19.953	0.00	208.930	0.00	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	0.00	239.883	0.00	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	0.00	275.423	0.00	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.00	30.200	0.00	316.228	0.00	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	0.00	363.078	0.00	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.00	39.811	0.00	416.869	0.00	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.00	45.709	0.00	478.630	0.00	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.00	52.481	0.00	549.541	0.09	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.00	60.256	0.00	630.957	1.13	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.00	69.183	0.00	724.436	4.38	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.00	79.433	0.00	831.764	10.31	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.00	91.201	0.00	954.993	17.20	10000.000	0.00
0.091	0.00	0.955	0.00	10.000	0.00	104.713	0.00	1096.478	21.45		
0.105	0.00	1.096	0.00	11.482	0.00	120.226	0.00	1258.925	20.49		

Operator notes:

Malvern Instruments Ltd.
Malvern, UK

Mastersizer 2000 Ver. 5.60
Serial Number : MAL1007548

File name: 13 April 2015
Record Number: 24

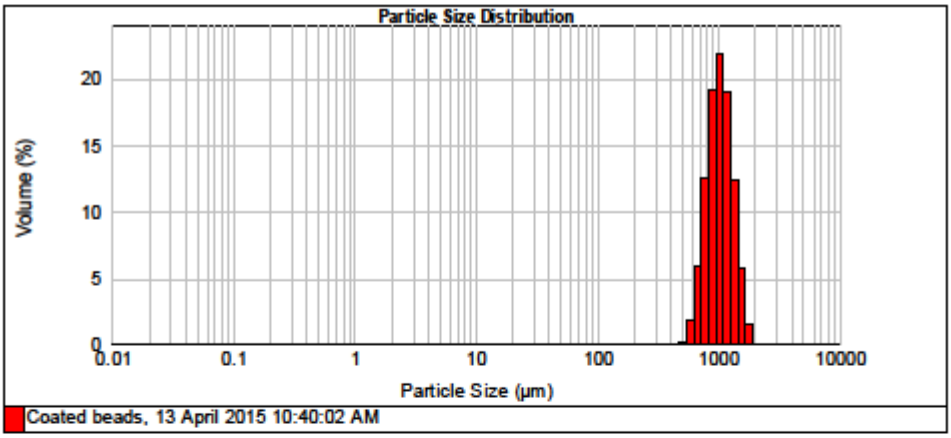
A.9: MicroceLac® 100 coated beads containing active ingredient furosemide

MASTERSIZER 2000

Result Analysis Report

Sample Name: Coated beads	**SOP Name:** Leandri (Beads)	**Measured:** 13 April 2015 10:40:02 AM	
Sample Source & type:	**Measured by:** Administrator	**Analysed:** 13 April 2015 10:40:03 AM	
Sample bulk lot ref: M-CF (Sample 1)	**Result Source:** Measurement		
Particle Name: Titanium Dioxide	**Accessory Name:** Hydro 2000MU (A)	**Analysis model:** General purpose	**Sensitivity:** Normal
Particle RI: 2.741	**Absorption:** 0.1	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 1.30 %
Dispersant Name: Water	**Dispersant RI:** 1.330	**Weighted Residual:** 11.150 %	**Result Emulation:** Off
Concentration: 0.1840 %Vol	**Span :** 0.630	**Uniformity:** 0.193	**Result units:** Volume
Specific Surface Area: 0.00606 m²/g	**Surface Weighted Mean D[3,2]:** 990.293 um	**Vol. Weighted Mean D[4,3]:** 1048.624 um	

d(0.1): 747.756 um **d(0.5):** 1019.498 um **d(0.9):** 1389.985 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.00	120.226	0.00	1258.925	12.31
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.00	138.038	0.00	1445.440	5.67
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.00	158.489	0.00	1659.587	1.47
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.00	181.970	0.00	1905.461	0.09
0.017	0.00	0.182	0.00	1.905	0.00	19.953	0.00	208.930	0.00	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	0.00	239.883	0.00	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	0.00	275.423	0.00	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.00	30.200	0.00	316.228	0.00	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	0.00	363.078	0.00	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.00	39.811	0.00	416.869	0.00	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.00	45.709	0.00	478.630	0.00	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.00	52.481	0.00	549.541	0.18	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.00	60.256	0.00	630.957	1.72	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.00	69.183	0.00	724.436	5.88	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.00	79.433	0.00	831.764	12.60	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.00	91.201	0.00	954.993	19.29	10000.000	
0.091	0.00	0.955	0.00	10.000	0.00	104.713	0.00	1096.478	21.91		
0.105	0.00	1.096	0.00	11.482	0.00	120.226	0.00	1258.925	18.98		

Operator notes:

Malvern Instruments Ltd.
Malvern, UK

Mastersizer 2000 Ver. 5.60
Serial Number : MAL1007548

File name: 13 April 2015
Record Number: 47

A.10: MicroceLac® 100 uncoated beads containing active ingredient pyridoxine

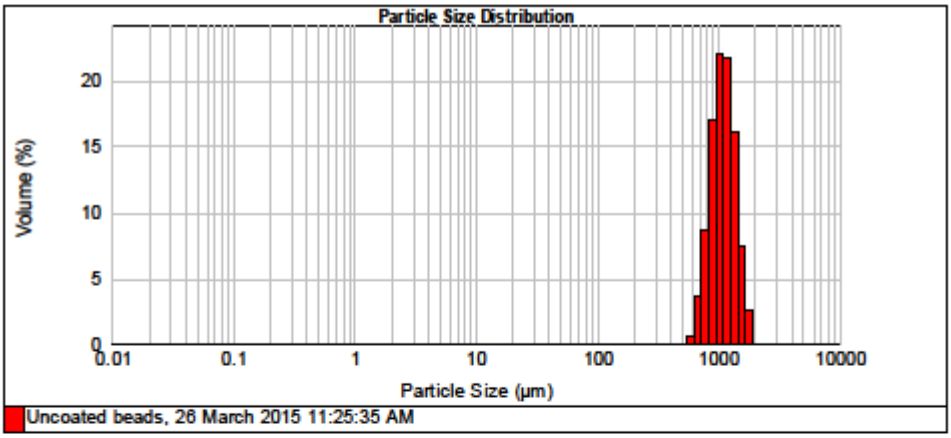
MALVERN INSTRUMENTS

MASTERSIZER 2000

Result Analysis Report

Sample Name: Uncoated beads	**SOP Name:** Leandri (Beads)	**Measured:** 26 March 2015 11:25:35 AM	
Sample Source & type:	**Measured by:** Administrator	**Analysed:** 26 March 2015 11:25:37 AM	
Sample bulk lot ref: MP 1 (Sample 1)	**Result Source:** Measurement		
Particle Name: Titanium Dioxide	**Accessory Name:** Hydro 2000MU (A)	**Analysis model:** General purpose	**Sensitivity:** Normal
Particle RI: 2.741	**Absorption:** 0.1	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 0.77 %
Dispersant Name: Alcohol	**Dispersant RI:** 1.320	**Weighted Residual:** 7.050 %	**Result Emulation:** Off
Concentration: 0.1159 %Vol	**Span :** 0.598	**Uniformity:** 0.192	**Result units:** Volume
Specific Surface Area: 0.0057 m²/g	**Surface Weighted Mean D[3,2]:** 1051.934 um	**Vol. Weighted Mean D[4,3]:** 1108.720 um	

d(0.1): 800.462 um d(0.5): 1082.932 um d(0.9): 1447.821 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.00	120.226	0.00	1258.925	16.09
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.00	138.038	0.00	1445.440	7.52
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.00	158.489	0.00	1659.587	2.58
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.00	181.970	0.00	1905.461	0.02
0.017	0.00	0.182	0.00	1.905	0.00	19.953	0.00	208.930	0.00	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	0.00	239.883	0.00	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	0.00	275.423	0.00	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.00	30.200	0.00	316.228	0.00	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	0.00	363.078	0.00	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.00	39.811	0.00	416.869	0.00	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.00	45.709	0.00	478.630	0.04	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.00	52.481	0.00	549.541	0.53	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.00	60.256	0.00	630.957	3.73	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.00	69.183	0.00	724.436	8.64	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.00	79.433	0.00	831.764	17.05	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.00	91.201	0.00	954.993	22.06	10000.000	
0.091	0.00	0.955	0.00	10.000	0.00	104.713	0.00	1096.478	21.75		
0.105	0.00	1.096	0.00	11.482	0.00	120.226	0.00	1258.925	21.75		

Operator notes:

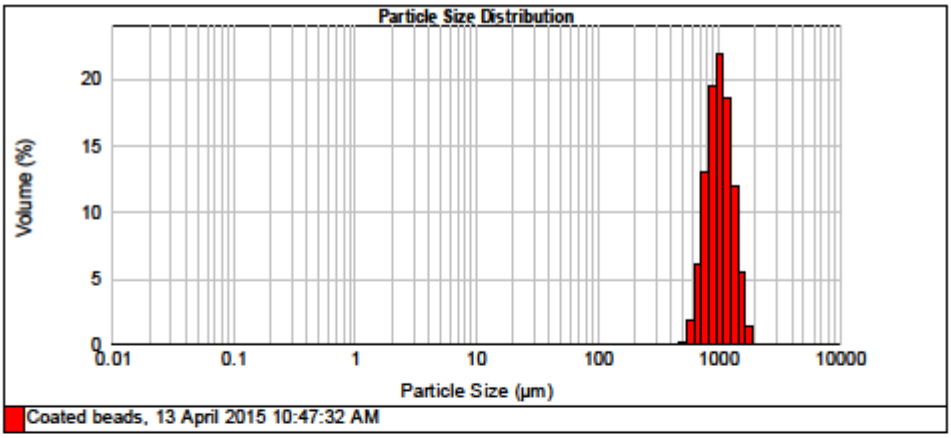
A.11: MicroceLac® 100 coated beads containing active ingredient pyridoxine

MASTERSIZER 2000

Result Analysis Report

Sample Name: Coated beads	**SOP Name:** Leandri (Beads)	**Measured:** 13 April 2015 10:47:32 AM	
Sample Source & type:	**Measured by:** Administrator	**Analysed:** 13 April 2015 10:47:33 AM	
Sample bulk lot ref: M-CP (Sample 1)	**Result Source:** Measurement		
Particle Name: Titanium Dioxide	**Accessory Name:** Hydro 2000MU (A)	**Analysis model:** General purpose	**Sensitivity:** Normal
Particle RI: 2.741	**Absorption:** 0.1	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 1.52 %
Dispersant Name: Water	**Dispersant RI:** 1.330	**Weighted Residual:** 12.704 %	**Result Emulation:** Off
Concentration: 0.2139 %Vol	**Span :** 0.630	**Uniformity:** 0.192	**Result units:** Volume
Specific Surface Area: 0.0061 m²/g	**Surface Weighted Mean D[3,2]:** 984.056 um	**Vol. Weighted Mean D[4,3]:** 1042.043 um	

d(0.1): 743.010 um d(0.5): 1012.852 um d(0.9): 1381.194 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.00	120.226	0.00	1258.925	11.93
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.00	138.038	0.00	1445.440	5.39
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.00	158.489	0.00	1659.587	1.38
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.00	181.970	0.00	1905.461	0.08
0.017	0.00	0.182	0.00	1.905	0.00	19.953	0.00	208.930	0.00	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	0.00	239.883	0.00	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	0.00	275.423	0.00	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.00	30.200	0.00	316.228	0.00	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	0.00	363.078	0.00	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.00	39.811	0.00	416.869	0.00	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.00	45.709	0.00	478.630	0.21	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.00	52.481	0.00	549.541	1.84	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.00	60.256	0.00	630.957	6.13	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.00	69.183	0.00	724.436	12.95	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.00	79.433	0.00	831.764	19.50	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.00	91.201	0.00	954.993	21.92	10000.000	
0.091	0.00	0.955	0.00	10.000	0.00	104.713	0.00	1096.478	18.66		
0.105	0.00	1.096	0.00	11.482	0.00	120.226	0.00	1258.925			

Operator notes:

Malvern Instruments Ltd.
Malvern, UK

Mastersizer 2000 Ver. 5.60
Serial Number : MAL1007548

File name: 13 April 2015
Record Number: 52

A.12: RetaLac® uncoated beads containing active ingredient furosemide

MALVERN INSTRUMENTS

MASTERSIZER 2000

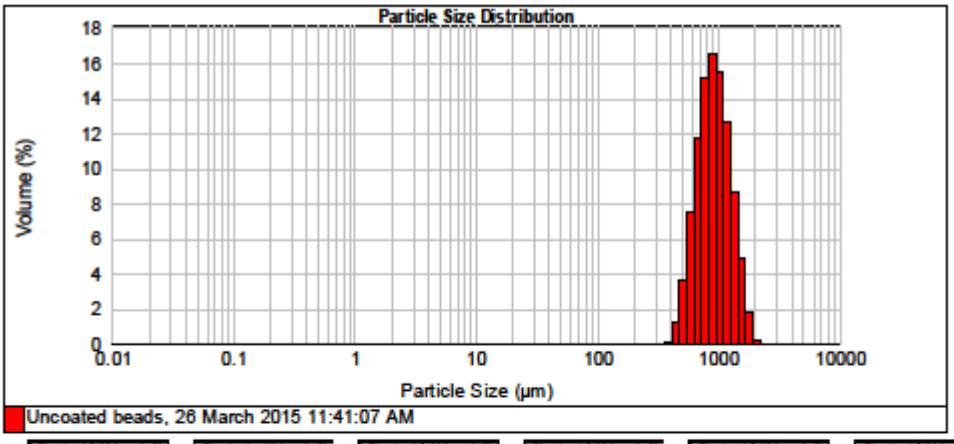
Result Analysis Report

Sample Name: Uncoated beads	**SOP Name:** Leandri (Beads)	**Measured:** 26 March 2015 11:41:07 AM	
Sample Source & type:	**Measured by:** Administrator	**Analysed:** 26 March 2015 11:41:08 AM	
Sample bulk lot ref: RF 1 (Sample 1)	**Result Source:** Measurement		

Particle Name: Titanium Dioxide	**Accessory Name:** Hydro 2000MU (A)	**Analysis model:** General purpose	**Sensitivity:** Normal
Particle RI: 2.741	**Absorption:** 0.1	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 1.45 %
Dispersant Name: Alcohol	**Dispersant RI:** 1.320	**Weighted Residual:** 4.254 %	**Result Emulation:** Off

Concentration: 0.1791 %Vol	**Span :** 0.839	**Uniformity:** 0.262	**Result units:** Volume
Specific Surface Area: 0.00693 m²/g	**Surface Weighted Mean D[3,2]:** 866.084 um	**Vol. Weighted Mean D[4,3]:** 952.797 um	

d(0.1): 605.859 um **d(0.5):** 907.544 um **d(0.9):** 1367.168 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.00	120.226	0.00	1258.925	8.72
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.00	138.038	0.00	1445.440	4.90
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.00	158.489	0.00	1659.587	1.82
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.00	181.970	0.00	1905.461	0.26
0.017	0.00	0.182	0.00	1.905	0.00	19.953	0.00	208.930	0.00	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	0.00	239.883	0.00	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	0.00	275.423	0.00	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.00	30.200	0.00	316.228	0.00	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	0.00	363.078	0.15	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.00	39.811	0.00	416.869	1.25	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.00	45.709	0.00	478.630	3.73	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.00	52.481	0.00	549.541	7.52	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.00	60.256	0.00	630.957	11.75	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.00	69.183	0.00	724.436	15.15	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.00	79.433	0.00	831.764	16.57	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.00	91.201	0.00	954.993	15.57	10000.000	
0.091	0.00	0.955	0.00	10.000	0.00	104.713	0.00	1096.478	12.82		
0.105	0.00	1.096	0.00	11.482	0.00	120.226	0.00	1258.925			

Operator notes:

Malvern Instruments Ltd.
Malvern, UK

Mastersizer 2000 Ver. 5.60
Serial Number : MAL1007548

File name: 13 April 2015
Record Number: 31

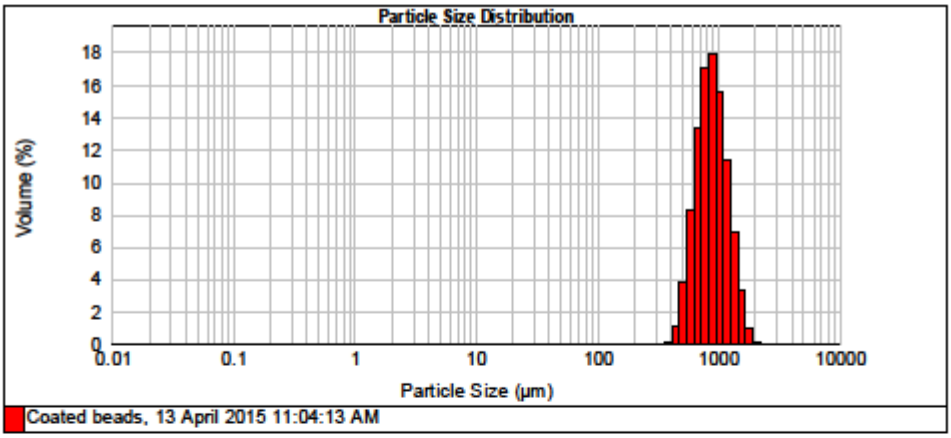
A.13: RetaLac® coated beads containing active ingredient furosemide

MASTERSIZER 2000

Result Analysis Report

Sample Name: Coated beads	**SOP Name:** Leandri (Beads)	**Measured:** 13 April 2015 11:04:13 AM	
Sample Source & type:	**Measured by:** Administrator	**Analysed:** 13 April 2015 11:04:14 AM	
Sample bulk lot ref: RCF (Sample 1)	**Result Source:** Measurement		
Particle Name: Titanium Dioxide	**Accessory Name:** Hydro 2000MU (A)	**Analysis model:** General purpose	**Sensitivity:** Normal
Particle RI: 2.741	**Absorption:** 0.1	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 1.88 %
Dispersant Name: Cyclohexane	**Dispersant RI:** 1.426	**Weighted Residual:** 4.464 %	**Result Emulation:** Off
Concentration: 0.2270 %Vol	**Span :** 0.782	**Uniformity:** 0.24	**Result units:** Volume
Specific Surface Area: 0.00713 m²/g	**Surface Weighted Mean D[3,2]:** 840.988 um	**Vol. Weighted Mean D[4,3]:** 914.660 um	

d(0.1): 603.095 um d(0.5): 872.928 um d(0.9): 1285.329 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.00	120.226	0.00	1258.925	6.92
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.00	138.038	0.00	1445.440	3.30
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.00	158.489	0.00	1659.587	1.00
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.00	181.970	0.00	1905.461	0.10
0.017	0.00	0.182	0.00	1.905	0.00	19.953	0.00	208.930	0.00	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	0.00	239.883	0.00	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	0.00	275.423	0.00	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.00	30.200	0.00	316.228	0.00	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	0.00	363.078	0.11	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.00	39.811	0.00	416.869	1.12	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.00	45.709	0.00	478.630	3.79	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.00	52.481	0.00	549.541	8.24	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.00	60.256	0.00	630.957	13.32	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.00	69.183	0.00	724.436	17.08	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.00	79.433	0.00	831.764	17.98	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.00	91.201	0.00	954.993	15.66	10000.000	
0.091	0.00	0.955	0.00	10.000	0.00	104.713	0.00	1096.478	11.44		
0.105	0.00	1.096	0.00	11.482	0.00	120.226	0.00	1258.925	11.44		

Operator notes:

Malvern Instruments Ltd.
Malvern, UK

Mastersizer 2000 Ver. 5.60
Serial Number : MAL1007548

File name: 13 April 2015
Record Number: 56

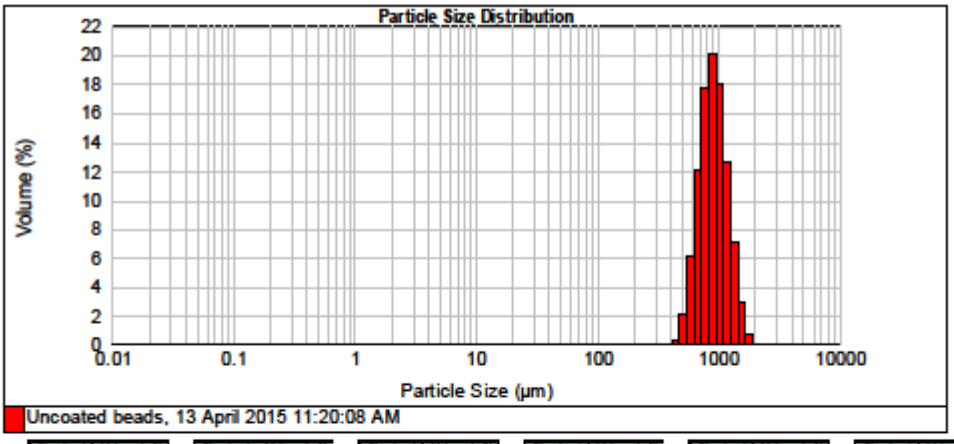
A.14: RetaLac® uncoated beads containing active ingredient pyridoxine

MASTERSIZER 2000

Result Analysis Report

Sample Name: Uncoated beads	**SOP Name:** Leandri (Beads)	**Measured:** 13 April 2015 11:20:08 AM	
Sample Source & type:	**Measured by:** Administrator	**Analysed:** 13 April 2015 11:20:09 AM	
Sample bulk lot ref: UCRP (Sample 1)	**Result Source:** Measurement		
Particle Name: Titanium Dioxide	**Accessory Name:** Hydro 2000MU (A)	**Analysis model:** General purpose	**Sensitivity:** Normal
Particle RI: 2.741	**Absorption:** 0.1	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 1.23 %
Dispersant Name: Cyclohexane	**Dispersant RI:** 1.426	**Weighted Residual:** 7.789 %	**Result Emulation:** Off
Concentration: 0.1533 %Vol	**Span :** 0.696	**Uniformity:** 0.219	**Result units:** Volume
Specific Surface Area: 0.00688 m²/g	**Surface Weighted Mean D[3,2]:** 872.572 um	**Vol. Weighted Mean D[4,3]:** 934.699 um	

d(0.1): 644.607 um d(0.5): 900.195 um d(0.9): 1271.347 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.00	120.226	0.00	1258.925	7.07
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.00	138.038	0.00	1445.440	2.89
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.00	158.489	0.00	1659.587	0.70
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.00	181.970	0.00	1905.461	0.04
0.017	0.00	0.182	0.00	1.905	0.00	19.953	0.00	208.930	0.00	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	0.00	239.883	0.00	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	0.00	275.423	0.00	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.00	30.200	0.00	316.228	0.00	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	0.00	363.078	0.01	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.00	39.811	0.00	416.869	0.33	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.00	45.709	0.00	478.630	2.07	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.00	52.481	0.00	549.541	6.13	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.00	60.256	0.00	630.957	12.14	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.00	69.183	0.00	724.436	17.78	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.00	79.433	0.00	831.764	20.12	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.00	91.201	0.00	954.993	17.98	10000.000	
0.091	0.00	0.955	0.00	10.000	0.00	104.713	0.00	1096.478	12.73		
0.105	0.00	1.096	0.00	11.482	0.00	120.226	0.00	1258.925			

Operator notes:

Malvern Instruments Ltd.
Malvern, UK

Mastersizer 2000 Ver. 5.60
Serial Number : MAL1007548

File name: 13 April 2015
Record Number: 63

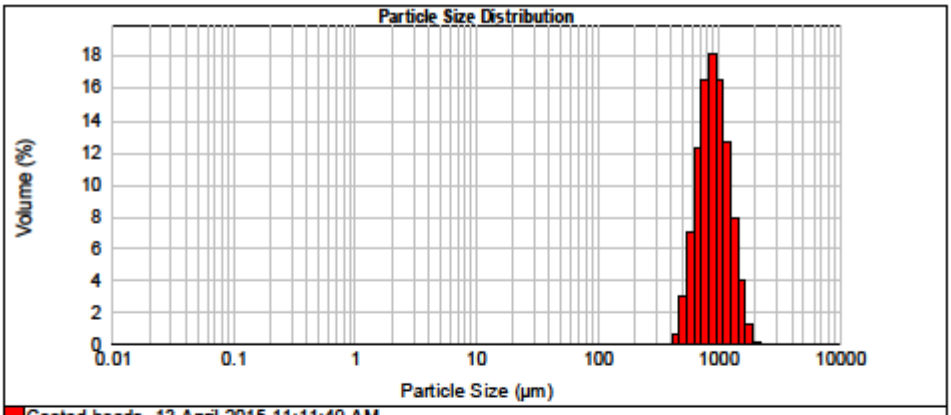
A.15: RetaLac® coated beads containing active ingredient pyridoxine

MASTERSIZER 2000

Result Analysis Report

Sample Name: Coated beads	**SOP Name:** Leandri (Beads)	**Measured:** 13 April 2015 11:11:49 AM	
Sample Source & type:	**Measured by:** Administrator	**Analysed:** 13 April 2015 11:11:50 AM	
Sample bulk lot ref: RCP (Sample 1)	**Result Source:** Measurement		
Particle Name: Titanium Dioxide	**Accessory Name:** Hydro 2000MU (A)	**Analysis model:** General purpose	**Sensitivity:** Normal
Particle RI: 2.741	**Absorption:** 0.1	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 1.77 %
Dispersant Name: Cyclohexane	**Dispersant RI:** 1.426	**Weighted Residual:** 3.984 %	**Result Emulation:** Off
Concentration: 0.2195 %Vol	**Span :** 0.773	**Uniformity:** 0.241	**Result units:** Volume
Specific Surface Area: 0.00692 m²/g	**Surface Weighted Mean D[3,2]:** 867.652 um	**Vol. Weighted Mean D[4,3]:** 942.010 um	

d(0.1): 623.408 um d(0.5): 900.922 um d(0.9): 1319.697 um



Size (µm)	Volume In %
0.010	0.00
0.011	0.00
0.013	0.00
0.015	0.00
0.017	0.00
0.020	0.00
0.023	0.00
0.026	0.00
0.030	0.00
0.035	0.00
0.040	0.00
0.046	0.00
0.052	0.00
0.060	0.00
0.069	0.00
0.079	0.00
0.091	0.00
0.105	0.00

Size (µm)	Volume In %
0.105	0.00
0.120	0.00
0.138	0.00
0.158	0.00
0.182	0.00
0.209	0.00
0.240	0.00
0.275	0.00
0.316	0.00
0.363	0.00
0.417	0.00
0.479	0.00
0.550	0.00
0.631	0.00
0.724	0.00
0.832	0.00
0.955	0.00
1.096	0.00

Size (µm)	Volume In %
1.096	0.00
1.259	0.00
1.445	0.00
1.660	0.00
1.905	0.00
2.188	0.00
2.512	0.00
2.884	0.00
3.311	0.00
3.802	0.00
4.365	0.00
5.012	0.00
5.754	0.00
6.607	0.00
7.586	0.00
8.710	0.00
10.000	0.00
11.482	0.00

Size (µm)	Volume In %
11.482	0.00
13.183	0.00
15.136	0.00
17.378	0.00
19.953	0.00
22.909	0.00
26.303	0.00
30.200	0.00
34.674	0.00
39.811	0.00
45.709	0.00
52.481	0.00
60.256	0.00
69.183	0.00
79.433	0.00
91.201	0.00
104.713	0.00
120.226	0.00

Size (µm)	Volume In %
120.226	0.00
138.038	0.00
158.489	0.00
181.970	0.00
208.930	0.00
239.883	0.00
275.423	0.00
316.228	0.00
363.078	0.07
416.869	0.67
478.630	3.01
549.541	7.08
630.957	12.20
724.436	16.48
831.764	18.12
954.993	16.54
1096.478	12.60
1258.925	7.95

Size (µm)	Volume In %
1258.925	7.95
1445.440	3.93
1659.587	1.22
1905.461	0.12
2187.762	0.00
2511.886	0.00
2884.032	0.00
3311.311	0.00
3801.894	0.00
4365.158	0.00
5011.872	0.00
5754.399	0.00
6606.934	0.00
7585.776	0.00
8709.636	0.00
10000.000	

Operator notes:

Malvern Instruments Ltd.
Malvern, UK

Mastersizer 2000 Ver. 5.60
Serial Number : MAL1007548

File name: 13 April 2015
Record Number: 59

ANNEXURE B

TABLET TEST DATA FOR UNCOATED AND COATED MINI-MUPS

Table B.1: Tablet mass variation of the mini-MUPS prepared from uncoated beads with active ingredients furosemide and pyridoxine

Table B.2: Tablet mass variation of the mini-MUPS prepare from coated beads with active ingredients furosemide and pyridoxine

Table B.3: Hardness, diameter and thickness for Avicel® PH 101 mini-MUPS prepared from uncoated beads with active ingredients furosemide and pyridoxine

Table B.4: Hardness, diameter and thickness for MicroceLac® 100 mini-MUPS prepared from uncoated beads with active ingredients furosemide and pyridoxine

Table B.5: Hardness, diameter and thickness for RetaLac® mini-MUPS prepared from uncoated beads with active ingredients furosemide and pyridoxine

Table B.6: Hardness, diameter and thickness for mini-MUPS prepared from coated beads with active ingredients furosemide and pyridoxine

Table B.1: Tablet mass variation of the mini-MUPS prepared from uncoated beads with active ingredients furosemide and pyridoxine *(%RSD is indicated in parentheses)*

Formulation	Average (mg)	Compliance
A0F0	87.86 (26.06)	*Fail
A0F3	96.62 (2.05)	Pass
A0F5	99.14 (2.53)	Pass
A0P0	93.19 (18.52)	*Fail
A0P5	92.90 (5.56)	Fail
A0.05P3	88.74 (4.39)	Pass
A0.05P0	96.41 (4.13)	Pass
M0.05F0	100.52 (9.21)	Pass
M0.05F3	98.02 (1.52)	Pass
M0.05F5	97.68 (6.75)	Pass
M0.1F5	100.10 (2.83)	Pass
M0P0	106.97 (2.47)	Pass
M0.05P0	96.37 (6.29)	Pass
M0.05P3	90.36 (4.93)	Fail
R0.05F0	96.25 (2.41)	Pass
R0.1F0	98.41 (1.12)	Pass
R0.1F3	98.34 (2.30)	Pass
R0P0	95.15 (2.96)	Pass
R0.05P0	101.06 (1.36)	Pass
R0.1P0	98.32 (2.04)	Pass
R0.1P3	104.42 (1.34)	Pass

**Fail = Deviated by more than 15% of the average mass*

Table B.2: Tablet mass variation of the mini-MUPS prepare from coated beads with active ingredients furosemide and pyridoxine *(%RSD is indicated in parentheses)*

Formulation	Average (mg)	Compliance
A0F3	102.66 (4.86)	Fail
A0P0	103.73 (5.89)	*Fail
M0.05F3	102.46 (3.87)	Pass
M0.05P0	100.35 (3.89)	Pass
R0.1F3	99.79 (1.53)	Pass
R0.1P3	99.48 (2.41)	Pass

**Fail = Deviated by more than 15% of the average mass*

Table B.3: Hardness, diameter and thickness for Avicel® PH 101 mini-MUPS prepared from uncoated beads with active ingredients furosemide and pyridoxine *(%RSD is indicated in parentheses)*

Formulation	Hardness (N)	Diameter (mm)	Thickness (mm)
A0F0	23.25 (46.71)	6.02 (0.20)	3.15 (4.50)
A0F3	67.40 (13.51)	6.01 (0.12)	3.19 (2.29)
A0F5	79.30 (15.46)	6.00 (0.10)	3.24 (1.61)
A0P0	30.70 (22.67)	5.94 (0.15)	3.32 (3.94)
A0P5	0.00 (100.00)	5.97 (0.27)	3.22 (6.68)
A0.05P3	31.40 (31.39)	5.88 (0.43)	3.16 (4.09)
A0.05P0	12.17 (29.14)	5.91 (0.31)	3.19 (6.86)

Table B.4: Hardness, diameter and thickness for MicroceLac® 100 mini-MUPS prepared from uncoated beads with active ingredients furosemide and pyridoxine *(%RSD is indicated in parentheses)*

Formulation	Hardness (N)	Diameter (mm)	Thickness (mm)
M0.05F0	23.00 (32.99)	6.03 (0.13)	3.19 (2.01)
M0.05F3	37.20 (15.82)	6.06 (0.20)	3.11 (2.99)
M0.05F5	55.60 (16.11)	6.04 (0.10)	3.18 (2.58)
M0.1F5	33.60 (30.39)	6.03 (0.22)	3.18 (0.91)
M0P0	99.40 (11.32)	6.00 (0.22)	3.40 (1.97)
M0.05P0	89.30 (11.91)	5.96 (0.17)	3.14 (3.50)
M0.05P3	156.20 (9.23)	5.99 (0.17)	2.85 (7.08)

Table B.5: Hardness, diameter and thickness for RetaLac® mini-MUPS prepared from uncoated beads with active ingredients furosemide and pyridoxine *(%RSD is indicated in parentheses)*

Formulation	Hardness (N)	Diameter (mm)	Thickness (mm)
R0.05F0	76.80 (7.86)	5.97 (0.18)	3.50 (3.97)
R0.1F0	66.80 (22.16)	6.03 (0.17)	3.51 (1.45)
R0.1F3	70.20 (23.39)	5.99 (0.18)	3.81 (25.90)
R0P0	121.10 (8.67)	6.00 (0.13)	3.47 (2.22)
R0.05P0	114.60 (12.66)	6.00 (0.15)	3.63 (1.63)
R0.1P0	89.30 (5.95)	6.01 (0.27)	3.65 (1.40)
R0.1P3	111.90 (6.14)	6.00 (0.13)	3.98 (24.20)

Table B.6: Hardness, diameter and thickness for mini-MUPS prepared from coated beads with active ingredients furosemide and pyridoxine *(%RSD is indicated in parentheses)*

Formulation	Hardness (N)	Diameter (mm)	Thickness (mm)
A0F3	25.20 (23.20)	6.05 (0.47)	2.87 (33.67)
A0P0	0.00 (100)	6.03 (0.78)	3.37 (1.94)
M0.05F3	33.90 (15.13)	3.37 (1.94)	3.14 (2.96)
M0.05P0	21.50 (30.08)	3.14 (2.96)	3.17 (2.52)
R0.1F3	42.40 (11.98)	3.17 (2.52)	3.42 (1.44)
R0.1P3	66.10 (18.81)	3.42 (1.44)	3.47 (1.70)

ANNEXURE C

DISSOLUTION STUDIES

Table C.1: Percentage drug dissolution for the optimised uncoated for the active ingredients furosemide and pyridoxine

Table C.2: Percentage drug dissolution for the coated formulations for the active ingredients furosemide and pyridoxine

Table C.3: Percentage drug dissolution for the mini-MUPs-in-capsule dosage forms for the active ingredients furosemide and pyridoxine

Table C.1: Percentage drug dissolution for the optimised mini-MUPS prepared from uncoated beads for the active ingredients furosemide and pyridoxine

Average % in solution						
Time (min)	**Avi0F3**	**Avi0P0**	**Mic0.05F3**	**Mic0.05P0**	**Ret0.1F3**	**Ret0.1P3**
0	0.00	0.00	0.00	0.00	0.00	0.00
5	4.99	72.25	4.21	24.33	18.55	7.02
13	8.74	82.99	4.83	36.01	19.69	7.28
21	13.66	85.09	6.55	64.90	21.55	16.33
30	17.38	85.46	10.10	77.36	25.88	24.24
60	17.41	85.46	10.78	86.13	30.10	34.29
90	17.41	85.46	10.79	86.27	35.62	50.16
120	17.41	85.46	10.79	86.23	40.43	62.21
150	49.87	92.83	91.78	97.68	97.84	95.29
180	73.77	94.82	99.95	98.59	98.04	96.05
240	96.55	99.97	100.00	99.99	99.36	96.12
300	99.98	100.00	100.00**	100.00	100.00	99.98
360	100.00	100.00**	100.00**	100.00**	100.00**	100.00
MDT (min)*	16.99	5.30	13.85	6.43	12.22	12.90
f_1*	-	-	17.91	12.53	27.52	36.29
f_2*	-	-	41.99	34.45	36.83	17.98

** f_1 = Difference factor; f_2 = Similarity factor; MDT = Mean dissolution time. ** The dark background = no further dissolution*

Table C.2: Percentage drug dissolution for the mini-MUPS prepared from coated beads for the active ingredients furosemide and pyridoxine

Average % in solution						
Time (min)	**Avi0F3**	**Avi0P0**	**Mic0.05F3**	**Mic0.05P0**	**Ret0.1F3**	**Ret0.1P3**
0	0.00	0.00	0.00	0.00	0.00	0.00
5	1.19	50.51	1.11	28.56	2.54	11.75
13	1.19	91.13	1.11	53.77	5.18	16.05
21	1.19	91.88	4.49	55.36	11.72	26.06
30	1.19	93.19	6.47	68.16	17.20	31.12
60	7.78	95.07	7.58	70.19	28.22	39.13
90	9.27	95.19	9.48	72.47	28.51	48.12
120	15.29	97.16	12.37	75.45	31.03	63.55
150	76.14	99.43	92.68	99.44	99.45	94.57
180	93.06	100.00	92.96	100.00	100.00	99.97
240	99.96	100.00**	99.96	100.00**	100.00**	100.00
300	100.00	100.00**	100.00	100.00**	100.00**	100.00**
MDT (min)*	15.69	3.99	15.07	7.17	13.10	13.83
f_1*	-	-	5.67	20.82	23.23	13.33
f_2*	-	-	63.92	31.58	44.32	49.97

** f_1 = Difference factor; f_2 = Similarity factor; MDT = Mean dissolution time. ** The dark background = no further dissolution*

Table C.3: Percentage drug dissolution for the mini-MUPS-in-capsule dosage forms for the active ingredients furosemide and pyridoxine

Average % in solution						
Time (min)	**Avi0F3**	**Avi0P0**	**Mic0.05F3**	**Mic0.05P0**	**Ret0.1F3**	**Ret0.1P3**
0	0.00	0.00	0.00	0.00	0.00	0.00
5	9.74	22.19	0.70	5.60	9.04	1.46
13	10.66	22.41	1.22	13.70	6.68	12.23
21	11.21	22.96	2.10	17.18	12.40	24.98
30	12.45	22.96	3.14	17.33	19.07	30.64
60	13.00	23.10	3.96	17.37	32.60	30.69
90	14.02	23.33	6.56	17.82	35.67	39.15
120	15.33	23.33	7.36	18.01	35.27	39.23
150	47.11	93.88	78.01	87.32	93.64	84.81
180	62.88	93.86	92.16	87.38	93.49	90.09
240	78.07	93.86	99.96	87.38	99.96	99.94
300	78.62	99.97	100.00	87.38	100.00	100.00
360	80.11	100.00	100.00**	97.60	100.00**	100.00**
420	91.88	100.00**	100.00**	99.99	100.00**	100.00**
480	99.23	100.00**	100.00**	100.00	100.00**	100.00**
540	100.00	100.00**	100.00**	100.00**	100.00**	100.00**
MDT (min)*	21.24	13.16	16.07	14.90	15.34	13.83
f_1*	-	-	36.72	11.83	42.30	13.33
f_2*	-	-	40.70	55.12	35.96	49.97

** f_1 = Difference factor; f_2 = Similarity factor; MDT = Mean dissolution time. ** The dark background = no further dissolution*

ANNEXURE D

ACCELERATED STABILITY TESTING

Table D.1: Physical properties for Avicel® PH 101/furosemide mini-MUPS after exposure to 6 months accelerated stability testing

Table D.2: Physical properties for MicroceLac® 100/furosemide mini-MUPS after exposure to 6 months accelerated stability testing

Table D.3: Physical properties for RetaLac®/furosemide mini-MUPS after exposure to 6 months accelerated stability testing

Table D.4: Physical properties for Avicel® PH 101/pyridoxine mini-MUPS after exposure to 6 months accelerated stability testing

Table D.5: Physical properties for MicroceLac® 100/pyridoxine mini-MUPS after exposure to 6 months accelerated stability testing

Table D.6: Physical properties for RetaLac®/pyridoxine mini-MUPS after exposure to 6 months accelerated stability testing

Table D.7: Complaince of furosemide formulations to mass variation over 6 months of accelerated stability testing in different condition chambers

Table D.8: Complaince of pyridoxine formulations to mass variation over 6 months of accelerated stability testing in different condition chambers

Table D.1: Physical properties for Avicel® PH 101 / furosemide mini-MUPS after exposure to 6 months accelerated stability testing *(%RSD is indicated in parentheses)*

				Chamber 1: 25°C / 60% RH*				Chamber 2: 30°C / 70% RH*				Chamber 3: 40°C / 75% RH*			
				Time (months)											
				1	2	3	6	1	2	3	6	1	2	3	6
Mini-MUPS containing furosemide as active ingredient	A0F3 uncoated beads	Physical properties	Assay (mg/100mg)	8.68	11.14	4.98	6.99	8.37	10.25	5.51	6.88	9.28	11.37	4.84	6.89
			Mass variation (mg)	99.05 (9.01)	103.04 (5.79)	102.37 (5.54)	97.76 (20.86)	102.77 (5.68)	106.97 (5.84)	101.19 (7.33)	98.98 (23.16)	101.32 (7.47)	103.83 (5.94)	102.80 (6.94)	98.10 (8.24)
			Tensile strength ($N.mm^{-2}$)	1.88 (31.86)	2.11 (37.73)	2.36 (30.55)	2.12 (22.04)	2.23 (23.17)	2.29 (54.41)	2.13 (24.68)	2.02 (33.56)	2.60 (17.56)	2.49 (29.72)	3.14 (22.38)	2.58 (33.38)
			Friability (%)	0.91	5.49	3.84	1.98	3.80	5.61	2.53	2.02	2.02	0.42	2.52	1.76
			Disintegration time (min)	5.96 (17.64)	6.18 (30.70)	12.04 (39.36)	9.36 (54.19)	7.23 (12.46)	11.8 (36.48)	10.51 (40.37)	13.47 (17.64)	6.61 (6.53)	11.28 (40.28)	11.89 (42.46)	12.84 (26.56)
	A0F3 coated beads		Assay (mg/100mg)	8.84	10.71	5.44	8.00	9.61	9.13	6.17	7.71	9.39	10.27	5.39	6.77
			Mass variation (mg)	104.48 (4.32)	105.06 (4.53)	98.16 (22.22)	103.5 (4.96)	103.18 (4.39)	99.73 (21.78)	105.44 (4.63)	102.10 (6.43)	105.60 (3.97)	105.70 (2.30)	105.10 (3.34)	106.67 (3.84)
			Tensile strength ($N.mm^{-2}$)	0.98 (20.65)	2.91 (46.64)	3.7 (103.9)	1.71 (45.38)	0.99 (36.77)	1.02 (24.02)	0.89 (20.68)	0.85 (17.87)	0.92 (34.41)	0.14 (27.85)	0.14 (16.98)	0.13 (18.92)
			Friability (%)	18.17	16.70	17.29	34.14	16.10	18.19	19.68	26.08	22.24	21.60	22.47	18.51
			Disintegration time (min)	5.33 (14.65)	5.35 (29.58)	4.19 (13.41)	3.76 (13.26)	4.29 (4.74)	4.02 (19.04)	3.36 (31.25)	2.89 (14.32)	4.88 (24.19)	3.97 (26.95)	4.49 (39.36)	2.63 (15.65)

**RH= Relative humidity*

Table D.2: Physical properties for MicroceLac® 100/furosemide mini-MUPS after exposure to 6 months accelerated stability testing *(%RSD is indicated in parentheses)*

				Chamber 1: 25°C / 60% RH**				Chamber 2: 30°C / 70% RH**				Chamber 3: 40°C / 75% RH**			
				Time (months)											
				1	2	3	6	1	2	3	6	1	2	3	6
Mini-MUPS containing furosemide as active ingredient	M0.05F3 uncoated-beads	Physical properties	Assay (mg/100mg)	10.77	14.16	9.36	9.60	11.66	13.29	8.23	9.17	11.54	14.47	7.38	9.83
			Mass variation (mg)	106.87 (5.80)	108.47 (5.38)	106.82 (6.66)	108.32 (6.34)	105.83 (5.70)	107.9 (6.08)	107.96 (5.84)	106.95 (4.73)	107.05 (5.58)	111.03 (4.23)	106.57 (5.91)	105.92 (5.25)
			Tensile strength ($N.mm^{-2}$)	2.51 (20.54)	2.60 (19.46)	2.22 (30.16)	2.71 (15.13)	2.43 (36.66)	2.59 (21.71)	2.69 (15.79)	2.67 (17.89)	3.21 (16.89)	3.15 (16.05)	4.03 (12.69)	3.85 (9.83)
			Friability (%)	0.09	0.04	0.11	0.06	0.06	0.06	0.13	0.02	0.14	0.03	0.01	0.00
			Disintegration time (min)	***ND**	***ND**	13.62 (6.86)	13.01 (9.04)	***ND**	***ND**	13.04 (14.08)	12.70 (3.00)	***ND**	***ND**	***ND**	12.82 (19.01)
	M0.05F3 coated beads		Assay (mg/100mg)	12.58	15.12	10.17	9.46	12.15	14.63	8.98	8.40	13.37	10.14	9.40	8.73
			Mass variation (mg)	102.11 (2.28)	101.13 (3.16)	101.13 (2.82)	99.89 (3.23)	99.73 (4.84)	101.98 (3.63)	101.94 (3.22)	103.01 (2.55)	101.31 (2.83)	101.85 (3.76)	97.18 (21.41)	102.06 (3.54)
			Tensile strength ($N.mm^{-2}$)	1.74 (12.55)	1.23 (34.05)	1.38 (21.22)	1.47 (17.87)	1.75 (16.47)	1.51 (25.31)	1.47 (18.22)	1.63 (17.52)	1.50 (13.60)	1.36 (21.97)	1.52 (20.65)	1.62 (13.41)
			Friability (%)	0.06	0.04	0.02	0.03	0.08	0.11	0.13	0.01	0.04	0.13	0.07	0.01
			Disintegration time (min)	***ND**	13.66 (6.33)	14.28 (7.17)	13.92 (5.40)	***ND**	14.03 (7.90)	14.87 (2.11)	14.49 (5.22)	***ND**	13.56 (8.85)	14.89 (1.84)	14.85 (2.45)

**ND= Considered non-disintegrating; **RH= Relative humidity*

Table D.3: Physical properties for RetaLac®/furosemide mini-MUPS after exposure to 6 months accelerated stability testing *(%RSD is indicated in parentheses)*

				Chamber 1: 25°C / 60% RH**				Chamber 2: 30°C / 70% RH**				Chamber 3: 40°C / 75% RH**			
				Time (months)											
				1	2	3	6	1	2	3	6	1	2	3	6
Mini-MUPS containing furosemide as active ingredient	R0.1F3 uncoated beads	Physical properties	Assay (mg/100mg)	8.59	13.16	9.07	11.26	11.54	11.56	9.01	11.04	10.98	12.61	8.91	11.27
			Mass variation (mg)	98.93 (1.87)	99.39 (2.97)	100.69 (3.00)	99.00 (2.47)	98.45 (2.34)	97.30 (3.52)	100.15 (3.98)	98.94 (2.77)	98.11 (2.13)	97.49 (3.78)	97.00 (3.43)	99.16 (3.61)
			Tensile strength ($N.mm^{-2}$)	3.53 (15.62)	3.08 (32.04)	3.07 (20.90)	3.29 (13.53)	3.73 (22.55)	3.11 (20.85)	3.71 (23.80)	3.36 (16.59)	3.32 (18.60)	2.84 (22.52)	3.12 (22.46)	3.41 (23.49)
			Friability (%)	0.09	0.06	0.10	0.00	0.02	0.05	0.12	0.00	0.07	0.07	0.18	0.02
			Disintegration time (min)	*ND	*ND	*ND	*ND	*ND	*ND	*ND	*ND	*ND	*ND	*ND	*ND
	R0.1F3 coated beads		Assay (mg/100mg)	10.07	14.51	9.13	10.69	10.07	12.20	9.67	11.15	12.85	13.24	9.53	10.33
			Mass variation (mg)	97.35 (3.19)	98.79 (2.24)	99.64 (2.14)	100.12 (1.32)	100.21 (1.89)	99.1 (1.94)	100.21 (2.14)	99.43 (1.91)	99.69 (1.46)	100.81 (2.17)	99.57 (2.16)	100.71 (1.48)
			Tensile strength ($N.mm^{-2}$)	3.53 (12.43)	3.08 (19.09)	3.07 (11.65)	3.29 (23.27)	2.10 (20.32)	1.75 (32.08)	2.04 (9.33)	1.93 (10.98)	2.21 (14.67)	1.88 (26.68)	1.93 (17.01)	2.30 (13.90)
			Friability (%)	0.01	0.04	0.13	0.00	0.01	0.03	9.65	0.00	0.09	0.02	0.05	0.00
			Disintegration time (min)	*ND	*ND	*ND	*ND	*ND	*ND	*ND	*ND	*ND	*ND	*ND	*ND

**ND= Considered non-disintegrating; **RH= Relative humidity*

Table D.4: Physical properties for Avicel® PH 101/pyridoxine mini-MUPS after exposure to 6 months accelerated stability testing *(%RSD is indicated in parentheses)*

				Chamber 1: 25°C / 60% RH**				Chamber 2: 30°C / 70% RH**				Chamber 3: 40°C / 75% RH**			
				Time (months)											
				1	2	3	6	1	2	3	6	1	2	3	6
Mini-MUPS containing pyridoxine as active ingredient	A0P0 uncoated beads	Physical properties	Assay (mg/100mg)	10.87	8.36	6.29	5.70	11.44	10.16	6.36	5.34	11.26	7.83	6.48	5.53
			Mass variation (mg)	103.96 (2.95)	101.28 (3.58)	103.67 (3.22)	103.96 (2.31)	103.83 (4.26)	103.59 (3.16)	103.56 (2.81)	103.56 (2.81)	102.54 (3.46)	102.15 (2.99)	101.47 (2.63)	103.11 (2.56)
			Tensile strength ($N.mm^{-2}$)	1.41 (18.92)	1.22 (14.49)	1.29 (17.65)	1.23 (6.78)	1.08 (18.32)	0.78 (17.22)	0.95 (18.12)	0.89 (26.39)	0.00 (100.00)	0.77 (20.97)	0.54 (19.06)	0.89 (17.93)
			Friability (%)	11.08	11.58	11.08	24.84	10.92	19.02	12.80	12.52	29.67	38.69	49.50	31.15
			Disintegration time (min)	2.40 (0.00)	3.88 (7.39)	3.54 (20.84)	5.47 (16.97)	2.45 (0.00)	2.74 (18.68)	3.54 (11.53)	4.87 (10.36)	2.50 (0.00)	1.73 (32.40)	1.90 (27.54)	2.08 (40.01)
	A0P0 coated beads		Assay (mg/100mg)	9.75	9.86	5.77	5.91	10.91	10.32	6.45	6.46	10.26	8.48	6.12	5.54
			Mass variation (mg)	100.64 (6.77)	89.81 (23.46)	99.49 (8.93)	99.86 (7.70)	99.12 (6.19)	89.89 (25.04)	97.34 (10.72)	101.59 (8.02)	97.42 (10.63)	95.74 (13.74)	103.33 (5.28)	103.92 (3.69)
			Tensile strength ($N.mm^{-2}$)	***NV**	***NV**	***NV**	***NV**	***NV**	***NV**	***NV**	***NV**	***NV**	***NV**	***NV**	***NV**
			Friability (%)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
			Disintegration time (min)	2.54 (36.39)	1.68 (51.09)	2.40 (60.78)	2.22 (54.84)	2.47 (34.55)	2.44 (42.17)	1.65 (28.57)	1.18 (76.39)	3.27 (0.00)	1.74 (62.33)	1.06 (24.70)	2.43 (30.56)

**NV= No value; **RH= Relative humidity*

Table D.5: Physical properties for MicroceLac® 100/pyridoxine mini-MUPS after exposure to 6 months accelerated stability testing *(%RSD is indicated in parentheses)*

				Chamber 1: 25°C / 60% RH**				Chamber 2: 30°C / 70% RH**				Chamber 3: 40°C / 75% RH**			
				Time (months)											
				1	2	3	6	1	2	3	6	1	2	3	6
Mini-MUPS containing pyridoxine as active ingredient	M0.05P0 uncoated beads	Physical properties	Assay (mg/100mg)	9.92	8.69	7.78	5.48	10.58	10.38	7.91	5.44	10.56	15.32	7.68	5.49
			Mass variation (mg)	105.74 (4.25)	106.08 (3.81)	105.45 (4.29)	104.81 (2.94)	105.56 (3.48)	105.03 (2.58)	105.77 (4.26)	99.41 (22.68)	106.05 (3.41)	104.45 (2.88)	105.39 (3.47)	103.23 (3.04)
			Tensile strength ($N.mm^{-2}$)	2.59 (14.16)	2.29 (13.53)	2.55 (10.59)	2.62 (11.95)	2.30 (10.11)	2.09 (13.61)	2.56 (12.15)	2.63 (10.06)	1.96 (39.98)	1.98 (22.83)	1.92 (31.62)	1.91 (53.49)
			Friability (%)	0.30	0.33	0.28	0.04	0.38	0.16	0.46	0.02	0.32	0.16	0.37	0.00
			Disintegration time (min)	13.50 (17.21)	***ND**	14.26 (8.75)	***ND**	14.18 (14.16)	***ND**	***ND**	***ND**	14.20 (13.86)	***ND**	***ND**	***ND**
	M0.05P0 coated beads		Assay (mg/100mg)	10.43	8.67	6.89	5.66	9.49	10.82	6.58	5.67	9.49	10.82	6.58	5.67
			Mass variation (mg)	100.20 (4.08)	100.16 (2.73)	98.83 (3.97)	99.06 (3.14)	101.81 (2.98)	100.15 (2.33)	100.06 (2.72)	100.31 (3.93)	100.6 (3.24)	99.86 (4.22)	99.72 (4.54)	101.41 (3.50)
			Tensile strength ($N.mm^{-2}$)	1.05 (10.02)	0.79 (38.13)	1.04 (25.05)	1.16 (29.27)	1.05 (19.75)	0.78 (25.70)	1.04 (29.37)	1.11 (24.84)	1.81 (26.17)	1.14 (49.78)	3.81 (40.57)	9.32 (5.68)
			Friability (%)	0.18	0.28	0.15	0.11	2.53	0.06	0.09	0.1	0.14	0.19	0.29	0.12
			Disintegration time (min)	14.18 (9.00)	10.65 (20.07)	10.56 (23.02)	9.66 (18.70)	***ND**	9.37 (9.87)	12.00 (28.54)	11.25 (19.21)	14.53 (8.01)	10.01 (13.41)	14.37 (6.79)	***ND**

**ND= Considered non-disintegrating; **RH= Relative humidity*

Table D.6: Physical properties for RetaLac®/pyridoxine mini-MUPS after exposure to 6 months accelerated stability testing *(%RSD is indicated in parentheses)*

				Chamber 1: 25°C / 60% RH**				Chamber 2: 30°C / 70% RH**				Chamber 3: 40°C / 75% RH**			
				Time (months)											
				1	2	3	6	1	2	3	6	1	2	3	6
Mini-MUPS containing pyridoxine as active ingredient	R0.1P3 uncoated beads	Physical properties	Assay (mg/100mg)	11.75	13.00	6.65	4.34	10.10	12.99	7.05	4.41	9.87	12.03	6.90	3.96
			Mass variation (mg)	92.87 (5.56)	96.21 (3.18)	97.39 (3.00)	93.81 (4.49)	94.49 (4.38)	95.97 (4.39)	96.16 (3.51)	95.41 (4.62)	96.78 (2.67)	95.37 (4.23)	94.87 (4.16)	96.19 (3.95)
			Tensile strength ($N.mm^{-2}$)	1.40 (20.28)	0.94 (40.37)	1.33 (18.6)	1.11 (24.19)	1.24 (28.85)	1.08 (35.56)	1.21 (34.09)	1.39 (27.33)	1.15 (31.27)	1.09 (40.75)	1.98 (26.23)	1.40 (23.94)
			Friability (%)	6.21	0.55	0.35	0.68	14.05	1.92	3.03	4.33	4.54	4.38	2.83	0.16
			Disintegration time (min)	*ND	*ND	*ND	*ND	*ND	*ND	*ND	*ND	*ND	*ND	*ND	*ND
	R0.1P3 coated beads		Assay (mg/100mg)	10.27	10.63	6.07	5.30	10.52	11.25	11.25	5.14	9.99	11.45	6.66	5.01
			Mass variation (mg)	99.66 (2.20)	99.65 (2.10)	99.86 (1.97)	100.35 (2.35)	100.22 (2.15)	99.13 (1.01)	99.05 (2.04)	99.42 (1.68)	99.5 (2.07)	99.22 (1.84)	100.34 (1.90)	100.85 (2.71)
			Tensile strength ($N.mm^{-2}$)	2.75 (11.95)	2.12 (24.85)	2.67 (17.26)	2.53 (12.51)	2.85 (8.98)	2.32 (18.29)	2.48 (16.35)	2.33 (10.35)	1.66 (37.54)	1.44 (41.28)	2.31 (26.06)	3.10 (12.12)
			Friability (%)	0.00	0.00	0.08	0.75	0.01	0.04	0.05	0.01	0.01	0.02	0.02	0.00
			Disintegration time (min)	*ND	*ND	*ND	*ND	*ND	*ND	*ND	*ND	*ND	*ND	*ND	*ND

**ND= Considered non-disintegrating; **RH= Relative humidity*

Table D.7: Compliance of furosemide formulations to mass variation over 6 months of accelerated stability testing in different condition chambers

				Chamber 1: 25°C / 60% RH**	Chamber 2: 30°C / 70% RH**	Chamber 3: 40°C / 75% RH**
Uncoated beads	A0F3	Time (months)	1	*Fail	Fail	Fail
			2	Fail	Fail	Fail
			3	Fail	*Fail	Fail
			6	*Fail	*Fail	*Fail
Coated beads	A0F3		1	Pass	Fail	Pass
			2	Pass	*Fail	Pass
			3	*Fail	Pass	Pass
			6	Fail	Fail	Pass
Uncoated beads	M0.05F3		1	Fail	Pass	Fail
			2	Fail	Fail	Pass
			3	Fail	Fail	Fail
			6	Fail	Pass	Fail
Coated beads	M0.05F3		1	Pass	Pass	Pass
			2	Pass	Pass	Pass
			3	Pass	Pass	*Fail
			6	Pass	Pass	Pass
Uncoated beads	R0.1F3		1	Pass	Pass	Pass
			2	Pass	Pass	Pass
			3	Pass	Pass	Pass
			6	Pass	Pass	Pass
Coated beads	R0.1F3		1	Pass	Pass	Pass
			2	Pass	Pass	Pass
			3	Pass	Pass	Pass
			6	Pass	Pass	Pass

**Fail = Deviated by more than 15% of the average mass; **RH= Relative humidity*

Table D.8: Compliance of pyridoxine formulations to mass variation over 6 months of accelerated stability testing in different condition chambers

				Chamber 1: 25°C / 60% RH**	Chamber 2: 30°C / 70% RH**	Chamber 3: 40°C / 75% RH**
Uncoated beads	A0P0	Time (months)	1	Pass	Pass	Pass
			2	Pass	Pass	Pass
			3	Pass	Pass	Pass
			6	Pass	Pass	Pass
Coated beads	A0P0		1	*Fail	Fail	*Fail
			2	*Fail	*Fail	*Fail
			3	*Fail	*Fail	Fail
			6	*Fail	*Fail	Pass
Uncoated beads	M0.05P0		1	Pass	Pass	Pass
			2	Pass	Pass	Pass
			3	Pass	Pass	Pass
			6	Pass	*Fail	Pass
Coated beads	M0.05P0		1	Pass	Pass	Pass
			2	Pass	Pass	Pass
			3	Pass	Pass	Pass
			6	Pass	Pass	Pass
Uncoated beads	R0.1P3		1	Fail	Pass	Pass
			2	Pass	Pass	Pass
			3	Pass	Pass	Pass
			6	Pass	Pass	Pass
Coated beads	R0.1P3		1	Pass	Pass	Pass
			2	Pass	Pass	Pass
			3	Pass	Pass	Pass
			6	Pass	Pass	Pass

**Fail = Deviated by more than 15% of the average mass; **RH= Relative humidity*