# FORMULATION AND EVALUATION OF A GASTRORETENTIVE DRUG DELIVERY SYSTEM OF RANITIDINE HYDROCHLORIDE

by

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**2019**

# DEDICATION

I would like to dedicate this work to my beloved parents,

Lenny and Patrick.

# DECLARATION

I, **Princess Nkuna**, hereby declare that the work on which this study is based is original, except where acknowledgements indicate otherwise.

This dissertation is submitted for the degree Masters of Pharmacy (Pharmaceutics) at the University of Limpopo. Neither the whole work nor any part of it has been submitted before for any degree or examination at this or any other university.

Signed……..……on the……day of…….

# ACKNOWLEDGEMENTS

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# ABSTRACT

Various approaches have been developed to retain dosage forms in the gastrointestinal tract. One of the commonly used approaches is the use of microspheres. Due to their intrinsic low density and small size, they are distributed throughout the gastrointestinal tract which improves drug absorption thus improving bioavailability. Ranitidine hydrochloride, an antiulcer drug is poorly absorbed from the lower gastrointestinal tract and has a short half-life of 2.5-3 hours. The aim of this study was to formulate and evaluate gastroretentive microspheres of ranitidine hydrochloride in order to extend gastric retention in the upper gastrointestinal tract, which may result in enhanced absorption and thus improved bioavailability.

Pre-formulation studies were conducted to develop and validate the analytical method to identify and quantify ranitidine hydrochloride; to select the suitable polymers for further formulation development and; to determine the compatibility of the chosen polymers with ranitidine hydrochloride. The analytical method was validated and found to be sensitive, linear, precise and accurate. Preliminary formulations lead to the selection of ethyl cellulose and PEG 4000 as polymers and solvent evaporation as the method of manufacture. Compatibility studies were determined by DSC/TGA, FTIR and short-term accelerated studies and no incompatibilities were observed.

Two prototype formulations of the preliminary formulations F24 and F26 were manufactured comprised of varying drug: polymer concentration. The microspheres were evaluated for morphology, particle size, flow properties, percentage yield, buoyancy and *in vitro* drug release.

Both formulations resulted in spherical microspheres with good flow properties, high yield and buoyancy studies revealed that the microspheres would float immediately upon contact with the dissolution media and floating would continue for more than 8 hours. *In vitro* drug release studies revealed that polymer concentration greatly affected drug release. Dissolution kinetic studies revealed that formulation F24 and

F26 were best described by the Korsmeyer-Peppas and Higuchi kinetic models respectively. Formulation F26 was considered the best formulation, which comprised of a drug: PEG 4000 ratio of 1:2 w/w, as it yielded better in better drug encapsulation, better buoyancy results and had complete drug release.

Key words: Ranitidine hydrochloride, microspheres, ethyl cellulose, PEG 4000

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# ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| **cAMP** | Cyclic adenosine monophosphate |
| **DSC** | Differential scanning calorimetry |
| **ECL** | Enterochromaffin-like cell |
| **Ethocel** | Ethyl cellulose |
| **FDDS** | Floating drug delivery system |
| **FTIR** | Fourier-transformed infrared spectroscopy |
| **GRDDS** | Gastroretentive drug delivery system |
| **GI** | Gastrointestinal |
| **GIT** | Gastrointestinal tract |
| **GRT** | Gastric retention time |
| **$H_2$** | Histamine 2 |
| **HBS** | Hydro-dynamically balanced system |
| **HCl** | Hydrochloric acid |
| **HPLC** | High-performance liquid chromatography |
| **HPMC** | Hydroxypropylmethylcellulose |
| **ICH** | International council of harmonisation |
| **IR** | Infrared |
| **LOD** | Limit of detection |
| **LOQ** | Limit of quantification |
| **MMC** | Migrating myoelectric cycle |
| **MPD** | Median particle diameter |
| **PEG** | Polyethylene glycol |

| | |
|---|---|
| **$R^2$** | Regression coefficient |
| **RSD** | Relative standard deviation |
| **SEM** | Scanning electron microscopy |
| **TGA** | Thermogravimetric analysis |
| **TLC** | Thin layer chromatography |
| **USP** | United states pharmacopoeia |
| **UV-vis** | Ultraviolet-visible |
| **WHO** | World health organisation |

# CHAPTER 1
# INTRODUCTION

## 1.1 INTRODUCTION

This chapter provides an overview of the research project. It also includes the background of the study, the problem statement and significance of the study. The aim and objectives of the study are also defined.

## 1.2 BACKGROUND AND JUSTIFICATION

The oral route of administration is the most preferred route of drug administration to the systemic circulation despite numerous recent advancements in drug delivery. This is due to the ease of administration, low cost of therapy, patient compliance and flexibility in formulation (Chhetri & Thapa, 2014). The major drawback of this method is that not all drug candidates are absorbed uniformly throughout the gastrointestinal tract (Nayak, Malakar & Sen, 2010).

Over the past few decades, several oral drug delivery systems have been developed to act as drug reservoirs from which the active substance is released over a specific period at a predetermined and controlled rate (Gopalakrishnan & Chenthilnathan, 2011). These systems are advantageous as they minimise fluctuations of plasma drug concentration, enable complete absorption of the drug even in cases of vigorous intestinal movement and improves the bioavailability of drugs that are absorbed from a specific region of the gastrointestinal tract (GIT) (Narang, 2011).

The gastroretentive drug delivery systems (GRDDS) are retained in the stomach thereby assisting in improving the oral sustained delivery of drugs that have an absorption window in a particular region of the GIT. These systems help in continuously releasing the drug before it reaches the absorption window, thus ensuring

optimal bioavailability (Ingale, Thakare, Tekade & Patil, 2014). Various approaches to prolong gastric time have been designed and developed including the floating drug delivery system (FDDS), bio- or mucoadhesive system, expandable, un-foldable system and high-density systems (Makwana, Sameja, Parekh & Pandya, 2012).

FDDS also referred to as the hydro-dynamically balanced system (HBS) are systems that have a bulk density less than that of gastric fluids and so remain buoyant in the stomach without being affected by gastric emptying rate for a prolonged period (Sharma, Agrawal, Guptam & Khinchi, 2011). While the system is floating on the gastric contents, the drug will be released slowly at the desired rate from the system. After the release of the drug, the residual system is emptied from the stomach. This results in prolonged gastric retention time (GRT) and better control of the fluctuations in plasma drug concentration (Gopalakrishnan & Chenthilnathan, 2011).

Hollow microspheres also known as microballoons are spherical empty particles without a core (Singh & Chaudhary, 2011). Microspheres are currently one of the most promising buoyant systems as they combine the advantages of a multi-particulate system and good floating properties.

Microspheres are prepared by solvent evaporation method (Chaturvedi, Sharma & Sharadvisht, 2012). The polymers commonly used to develop these systems include polycarbonate, cellulose acetate, calcium alginate, Eudragit S and agar. Buoyancy and the rate of drug release from the dosage form are dependent on the number of polymers, the plasticizer polymer ratio and the solvent used for formulation (Jain, Awasthi, Jain & Agrawal, 2005). The GRT of the microspheres can be increased for more than 12 hours in dissolution media containing a surfactant (Chaturvedi *et al.*, 2012) which is also due to the incorporation of the porous structural element (Jain *et al.*, 2005).

Ranitidine hydrochloride is a selective and competitive histamine $H_2$-receptor antagonist (Sajal, Karki, Puttegowda & Ghosh, 2014). It is commonly used to treat gastric ulcers, gastroesophageal reflux disease (a condition in which a backward flow of stomach acid causes heartburn and injury of the oesophagus), and other medical conditions in which there is too much stomach acid production, such as Zollinger-Ellison syndrome (Dhankar, Garg, Dhamija & Awasthi, 2014).

Ranitidine hydrochloride is primarily absorbed in the initial part of the small intestine (Abdou, 2015), and it has an oral bioavailability of 50% (Janardhan, Lingam, Mohan & Venkateswarlu, 2008). It has a half-life of approximately 2.5 to 3 hours (Jaiswal, Bhattachyra, Yadav, Singh, Chandra & Jain, 2009). The short half-life of the drug and its instability in the in the colonic environment (due to colonic metabolism) favours the development of a gastroretentive delivery system for ranitidine hydrochloride (Narang, 2011).

## 1.3 PROBLEM STATEMENT

Oral administration is the most convenient way of all the drug delivery routes (Zhao, Maniglio, Chen, Chen & Migliaresi, 2016), but it has many disadvantages such as gastric acid/enzyme degradation, first pass metabolism and short half-lives due to rapid drug elimination from the systemic circulation (Chaturvedi *et al*., 2012). In the formulation of a site-specific orally administered controlled release dosage form, it is desirable to achieve a prolonged gastric residence time for drug delivery. Prolonged gastric retention improves bioavailability, increases the duration of drug release, reduces drug waste, and improves the drug solubility for drugs that are less soluble in a high pH environment (Sarojini & Manavalan, 2012).

Ranitidine hydrochloride, a histamine $H_2$-receptor antagonist is an antiulcer drug that is used in treating peptic ulceration, erosive esophagitis and Zollinger Ellison syndrome (Singh & Chaudhary, 2011; Sajal *et al*., 2014). It is poorly absorbed from

the lower gastrointestinal tract resulting in low oral bioavailability of 50% (Jaiswal *et al*., 2009), it is also unstable in the colon (Narang, 2011) and it has a short half-life of 2.5-3 hours (Singh & Chaudhary, 2011). The main objective of the study was to develop gastroretentive encapsulated microspheres of ranitidine hydrochloride in order to achieve extended retention in the upper gastrointestinal tract, which would result in enhanced absorption and thereby improved bioavailability.

## 1.4 AIM OF THE STUDY

The aim of this study was to formulate and evaluate gastroretentive microspheres system of ranitidine hydrochloride.

## 1.5 OBJECTIVES OF THE STUDY

To achieve the aim of this study, the objectives were set as follows:

- To investigate the compatibility of ranitidine hydrochloride with the selected excipients.
- To develop gastroretentive encapsulated microspheres of ranitidine hydrochloride.
- To characterise the physical properties and quality of the microspheres.
- To determine the buoyancy of the encapsulated microspheres.
- To evaluate the *in vitro* dissolution properties of ranitidine hydrochloride from the encapsulated microspheres.

## 1.6 SIGNIFICANCE OF THE STUDY

The success of this project in the formulation of microspheres will advance current knowledge about gastroretentive formulations. It will also decrease morbidity and mortality in South Africa resulting from peptic ulcers through the availability of a dosage form that will be retained in the upper gastrointestinal tract which may enhance absorption and thereby improve bioavailability.

# CHAPTER 2
# LITERATURE REVIEW

## 2.1 INTRODUCTION

In this chapter, literature is reviewed and the collected knowledge is divided into manageable and sequential sections. Ranitidine hydrochloride has been extensively studied; physicochemical properties, pharmacology, commercial availability and suitable analytical methods have been described. Various approaches to GRDDS and factors affecting these systems are fully discussed. Microspheres, being the focus of this study are discussed with emphasis on the various methods of manufacture and the materials used therein.

## 2.2 RANITIDINE HYDROCHLORIDE

Ranitidine hydrochloride is a white to pale yellow crystalline powder with a bitter taste and a sulphur-like odour. It has a chemical formula of $C_{13}H_{22}N_4O_3S$-HCl, a molecular weight of 350.87 g/mol, and the chemical name is (*N*-(2[({5-[(dimethylamino) methyl]-2-furanyl}methyl)thio]ethyl}-*N′*-methyl-2-nitro-1,1-ethenediaminehydrochloride) PubChem, 2006; Hossain, 2016). The chemical structure of ranitidine hydrochloride is depicted in Figure 2.1.

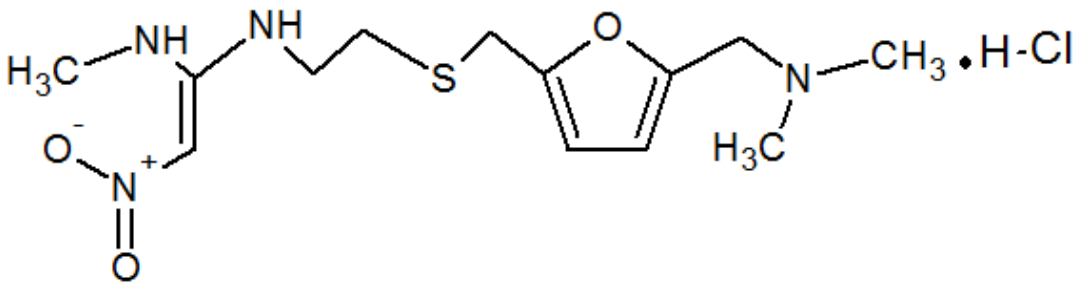



Figure 2.1: Molecular structure of ranitidine hydrochloride (Chakraborty, Mubeen, Lalitha & Kimbahune, 2015).

### 2.2.1 Chemical synthesis

The chemical synthesis of ranitidine is depicted in Figure 2.2 below.

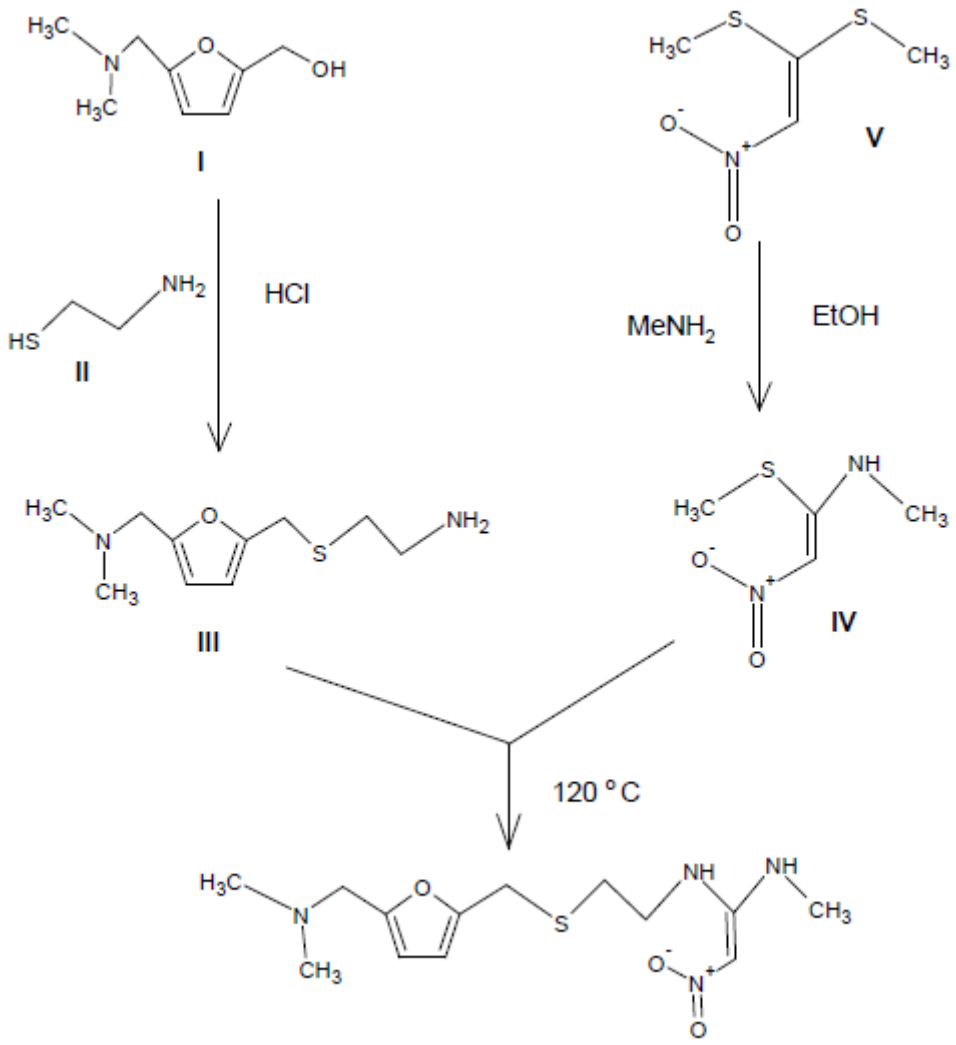



Figure 2.2: Schematic representation of ranitidine synthesis (Hossain, 2016).

The reaction of 5-dimethylaminomethyl-2-furanylmethanol (I) with 2-mercaptoethylamine (II) by means of aqueous hydrochloride gives 2-[(5-dimethylamino-methyl-2-furanyl) methylthio] ethaneamine (III), which is then condensed with N-methyl-1-methylthio-2-nitrotheneamine (IV) by heating at 120˚C. Compound (IV) is obtained by reaction of 1,1-bis(methylthio)-2-nitroethene (V) with methylamine in refluxing ethanol (Hossain, 2016).

### 2.2.2 Physicochemical properties

Ranitidine hydrochloride is very soluble in water with a solubility of 24.7 mg/mL. It is also soluble in different solvents that are listed in Table 2.1 and their solubility is described (King'ori, 2011).

Table 2.1: Solubility of ranitidine in various solvents

| **Solvent** | **Solubility** |
|---|---|
| Acetic acid | Freely soluble |
| Water | Very soluble |
| Methanol | Soluble |
| Ethanol | Sparingly soluble |
| Ethyl acetate | Very slightly soluble |
| Isopropanol | Very slightly soluble |
| Dioxane | Insoluble |
| Chloroform | Insoluble |

The melting point of ranitidine hydrochloride depends on the polymorphic form from which the compound was crystallized. Ranitidine hydrochloride crystals derived from polymorph Form 1 have a melting range of 135 – 136˚C and the crystals derived from polymorph Form 2 have a melting range of 143 – 144˚C. Ranitidine has a partition coefficient (water/ *n*- octanol) of 0.2 indicating the hydrophilic nature of the drug. The dissociation constant determined by potentiometric pH titration was 8.65 at 25˚C indicating the basic nature of the drug. The basic nature of ranitidine hydrochloride means it is more soluble in acidic solutions where the ionised form of the drug is predominant (King'ori, 2011; Neetu, Jalwal & Neha, 2013).

### 2.2.3 Clinical Pharmacology

Clinical pharmacology is defined as the study of drugs in humans. It is concerned with various fundamental areas that include the relationship between dose and biological

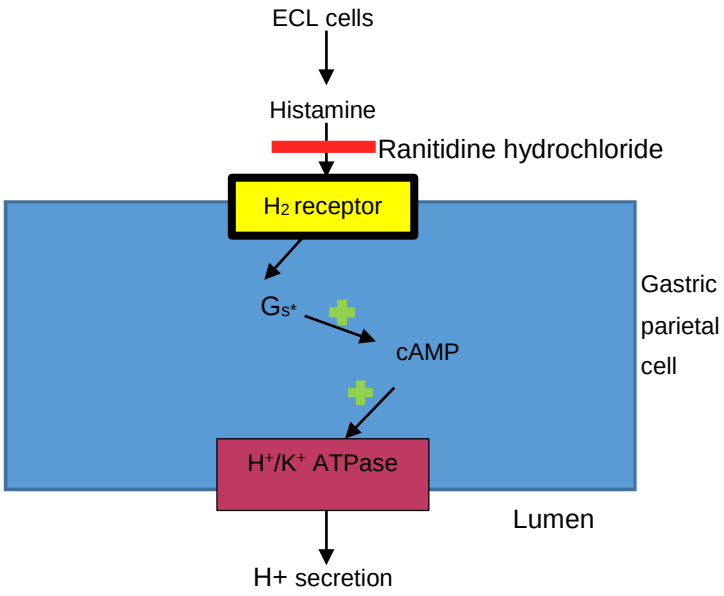
effect; the localization of the site of action of a drug; the mechanism of action of a drug and drug interactions (Atkinson, Huang, Lertora & Markey, 2012).

#### **2.2.3.1** Classification

Ranitidine hydrochloride which is a salt form of ranitidine is classified as an $H_2$ (histamine-2) - receptor antagonist which reduces gastric acid secretion by blocking the action of histamine at the $H_2$- receptors in the parietal cells of the stomach. Other drugs in this classification include cimetidine and famotidine (Rossiter, 2016).

#### **2.2.3.2** Mechanism of action

Histamine is released by the enterochromaffin-like cell (ECL) found in the gastric mucosa that aids in the production of gastric acid. Histamine binds to $H_2$-receptors located on the parietal cells resulting in an increase of intracellular cyclic adenosine monophosphate (cAMP), which in return activates the $H^+$ / $K^+$ ATPase. Activation of the ATPase shifts hydrogen ions ($H^+$) into the lumen of the stomach in exchange of the potassium ions ($K^+$) thus increasing stomach acid production. $H_2$-receptor blockers are competitive antagonists of histamine at the parietal cell $H_2$ receptor inhibiting the stimulation of the parietal cell, reducing cAMP levels and ultimately reducing the production of stomach acid as depicted in Figure 2.3 (Hossain, 2016; van Wering and Benninga, 2017).




*Gs = G protein subunit

Figure 2.3: Mechanism of action of ranitidine hydrochloride (Washabau, 2013).

### 2.2.3.3 Indications

According to McQuaid (2011), ranitidine hydrochloride is indicated as treatment in the following conditions: heartburn, hyperacidity, peptic ulcer disease, reflux oesophagitis and Zollinger-Ellison syndrome.

### 2.2.3.4 Dosage

The dose of ranitidine hydrochloride is given as follows (Rossiter, 2016):

- For the prevention of heartburn and hyperacidity: 75mg orally, 30 to 60 minutes before a meal that is suspected to cause symptoms with a maximum dose of 300mg/day.
- For the relief of heartburn and hyperacidity: 75mg once or twice a day as required with a maximum dose of 300mg/24 hours for a period of no longer than 14 days.
- For the management of peptic ulcer disease and reflux oesophagitis: 150mg taken orally twice a day or 300mg taken orally at bedtime for a period of 4 to 8

weeks; treatment up to 12 weeks may be necessary for reflux. If injected intravenously, a maintenance dose of 150mg at bedtime in which 50mg is administered every 6 to 8 hours by slow injection over not less than 2 minutes or administered by infusion over 2 hours, suitably diluted with 0.9% sodium chloride or 5% dextrose.

- For the management of Zollinger-Ellison syndrome: Start with 150mg, twice to thrice a day which is increased as necessary to 600-900mg/day.
- The paediatric dose is 2 to 4mg/kg taken orally, twice a day with a maximum dose of 300mg/day.

#### **2.2.3.5** Side effects

On administration of ranitidine hydrochloride, patients may rarely experience the following: cardiovascular system related side effects such as bradycardia, atrioventricular block and cardiac arrest. Central nervous system-related side effects such as confusion, loss of colour vision, aggressiveness, hallucinations, tiredness, dizziness and a severe headache. Gastrointestinal related side effects namely diarrhoea and hepatotoxicity. Ocular related side effects namely increased intraocular pressure. Dermatological side effects such as rash and hypersensitivity. Lastly general side effects such as fever, myalgia, agranulocytosis, neutropenia, thrombocytopenia, and leukopenia, possible transient and reversible changes in liver function tests (McQuaid, 2011).

#### **2.2.3.6** Contraindications

Ranitidine hydrochloride is contraindicated in patients with known hypersensitivity to the drug or any excipient that may be used in the formulation of a dosage form (Rossiter, 2016).

#### 2.2.3.7 Drug interactions

Ranitidine hydrochloride has the potential to alter the pharmacokinetic profile i.e. the absorption, distribution, metabolism and elimination of other drugs through the following mechanisms (McQuaid, 2011; Rossiter, 2016):

- *Inhibition of cytochrome P450-linked mixed function oxygenase system*

When administered at therapeutic doses, ranitidine hydrochloride does not potentiate the action of drugs such as diazepam, lidocaine, phenytoin, propranolol and theophylline which are inactivated by this enzyme system, however, there have been reports of altered prothrombin time associated with the concurrent administration of ranitidine hydrochloride and warfarin.

- *Competition for renal tubular secretion*

Ranitidine hydrochloride which is partially eliminated via the cationic system may affect the clearance of other drugs eliminated by this route. High doses of ranitidine, such as those administered for the treatment of Zollinger-Ellison syndrome, may reduce the elimination of procainamide and *N*-acetylprocainamide causing an increase in the plasma levels of these drugs.

- *Alteration of gastric pH*

The alteration of gastric pH may increase or decrease the absorption of drugs which ultimately affects the bioavailability of the drugs. pH alteration increases the absorption of triazolam, midazolam, and glipizide, and it causes a decrease in absorption for ketoconazole, atazanavir and gefitnib.

### 2.2.4 Clinical pharmacokinetics

Clinical pharmacokinetics is defined as the study of "*what the body does to the drug*". It includes the rate and extent at which drugs are absorbed into the body and distributed to the body tissues, the rate and pathways by which drugs are metabolised, excreted from the body and toxicology (Bauer, 2011).

#### 2.2.4.1 Absorption

Ranitidine hydrochloride is rapidly absorbed following oral administration, peak plasma concentration levels of 300 to 550 ng/mL occurred one to three hours after the administration of a 150mg tablet. Oral ranitidine hydrochloride has a bioavailability of 50%. During the absorption phase, two distinct peaks or a plateau are a result of reabsorption of the drug excreted into the intestine. The absorption phase is not significantly influenced by the presence of food in the stomach at the time of oral administration nor is it influenced by regular intake of antacids (Brahma, Gunda, Kumar, Satyanarayana & Naga, 2015).

#### 2.2.4.2 Distribution

Ranitidine has a volume of distribution of 1.4L/kg and serum protein binding is approximately 15%. It crosses the placenta when used in pregnancy and is also distributed into breast milk (Kortejarvi, Yliperttula, Dressman, Junginger, Midha, Shah & Barends, 2005).

#### 2.2.4.3 Metabolism

Ranitidine hydrochloride is metabolized by the liver which produces three different metabolites namely: *N*-oxide metabolites which accounts for 4% of the administered dose, S-oxide metabolites which account for 1% of the administered dose and lastly, *N*-desmethyl metabolites which also accounts for 1% of the administered dose. The administered dose unaccounted for is found in stool (King'ori, 2011).

#### 2.2.4.4 Elimination

The main route of elimination for ranitidine is through urinary excretion in which 30% of the orally administered dose is collected as an unchanged drug in 24 hours. The drug has an elimination half-life of 2.5 to 3 hours with renal clearance of approximately 410mL/min which indicates active tubular excretion (Rossiter, 2016).

### 2.2.5 Toxicity

Toxicity is described as the lethal dose of a substance that kills 50% of the test population which is abbreviated as $LD_{50}$. The $LD_{50}$ of ranitidine hydrochloride is 77mg/kg, which was observed following oral administration in mice. Symptoms of toxicity include muscular tremors, vomiting, and rapid respiration (Akter, 2016).

### 2.2.6 Storage and handling

Precaution should be taken with the handling and storage of ranitidine hydrochloride. During handling, prolonged or repeated exposure should be avoided as well as inhalation of dust particles. Ranitidine hydrochloride should be stored in a dry place with a temperature range of 4 ˚C to 25 ˚C with permitted excursions of 30 ˚C. It should be stored in a tightly closed container, protected from light (Mangesh, Girish, Maria, Zhaheed & Aney, 2009; Caymen Chemical, 2015).

### 2.2.7 Commercial availability

Table 2.2 below indicates the brand names of ranitidine hydrochloride products that are available in South Africa. The manufacturer of each brand is also listed as well as the dosage forms and strength (Rossiter, 2016).

Table 2.2: Commercial products of ranitidine in South Africa

| Brand name | Manufacturer | Dosage form | Strength |
|---|---|---|---|
| Zantac® | GlaxoSmithKline | Tablets, effervescent tablets, syrup, injection | 75mg, 150mg 300mg, 50mg/2mL, 150mg/10mL |
| Histak® | Ranbaxy Be-Tabs | Tablets | 75mg, 150mg, 300mg |
| Gulf Ranitidine® and RaniHexal® | Sandoz | Tablets | 150mg, 300mg |
| Ultak® | Cipla Medpro | Tablets | 150mg, 300mg |
| CPL Alliance Ranitidine® | Akacia | Tablets | 300mg |
| Ranitidine Biotech® | Biotech | Tablets | 300mg |
| R-Loc® | Zydus | Injection | 50mg/2mL |

## 2.3 ANALYTICAL METHODS

According to Kelker, Tolg, Gunzler & Williams (2001), analytical chemistry or analysis is any examination of a chemical substance with the goal of gathering information about its constituents namely: their character (form, pattern or chemical bonding), quantity and quality (concentration and content), distribution (homogeneity) and structure (arrangement of atoms or molecules). Various analytical methods have been used for the identification and quantification of ranitidine hydrochloride in pharmaceutical dosage forms and biological fluids. Some important analytical procedures reported in scientific literature will be discussed in this section.

### 2.3.1 High Performance Liquid chromatography

McPolin (2009) defined chromatography as a technique used to identify and quantify drug substances by separating components of a mixture using the differences in time that each component will take to travel through a stationary phase when carried through a mobile phase. Liquid chromatography is distinguished into three types depending on the stationary phase into thin layer chromatography (TLC), paper chromatography and liquid column chromatography. Liquid column chromatography is further divided into two namely, open and closed systems. The closed system uses gravity to pass the mobile phase through the stationary phase and the closed system employs a pump to transport the mobile phase which is referred to as high-performance liquid chromatography (HPLC) (Lembke, Henze, Cabrera, Brunner & Muller, 2001).

Nanda, Potawale, Bhagwat, Deshmukh & Deshpande (2010) developed and validated a high-performance thin layer chromatography method for the simultaneous determination of ranitidine hydrochloride and dicyclomine hydrochloride in a combined tablet dosage form. The separation was carried out on Merck aluminium plates precoated with silica gel using methanol: water: acetic acid 8:2:0.1 (v/v/v). The separated spots were stained with iodine vapours and scanned at a wavelength of 410 nm. The retention factor was found to be 0.27 and 0.67 for ranitidine hydrochloride and dicyclomine hydrochloride respectively. The method was found to be linear over a range of 0.4 – 2.4 µg per spot for dicyclomine hydrochloride and 0.150 – 0.9 µg per spot for ranitidine hydrochloride.

Haque, Shahriar, Parvin & Isham (2011) developed and validated an HPLC method for the quantification of ranitidine hydrochloride, domperidone and naproxen in single and combined tablets. A $C_{18}$ column was used as the stationary phase and the mobile phase consisted of 0.1 M orthophosphoric acid solution and methanol (35:65 v/v). A constant flow rate of 1 mL/min was used and detection was carried at using an ultraviolet-visible (UV-vis) detector at a wavelength of 280 nm. Retention time was

found to be 2.70, 3.66 and 9.84 minutes for ranitidine hydrochloride, domperidone and naproxen respectively.

Nowakowska & Pikul (2012) used a TLC method to separate the thermal decomposition products of ranitidine hydrochloride. For the study, three standard samples were heated in a thermostat for 5 days in 60˚C, 8 days in 80˚C and 2 hours in 100˚C. Silica plates were used as the stationary phase and the mobile phase consisted of dimethyl sulfoxide, acetonitrile, methanol, ammonia, 2-propranol and 2-methoxyethanol in varying concentrations. The various mobile phases were useful in separating ranitidine and products of its thermal decomposition and thermodynamic dependence was shown.

Chakraborty *et al.,* (2015) developed an HPLC method for the quantitative analysis of the prescribed combinations of ranitidine hydrochloride, domperidone and magaldrate in tablet while using metronidazole as an internal standard. Chromatography was carried out on a $C_{18}$ column as the stationary phase and the mobile phase was methanol and potassium dihydrogen- o- phosphate at a current rate of 1 mL/min and measured at a wavelength of 227 nm. Retention time was found to be 2.68, 3.66 and 5.25 minutes for ranitidine hydrochloride, domperidone and magaldrate respectively.

### 2.3.2 Ultraviolet-visible spectrophotometry method

Spectrophotometry is a method that measures the amount of light a chemical substance absorbs by measuring the intensity of light as a light beam that passes through a sample solution. The principle of this method is based on the ability of every chemical compound to absorb, transmit or reflect light (electromagnetic radiation) over a certain range of wavelength. The measurement is carried out with the use of a spectrophotometer; with this, the concentration of a substance (with a known amount) can be determined by measuring the intensity of light detected (Filip, Macocian, Toderas & Caraban, 2012; Cioabla, 2013).

Salve, Gharge, Kirtawade, Dhabale & Burade (2010) developed a simple UV-vis spectrophotometric method for the estimation of ranitidine hydrochloride from tablet formulations. They found the absorption maxima of ranitidine hydrochloride in distilled water was 315 nm. The analysis obeyed Beer's law in a concentration range of 1-13 mcg/mL, which was validated by recovery studies. The recovery was more than 98% which concluded the method to be simple, accurate, precise, economical and robust.

Mukherjee, Bagchi & Raha (2015) developed a UV-vis spectrophotometric method using a simultaneous equation for the simultaneous determination of ranitidine hydrochloride and itopride in a binary mixture. Signals were measured at 315 and 258 nm corresponding to the absorbance maxima of ranitidine and itopride hydrochloric acid in double distilled water respectively. Linearity range was observed in a concentration range of 2 to 20 mcg/mL for both drugs. The method was validated statistically and accuracy was confirmed by a recovery study. The method was to be found rapid, simple, accurate and precise.

## 2.4 GASTRORETENTIVE DRUG DELIVERY SYSTEM

Gastroretentive drug delivery systems retain orally administered dosage forms in the upper GIT resulting in a prolonged contact time of the drug with the GI mucosa. This action leads to an increased bioavailability, which enhances therapeutic efficacy, reduced frequency of drug administration and thus improved patient compliance however, there are many physiological constraints which may limit the development of such delivery systems particularly gastric emptying time (Hossain, 2016; Tripathi, Ubaidulla & Khar, 2012). To understand this concept, the normal physiology is to be reviewed.

### 2.4.1 Anatomy and physiology of the stomach

The stomach is a hollow, muscular and dilated part of the GIT which has a structure resembling the letter "J" from the English alphabet. It is located between the

oesophagus and the duodenum in the upper left part of the abdominal cavity (Chaturvedi *et al.*, 2012). It is anatomically subdivided into four main regions, namely, the cardia, fundus, body and pylorus as indicated in Figure 2.4.

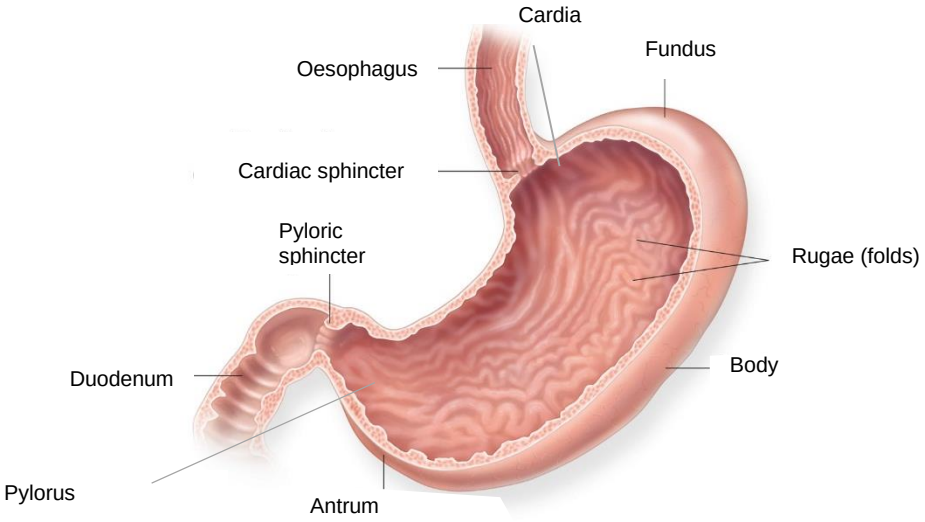



Figure 2.4: Structure of the stomach (Sarojini & Manavalan, 2012).

The main function of the stomach is to process and transport food. It serves as a short-term storage reservoir which enables human beings to consume a large meal rather quickly. Significant enzymatic digestion is initiated in the stomach, particularly of proteins. Vigorous contractions of gastric smooth muscle mix and grind food particles with gastric secretions causing the liquefaction of the food particles. As the liquefaction occurs, the product is slowly released into the small intestine for further processing (Narang, 2011). The proximal part made of fundus and body acts as a reservoir for undigested material, whereas the antrum is the main site for mixing motions and act as a pump for gastric emptying by propelling actions.

### 2.4.2 Gastric emptying process

Gastric emptying is the natural process in which the contents of the stomach are emptied which occurs during fasting as well as fed states; however, the pattern of motility is distinct in the two states. During the fasting state, an inter-digestive series

of electrical events take place, which cycles both through stomach and intestine every 2 to 3 hours. These events are called the inter-digestive myoelectric cycle or migrating myoelectric cycle (MMC), which is further divided into four phases as described by Sarojini & Manavalan (2012) and illustrated in Figure 2.5 below.

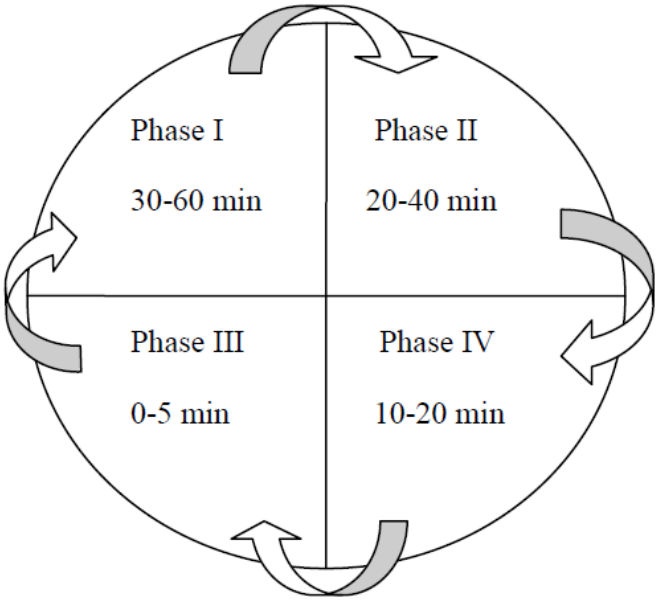



Figure 2.5: Motility pattern in the GIT (Narang, 2011).

**Phase I (Basal phase) -** Consists of rare contractions and it lasts for 30 to 60 minutes.

**Phase II (Pre-burst phase) -** Consists of intermittent action potential and contractions in which the intensity and frequency gradually increase as the phase progresses; this phase lasts for 20 to 40 minutes.

**Phase III (Burst phase) -** Consists of intense and regular contractions for a short period of 10 to 20 minutes. In this phase, all the undigested material is swept from the stomach into the small intestine, this has resulted in this phase to be also known as the “housekeeper wave”.

**Phase IV -** This phase occurs between phase III and Phase I of two consecutive cycles and it lasts for 0 to 5 minutes.

### 2.4.3 Factors affecting gastric emptying time

According to Saini, Garg, Pitliya & Pareek (2014), the following factors affect GRT and hence the gastric retention of drugs:

- *Fed or Unfed State*

During fasting conditions, the GI motility is characterized by a series of strong motor activity every 1.5 to 2 hours that sweep undigested material from the stomach; if the time of drug administration is concurrent with these activities then the dosage form can be expected to have a short GRT. However, during the fed state, these activities are delayed and GRT is considerably longer.

- *Density*

Dosage forms formulated to have increased GRT should have a density value that is less than the gastric content, therefore, a density of less than 1.0 gm/$cm^3$ is required to exhibit floating properties.

- *Size*

Dosage forms with a diameter greater than 7.5 mm are reported to have an increased GRT because the larger size would not allow the dosage form to quickly pass through the pyloric antrum into the intestine and it occurs with smaller sized dosage forms.

- *Concomitant administration of drugs*

Anticholinergic substances such as atropine, propantheline and opiates delay GRT while prokinetic substances such as metoclopramide and cisapride heighten the gastric emptying process.

- *Nature of the food*

Indigestible polymers of fatty acid salts can alter the motility pattern of the stomach in fed state hence GRT and prolonging drug release.

- *Calorie content of food*

Meals high in fat and protein content increase GRT to between four to ten hours.

- *Frequency of intake*

The intake of successive meals can increase GRT by over four hundred minutes as compared to a single meal due to the low frequency of migrating myoelectric complexes.

- *Gender*

Males generally have a faster gastric emptying rate than females.

- *Age*

Elderly people, particularly those over the age of 70 have longer GRT.

- *Others*

Psychological health of the patient affect GRT: stress increases gastric emptying rates while depression slows it down.

### 2.4.4 Candidates for gastroretentive drug delivery systems

Potential drug candidates for GRDDS are: drugs that are locally active in the stomach e.g. misoprostol, antacids; drugs that have a narrow absorption window in GIT e.g. levodopa, para-aminobenzoic acid, furosemide, riboflavin etc.; drugs that are unstable (i.e. degrade) in the intestinal or colonic environment e.g. captopril, ranitidine hydrochloride; drugs that disturb normal colonic microbes e.g. antibiotics against *Helicobacter pylori* e.g. amoxicillin trihydrate and lastly, drugs that exhibit low solubility at high pH values e.g. diazepam, chlordiazepoxide, and verapamil hydrochloride (Dehghan & Khan, 2009; Sarojini & Manavalan, 2012).

Drugs that are unsuitable candidates for GRDDS are drugs with limited acid solubility e.g. phenytoin; drugs that are unstable in the gastric environment e.g. erythromycin as well as drugs that are intended for selective release in the colon e.g. 5- aminosalicylic acid and corticosteroids (Makwana *et al*., 2012).

### 2.4.5 Advantages of gastroretentive drug delivery systems

Gastroretentive drug formulations have the following advantages (Jayanth, Lavanya, Datta & Babu, 2013). GRDDS:

- Enhance bioavailability of drugs that are absorbed in the upper part of the GIT.
- Sustain drug delivery and reduce the frequency of dosing of drugs with relatively short half-lives which improves patient compliance.
- Proved prolonged and sustained release of drugs from dosage forms which is beneficiary in targeted therapy e.g. targeted treatment in the stomach or small intestine.
- Minimize fluctuations of plasma drug levels and the optimal therapeutic plasma and tissue concentrations are maintained over a period, this then avoids sub-therapeutics and toxic concentration hence minimizing the risk of treatment failure and undesirable effects.

### 2.4.6 Disadvantages of gastroretentive drug delivery systems

Gastroretentive drug formulations have the following limitations (Chhetri *et al.*, 2014):

- These systems are not suitable for drugs that are not stable in an acidic environment nor drugs that are absorbed better from the lower part of the GIT.
- They present a difficulty in attaining the desired outcome and a problem of dose dumping.
- Gastric retention is affected by various factors such as gastric motility, pH and the presence of food; therefore, the dosage form should be able to withstand the forces associated with the peristaltic wave of the stomach.
- Formulation costs are higher.
- Difficulty in drug retrieval in the case of toxicity, poisoning or hypersensitivity reaction.

### 2.4.7 Types of gastroretentive drug delivery systems

According to Sarojini & Manavalan (2012), there are various approaches to achieve a gastroretentive drug delivery system. These approaches include high-density systems, expandable and magnetic systems and low-density systems. Low-density systems include systems that have low density due: to gas generation, expandable excipients and systems with intrinsic low density due to porosity.

#### **2.4.7.1** High- density systems

In this approach of gastroretention, the system has a density of approximately 3g/cm$^3$ and it causes gastroretention by being retained in the rugae of the stomach and is capable of withstanding its peristaltic movements. Figure 2.6 illustrates gastroretention achieved by a high-density system. Diluents such as barium sulphate, zinc oxide, titanium oxide and iron powder are employed to achieve such a high-density formulation. It has been reported that this delivery system did not significantly extend the GRT (Samar, 2010; Bhardwaj & Harikumar, 2013).

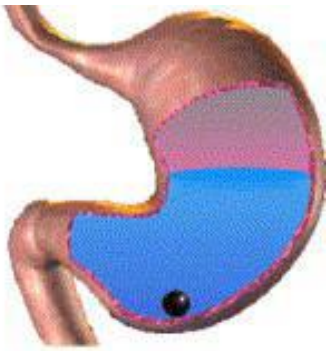


Figure 2.6: High-density gastroretentive drug delivery system (Tripathi *et al.,* 2012).

#### **2.4.7.2** Expandable systems

The expandable GRDDS systems are based on three configurations: firstly, a small configuration which enables convenient oral administration; secondly, an expanded form that is achieved in the stomach that prevents passage through the pyloric sphincter; and lastly, another small form that is achieved in the stomach when the

active ingredient has been released and retention is no longer required. The expansion is achieved by swelling or by unfolding in the stomach (Prinderre & Sauzet & Fuxen, 2011).

Unfoldable systems are made of biodegradable polymers and are available in different geometric forms like tetrahedron, ring or planner membrane (4- lobed discs or 4-limbed form) that are compressed within a capsule. The geometric forms are shown in Figure 2.7. Swellable systems are also retained in the GIT due to their mechanical properties. The swelling is a result of osmotic absorption of water (Nayak, Maji & Das, 2010).

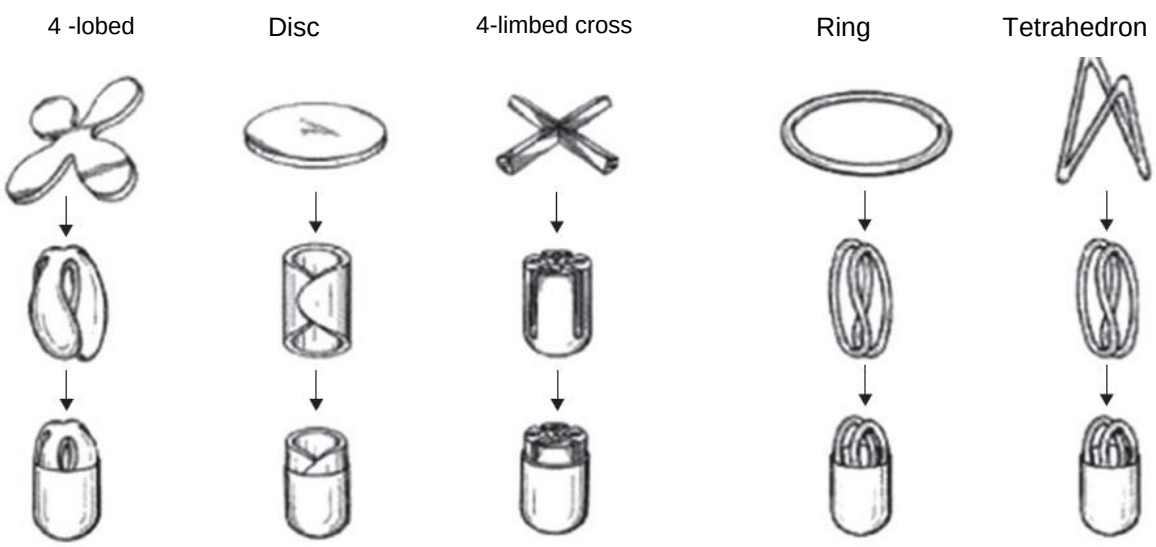



Figure 2.7: Different geometric forms of unfoldable systems (Prinderre *et al.,* 2011).

Expandable systems have limitations like storage of much easily hydrolyzed and biodegradable polymers. The relatively short-lived mechanical shape memory for unfolding is difficult to industrialize and is costly. Another major drawback with these systems is the brief obstruction, intestinal adhesion and gastropathy that may arise from the retention of a rigid and large single unit (Adibkia, Ghanbarzabeh, Mohammadi, Atashgah & Sabzevari, 2013).

#### 2.4.7.3 Magnetic system

In this gastroretentive technique, the dosage form contains a small magnetic and another magnet is to be placed on the abdomen over the position of the stomach at an exact degree which may retain the dosage form in the gastric region (Badoni, Ojha, Gnanarajan & Kothiyal, 2012). This delivery system contains magnetic responsive-ness that are integrated into various carriers such as magnetite, iron, cobalt, nickel and iron-boron. The magnetic compound along with the drug is injected into the general circulation and a magnetic field is applied at the target site to target delivery (Kudr, Haddad, Richtera, Heger, Cernak, Adam & Zitka, 2017).

The main advantage of this system is the ability to target tissue delivery which results in the reduction of free drug in the circulatory system which ultimately reduces the risk of side effects. The main disadvantage with this delivery system is that it requires an external magnet to be positioned with precision which leads to patient non-compliance (Ahmad, Minhas, Sohail, Faisal & Rashid, 2013).

#### 2.4.7.4 Bio-adhesive or mucoadhesive system

A bio-adhesive is a substance that interacts with biological materials and is capable of being retained on the biological substrate for a period. This gastroretentive technique is used to localize a delivery device within the stomach lumen. Bio-adhesion occurs in the presence of water in the stomach body cavity that enhances drug absorption at a specific site. The formulation of this system makes use of bio-adhesive polymers which have the ability to adhere to the epithelial surface of the GIT; these are usually macromolecular, hydrophilic gelling substances that have numerous hydrogen-bond forming groups such as carboxyl, hydroxyl, amide and sulphate groups e.g. cross-linked polyacrylic acids, sodium carboxymethylcellulose and sodium alginate and carrageenan (Samar, 2010).

The concept of bio-adhesion is gaining increasing interest in other routes of administration however this system is not very feasible as adhesion is prevented by

the acidic environment and thick mucus present in the stomach (Brahmbhatt, 2017). The high turnover rate of gastric mucus leads to difficulties in retaining a bio-adhesive system at site furthermore, specifically targeting the gastric mucus with bio-adhesive polymers is difficult as the polymers adhere to various surfaces they come into contact with. This may lead to the risk of oesophageal adherence which may cause drug-induced injuries (Samar, 2010).

#### **2.4.7.5** Floating or low-density system

This is a low-density system, also referred to as the floating drug delivery systems, that has sufficient buoyancy to float over the gastric contents and remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period. Figure 2.8 illustrates gastroretention achieved by a low-density system. As this system floats on the gastric contents, the drug is slowly released from the system. Following drug release, the residual system is emptied from the stomach. This type of system results in an increased GRT and control of the fluctuations in the drug's plasma concentration (Dwivedi & Kumar, 2011). Floating drug delivery systems are classified into two, namely the effervescent and non-effervescent system:

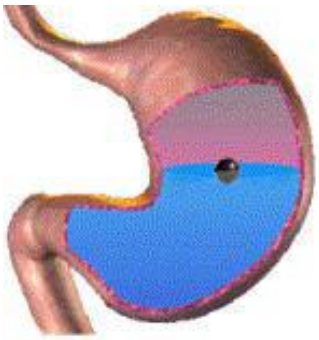


Figure 2.8: Low-density gastroretentive drug delivery system (Tripathi *et al.,* 2012).

- *Effervescent systems*

Effervescent systems are prepared to ensure that carbon dioxide is released upon arrival to the stomach, this then causes the formulation to float in the stomach as

shown in Figure 2.9. The formulation of this system makes use of matrices prepared by polymers with swelling properties such as HPMC or polysaccharides e.g. chitosan, and an effervescent component such as sodium bicarbonate, citric acid or tartaric acid (Dehghan & Khan, 2009).

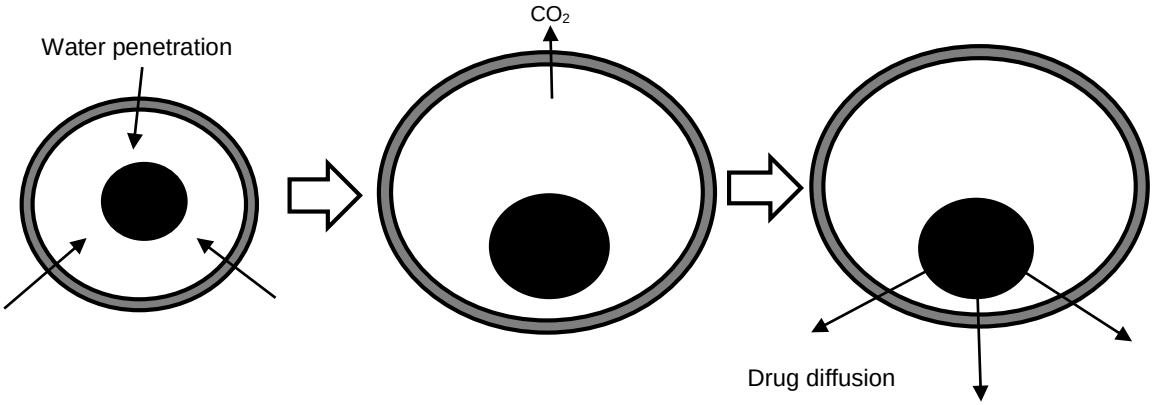



Figure 2.9: Drug release from an effervescent system (Chhetri & Thapa, 2014).

Effervescent floating systems have the main advantage of a rapid dissolution rate thus aiding in faster and better drug absorption. Limitations associated with this delivery system is that it requires high levels of fluids in the stomach; it is not suitable for drugs that have solubility or stability problems in the GIT and it is also not suitable for drugs that cause irritation to the gastric mucosa (Ahmed, Goyal & Sharma, 2014).

- *Non-effervescent systems*

The non-effervescent floating drug delivery systems are those that after swallowing, the formulation swells unrestrained via imbibition of gastric fluid to an extent that their exit is prevented from the stomach. The formulation of this system commonly employs the following excipients: gel forming or high swelling cellulose type hydrocolloids, polysaccharides and matrix forming polymers such as polycarbonate, polyacrylate, polymethacrylate and polystyrene (Patil, Hirlekar, Gide & Kadam, 2006). These systems are namely: the hydro-dynamically balanced systems, raft systems, alginate beads and microspheres that reviewed individually.

- *Hydro-dynamically balanced system*

This system is also known as the colloidal gel barrier systems in which the formulation contains the drug and gel-forming hydrocolloids which are meant to remain buoyant on the stomach content. When the hydrocolloid comes into contact with the gastrointestinal fluid, it hydrates and forms a colloid gel barrier around its surface as depicted in Figure 2.10.

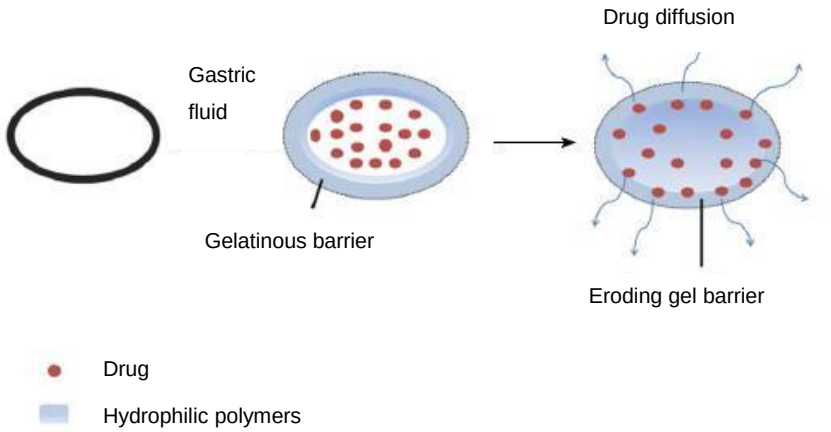



Figure 2.10: Hydro-dynamically balanced systems (Zhao, Lv, Zhang, Wang, Lv & Ma, 2014).

The formulation of this system involves incorporating high levels of one or more gel-forming, highly soluble cellulose type of hydrocolloids such as hydroxypropyl methylcellulose, polysaccharides as well as a matrix forming polymers such as polycarbophil, polystyrene and polyacrylate. These systems have been identified as unreliable and irreproducible in prolonging residence time in the stomach when orally administered due to their all or nothing emptying process (Gupta & Kothiyal, 2015).

- *Raft systems*

A raft is a flat structure that is made up of planks, logs or barrels that floats on water and is commonly used for transport or as a platform for swimmers. In this gastroretentive technique, a gel-forming solution swells upon contact with gastric contents/ fluids and it forms a viscous cohesive gel containing entrapped carbon

dioxide bubbles; this then forms a layer that floats on the stomach contents (Dolas, Hosmani & Somwanshi, 2015).

The main advantage of this system is that it does not interfere with the function of the pyloric sphincter and both rapid and long duration of action can be achieved. Major limitations of this system include instability problems as the system is formulated as a solution (this dosage form is susceptible to both chemical and microbial degradation) and special packaging and storage conditions are required to avoid the formation of the gel within the package and to maintain pH of the system (Pawar, Jadhav, Nikam, 2015).

- *Alginate beads*

This system is often referred to as “floating beads” as it is a multiple unit floating dosage form that is developed from freeze-dried calcium alginate. Spherical beads sized approximately 2.5 mm in diameter can be prepared by dropping a sodium alginate solution into aqueous solutions of calcium chloride, causing the precipitation of calcium alginate. The resulting beads are then separated, snap-frozen in liquid nitrogen and freeze-dried at 400˚C for twenty-four hours resulting in the formation of a porous system that can float for over twelve hours (Tripathi *et al.,* 2012).

The main advantage with alginate beads as a delivery system is easy manufacturing as the polymers can be designed with chemical functional groups and they gave the ability to tailor mechanical properties and degradability and the beads can be fabricated into various shapes (Tiwari, Singh & Sharma, 2013). The disadvantages of using sodium alginate are that it is costly and causes of side effects reducing patient compliance (Calvo & Santagapita, 2016).

- *Microspheres*

Microspheres are small spherical particles that have an approximate range of 1µm to 1000µm in diameter which are manufactured from various natural and synthetic polymers (Sahil, Akanksha, Premjeet & Kapoor, 2010). Microspheres provide a reliable means of site targeting with specificity.

The advantages of this system are that it: protects unstable and sensitive environment from prior environment; improves drug solubility, dispersibility and flowability; aids in ensuring safety when handling toxic material; improves bioavailability and thus improving therapeutic efficiency and lastly masks the odour or taste of a drug (Metkari, Kulkarni, Patil, Jadhav, Bamane & Kumbhar, 2014). With the benefits of this system considered, this delivery system was selected for this study and the manufacturing methods and materials are reviewed.

## 2.5 METHODS FOR MANUFACTURING MICROSPHERES

There are various methods to prepare hollow microspheres, below are a few preparation methods that have been employed to manufacture microspheres:

### 2.5.1 Spray-drying method

In this preparation method, a polymer is first dissolved in a suitable volatile organic solvent such as dichloromethane or acetone then the drug, in solid form, is dispersed into the polymer solution with the use of high-speed homogenization. The resultant dispersion is then atomized in a stream of hot air which leads to the formation of small droplets or fine mist from which the solvent evaporates instantly, causing the formation of the microspheres in a size range of 1-100 µm (Kadam & Survarna, 2015).

The spray-drying method has the main advantage of being a continuous one-step process thus offering good reproducibility and potential for scale-up productions unlike the other methods. The limitations with this method are that due to the use of high processing temperatures, organic solvents or water dry quickly reducing the time the polymers have to settle homogeneously resulting in the formation of an amorphous structure (Sipos, 2008).

### 2.5.2 Solvent evaporation method

This method of preparation is carried out in a liquid manufacturing vehicle. The microcapsule coating is dispersed in a volatile solvent which is immiscible with the liquid manufacturing vehicle then the core material which is to be encapsulated is dissolved or dispersed into the coating polymer solution. The core material mixture is dispersed in the liquid manufacturing vehicle phase by agitation which acquires the appropriate size microcapsule. If necessary, the mixture is then heated to evaporate the solvent. In this technique, if the core material is dissolved in the polymer solution then matrix-type microcapsules are formed (Sahil *et al.,* 2010). Figure 2.11 illustrates the manufacturing process.

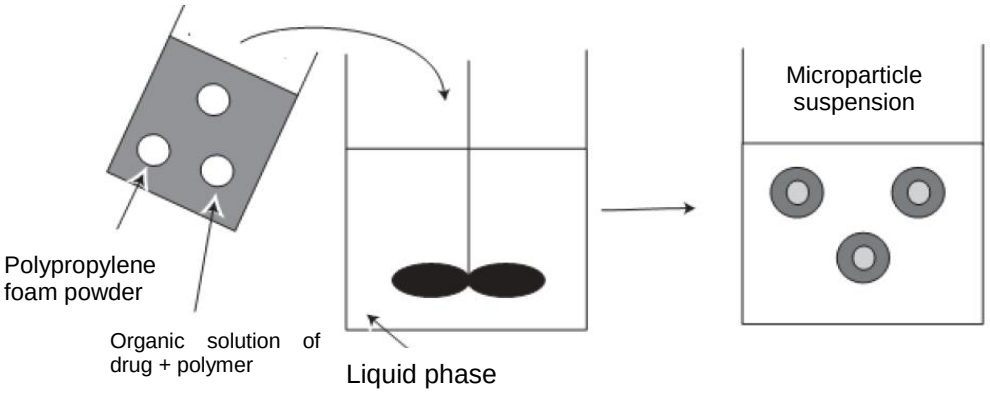



Figure 2.11: Solvent evaporation method of microsphere preparation (Saini *et al.,* 2014).

### 2.5.3 Inotropic gelation method

This preparation method is based on the cross-linking ability of electrolytes in the presence of counter ions to form beads. Despite the coating property and the ability for the natural polyelectrolytes to act as release rate retardants, these electrolytes contain certain anions on their chemical structure. These anions combine with the polyvalent cations hence forming a meshwork structure which induces gelation by binding mainly to the anion blocks. Hydrogel beads are then produced through dropping a drug-loaded polymeric solution into the aqueous solution of polyvalent cations (Saini *et al.,* 2014). Figure 2.12 schematically presents the manufacturing process.

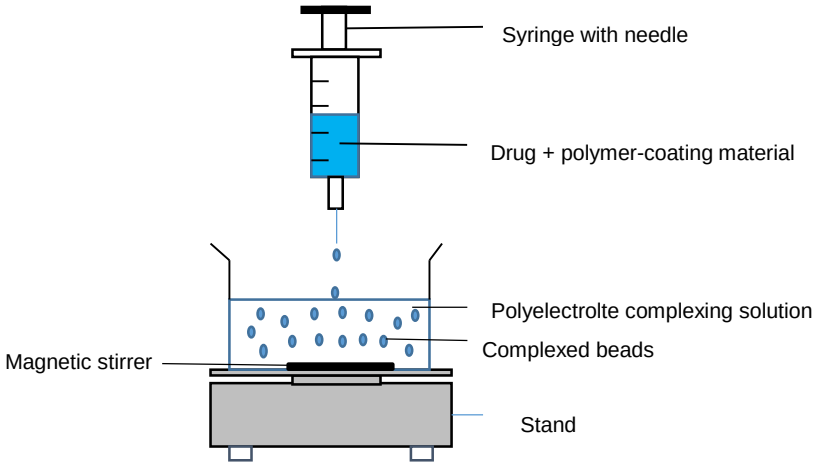



Figure 2.12: Inotropic gelation method of microsphere preparation (Saini *et al.,* 2014).

### 2.5.4 Emulsion-solvent diffusion method

In this technique, a solution or dispersion of the drug and the polymer is prepared in a solvent such as dichloromethane, ethanol, isopropanol or a combination of these. This solution or dispersion is then introduced into an aqueous solution of distilled water containing polyvinyl alcohol hence forming an oil-in-water type of emulsion. This emulsion is then subjected to agitation by a propeller type agitator in order to remove the organic solvent which then produces microspheres with a size range of 500 - 100 µm as diagrammatically shown in Figure 2.13 (Tripathi *et al*., 2012). The product is then washed and dried by vacuum oven at 40˚C for a day.

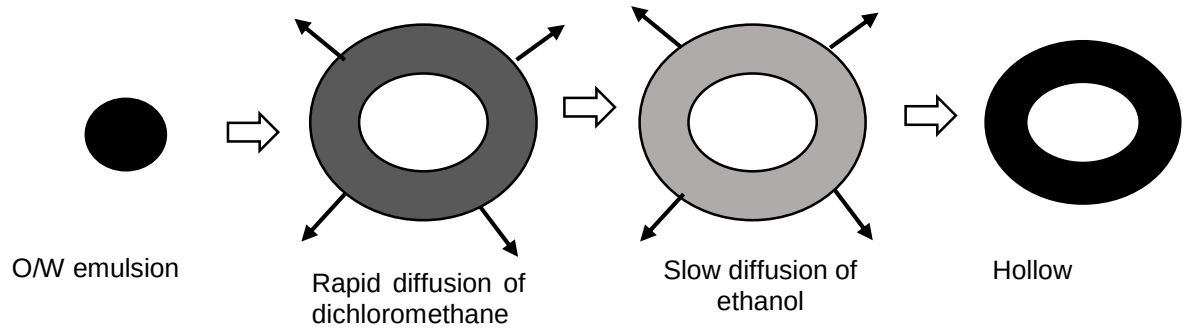



Figure 2.13: Solvent-emulsion diffusion method of microsphere preparation (Jayanth *et al.,* 2013).

This technique has been developed to achieve successful encapsulation and to prevent the degradation of the active pharmaceutical ingredient. The limitations associated with this technique is leakage of water soluble active pharmaceutical ingredients into the water phase and phase separation of the emulsion (Sipos, 2008).

## 2.6 MATERIALS FOR MANUFACTURING MICROSPHERES

According to Kadam & Survana (2015) microspheres are manufactured from two types of polymers. The first type of polymer used is the natural polymers which are obtained from various sources such as carbohydrates, proteins and chemically modified carbohydrates. The second type is the synthetic polymers, which are divided into two types: biodegradable polymers e.g. lactides, glycolides, poly anhydrides; and non-biodegradable polymers e.g. poly-methyl methacrylate, acrolein and epoxy polymers. A review of microspheres was conducted in an attempt to identify excipients that could be used during manufacturing.

Sato, Kawashima, Takeuchi & Yamamoto (2004) prepared hollow microspheres by the emulsion solvent diffusion using Eudragit S100, Eudragit L100, Eudragit L100-55, hydroxyl propyl methylcellulose (HPMC) and hydroxypropylmethylcellulose phthalates

polymers co-dissolved with the drugs (aspirin, salicylic acid, ethoxy benzamide, indomethacin and riboflavin). Although, the drugs exhibited various drug loading and drug release properties due to their differences in solubility; their study concluded that incorporating a polymer such as HPMC within the microspheres shells, the rate of drug release could be controlled while maintaining high buoyancy.

Radhika, Luqman & Borkhataria (2008) formulated delayed release microspheres of aceclofenac by solvent evaporation technique employing cellulose acetate phthalate, hydroxypropylmethylcellulose phthalate, Eudragit L100 and Eudragit S100 as enteric polymers. Effects of the various polymers were explored and the formulated microspheres were characterised for particle size, scanning electron microscope, percentage yield, drug entrapment and for *in vitro* release kinetics.

Jaiswal *et al*., (2009) developed oil entrapped floating alginate beads of ranitidine hydrochloride using sodium alginate as a polymer. The gel beads were prepared using a new emulsion gelation technique in which they gently mixed or homogenized oil and water phase containing the polymer which was then extruded into the calcium chloride solution. Unfortunately, the sodium alginate was not sufficient to sustain the drug release at gastric pH.

Darapu, Sundaramoorthy & Vetrichelvan (2011) prepared ranitidine hydrochloride loaded microspheres using HPMC, xanthan gum and Eudragit S100 as polymers. A solvent evaporation technique was employed and the formulations enhanced floatability and HPMC was concluded the suitable candidate for sustained release.

Ganesan & Kanth (2013) prepared microspheres with telmisartan as the model drug using emulsion solvent diffusion technique using HPMC and Eudragit RS100 as polymers. The best formulation showed an appropriate balance between buoyancy and drug release rate of 98.32% in 12 hours.

Dhankar *et al*., (2014) developed ranitidine hydrochloride loaded mucoadhesive microspheres using the water-in-oil emulsion method in which glutaraldehyde was used as a cross-linking agent. The process exhibited a high percentage of drug encapsulation (70%) and mucoadhesion (75%) and drug release was sustained for more than 12 hours.

Malipeddi, Awasthi & Dua (2016) formulated microspheres using metoprolol as a model drug using varying concentrations of ethyl cellulose and PEG 6000 as polymers. The microspheres were prepared by solvent evaporation method. The microspheres had good drug encapsulation properties of 91.2 to 95.7% and it was concluded that an increase in PEG results in a more permeable matrix that allows for increased drug release.

Pandey, Sah & Mahara (2016) formulated floating microspheres of nateglinide by oil-in-water emulsion solvent evaporation with ethyl cellulose and Eudragit S100 as release retarding polymers. The prepared microspheres showed prolonged drug release of 12 hours and remained buoyant for more than 12 hours.

## 2.7 SUMMARY

This chapter comprehensively reviewed ranitidine hydrochloride and GRDDS. Various analytical methods used to identify and quantify ranitidine hydrochloride have been reviewed namely, HPLC, TLC and UV-vis. Numerous approaches to GRDDS systems were reviewed and microspheres were selected for this project. An extensive literature review was explored to identify potential materials used in manufacturing microspheres and manufacturing methods. The next chapter provides information on the materials and methods used to obtain results.

# CHAPTER 3
# MATERIALS AND METHODS

## 3.1 INTRODUCTION

This chapter describes the materials and methods used in the preliminary formulations and in the manufacturing of ranitidine hydrochloride microspheres. It also features procedures used in encapsulating the microspheres into a dosage form. It provides a detailed list of the excipients used and their source.

## 3.2 STUDY METHODOLOGY AND DESIGN

The study followed a quantitative research method with a true experimental design in which data was collected and presented numerically and graphically based on averages recorded during the experiments.

## 3.3 STUDY MATERIALS

Table 3.1 lists all the materials used in the experimental phase of the study.

Table 3.1: Chemicals used in the preparation of microspheres

| Chemicals | Supplier of chemicals |
|---|---|
| Ranitidine hydrochloride (99,99%) | Orchev Pharma PVT. Ltd, India |
| Ethyl cellulose | Colorcon Limited, England |
| Eudragit S100 and L100 | Evonik Africa (Pty) Ltd, South Africa |
| HPMC K100 | Colorcon Limited, England |
| Magnesium Stearate | BDH Chemicals Ltd, England |
| Tween 20 | Sigma-Aldrich, South Africa |
| Tween 80 | Sigma-Aldrich, South Africa |
| Acetone | Rochelle Chemicals, South Africa |
| Ethanol | EthanolSA (Pty) Ltd, South Africa |
| Methanol | Rochelle Chemicals, South Africa |
| Liquid paraffin | Rochelle Chemicals, South Africa |
| Polyvinyl alcohol | Sigma-Aldrich, South Africa |
| 1 N Hydrochloric acid | E. Merck (Pty) Ltd, South Africa |
| Distilled water | Tshwane University of Technology, South Africa |

## 3.4 PRE-FORMULATION STUDIES

Pre-formulation studies are the first step in the rational development of dosage form of an active pharmaceutical ingredient. The main objective of pre-formulation studies is to develop a portfolio of information about the active pharmaceutical ingredient that is useful in developing the formulation (Prajapati, 2012).

Pre-formulation investigations conducted in this study included the validation of the analytical method, preliminary formulation studies, selection of polymers and the determination of compatibility between ranitidine and the chosen polymers.

### 3.4.1 Validation of analytical method

An analytical method is a procedure developed to test a defined characteristic of a drug substance or drug product against established acceptance criteria for that characteristic. Method validation is the process used to confirm that the analytical procedure employed to test a characteristic is suitable for its intended use (Huber, 2007; WHO, 2016).

The method was validated according to the International Council of Harmonisation (ICH) guidelines (2005) in terms of linearity, accuracy, precision and ruggedness, limits of detection (LOD) and limits of quantification (LOQ).

#### **3.4.1.1** Linearity

The linearity of an analytical procedure is defined as the ability to obtain results that are directly proportional to the concentration of drug used in a sample for example, the difference between a 10 mg concentration must be proportional to that of 5 mg looking at the absorbance obtained during the test (ICH, 2005).

Linearity in this study was confirmed by the construction of a calibration curve of ranitidine hydrochloride using 0.1 N hydrochloric acid (HCl) as a solvent at diluted concentrations of 10, 8, 6, 4, 2.0 and 1.0 µg/100mL. The absorbance of these drug solutions was determined at a wavelength of 314 nm against 0.1 N HCl as a blank using a Jenway 7315 UV-vis spectrophotometer (ThermoFisher Scientific, South Africa). This procedure was triplicated and the mean data was used to plot a calibration curve and the regression coefficient ($R^2$) was calculated using Microsoft Excel™.

- *Preparation of 0.1 N Hydrochloric acid*

To prepare 0.1 N of HCl, 500 mL of 1.0 N HCl was accurately measured using a measuring cylinder and diluted to 5000 mL with distilled water (Choudhary, 2013).

- *Preparation of stock solution*

The stock solution and dilutions for constructing the standard curve were prepared by accurately weighing 100 mg of ranitidine hydrochloride and dissolving in 100 mL of the various solvents to make a 1 mg/mL. 10mL of the above solution was further diluted to 1000 mL of the solvent to get a stock solution of 10 µm/mL concentration. The resulting solution was serially diluted with the solvent to get the concentration of 10, 8, 6, 4, 2.0 and 1.0 µg/100mL.

#### **3.4.1.2** Accuracy

The accuracy of an analytical method states the similarity between the value which is accepted either as a conventional true value or an accepted reference value and the found value. To determine the accuracy of a method, the reference material must be subjected to the analytical procedure (ICH, 2005). The diluted concentrations of 6, 8 and 10 µg/100mL were analysed in triplicate in this study to determine if all resulted in a similar absorbance or one closest to the first UV-vis reading.

#### **3.4.1.3** Precision

Validation of the assay methodology includes an investigation of precision. The precision of an analytical procedure expresses as the closeness of agreement between a series of measurements acquired from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered in three levels, these include repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be explored using artificially prepared samples or a sample solution. The precision of an analytical procedure is

usually stated as the variance, standard deviation or coefficient of variation of a series of measurements (ICH, 2005). The precision of the method was studied as intraday variations in which all the aliquots solutions of ranitidine hydrochloride were analysed three times in the same day.

#### **3.4.1.4** Robustness

Robustness is a measure to determine the ability of the analytical procedure to remain unchanged by slight or deliberate variations in method parameters used. It provides an indication of its reliability during a test procedure or analytical run (ICH, 2005). Robustness of this method was determined by having the aliquots from a homogenous slot by two analysts using the same operational and environmental conditions.

#### **3.4.1.5** Limit of detection and limit of quantification

Limit of detection and limit of quantification values are related but possess distinct definitions. LOD aims to define the smallest concentration of an analyte that can be detected with no guarantee about the bias or imprecision of the result by an assay. LOQ defines the concentration at which quantification is defined by bias and feasible precision goals and the concentration at which the analyte can be quantitated with a linear response (Shrivastava & Gupta, 2011). LOD and LOQ were calculated using Equation 1 and Equation 2 respectively.

$$LOD = \frac{3.3 \times S_y}{S} \qquad \text{(Equation 1)}$$

$$LOQ = \frac{10 \times S_y}{S} \qquad \text{(Equation 2)}$$

Where;

$S_y$ = Standard deviation of the response

S = slope of the calibration curve

## 3.5 PRELIMINARY FORMULATION DEVELOPMENT

Preliminary formulations of ranitidine hydrochloride loaded microspheres were formulated by emulsion-solvent diffusion method and solvent evaporation method. The polymers used during these trials included ethyl cellulose, Eudragit S100, Eudragit L100 and PEG 4000. The selected emulsifier was HPMC and the surfactants were Tween 20, Tween 80 and magnesium stearate. The selection of polymers, emulsifier and surfactants was based on literature review.

Both methods included weighing and co-dissolving different ratios of the drug and polymer into solvents (namely: acetone, ethanol, dichloromethane and methanol alone or in combination) and this was introduced to a vehicle phase for stirring for a specific time. Two mechanical stirrers were employed namely a Labotec ceramic magnetic stirrer (South Africa) and a Heidoph RZR 2021 three-bladed propeller overhead stirrer (Separations, South Africa). When the stirring time elapsed, the microspheres were filtered and washed before drying.

For the emulsion-solvent diffusion method, Eudragit S100 and L100 were used as polymers at different drug to polymer ratios. Magnesium stearate, Tween 20 and Tween 80 were used as surfactants at different quantities and HPMC was used as an emulsifying agent. The vehicle for this trial run was 1.5% polyvinyl alcohol in distilled water, maintained at 45˚C. Distilled water was used to wash the collected spheres prior to drying overnight at 40˚C in a Labcon laboratory oven (RSE projects, South Africa) overnight.

For the solvent evaporation method, ethyl cellulose and PEG 4000 were used as polymers. Different concentrations of ethyl cellulose solutions were experimented in conjunction with variations in drug: PEG 4000 ratios. The vehicle phase experimented with was liquid paraffin. Two organic solvents, n-hexane and petroleum ether were used to wash the collected microspheres. Drying was done in a laboratory oven at 40˚C overnight.

## 3.6 DRUG-EXCIPIENT COMPATIBILITY STUDIES

Compatibility between ranitidine hydrochloride and the selected polymers investigated using differential scanning calorimetry (DSC)/Thermogravimetric Analysis (TGA) and Fourier-transformed infrared (FTIR) spectroscopy and short-term accelerated stability studies.

### 3.6.1 Differential scanning calorimetry and thermogravimetric analysis

Murugendrappa, Khasim & Prasad (2000) and Gabbot (2007) defined DSC as a thermal analysis technique that observes how the heat capacity of material changes with temperature. A sample with a known mass is heated or cooled and the changes in its heat capacity are tracked as changes in heat flow. This tracking allows the detection of transitions such as melts, glass transitions, phase changes and curing which are illustrated by either an endothermic (energy being absorbed) or exothermic (energy being released) peak which are used to estabilish the purity of a compound and any conversion that may occur as a result of incompatibilities.

TGA is a test method with the capability of measuring the mass evolution of a milligram-scale sample as a function of temperature or time in a controlled atmosphere. These measurements are primarily to determine the composition of the specific sample and to predict their thermal stability at temperatures up to 1200$^{o}$C. The measurements also characterises materials that exhibit weight loss or gain due to decomposition, oxidation or dehydration (Dean, 2014; Mckinnon, 2014).

The TGA-DSC thermogram of ranitidine hydrochloride, the polymers individually and binary mixtures at the ratio of 1:1 (w/w) formed by ranitidine hydrochloride and the polymers. Samples of ranitidine and polymers (individually and as binary mixtures) weighing 5-8 mg were placed in sealed aluminum crimp cells (100 µl) and was heated to an end temperature, dependent on the melting point of the active pharmaceutical

ingredient and polymers, at a heating rate of 10˚C/min with a nitrogen gas flow of 35 mL/min.

### 3.6.2 Fourier-transformed infrared spectroscopy

Jaggi & Vij (2007) identified “Fourier spectroscopy” as a term used to describe the analysis of any varying signal into its constituent frequency components. FTIR spectroscopy is a well-established technique used to identify the functional groups in a molecule based on its vibration modes at different IR frequencies. The property of specificity in vibrational frequency constitutes the basis for detecting potential drug-excipient incompatibility.

The IR-spectrums of ranitidine hydrochloride, the polymers and two binary mixtures with a drug: polymer ratio of 1:1 (w/w) were recorded on a Nicolet Is5 FTIR Spectrometer iD5 ATR (ThermoFisher Scientific, South Africa) using solid plates over a range of 2000-400 $cm^{-3}$.

### 3.6.3 Short-term accelerated stability study

Short-term accelerated stability study is an analytical method used to determine drug and excipient/polymer interaction. This study involves storage of the drug and polymer blends with or without moisture at high temperatures to accelerate drug aging and interaction with excipients. The normal duration of the study is 3 - 4 weeks (Shahe, Chetty & Murthy, 2012).

Compatibility studies determined using DSC/TGA and FTIR only determine the presence of an incompatibility between the drug and polymer but does not indicate if the incompatibility is due to chemical or physical incompatibility. For this reason, short-term accelerated studies were conducted firstly, to confirm the absence or presence of incompatibility and secondly, to determine whether it is a physical or chemical incompatibility (Corvi, Cirri & Mura, 2006).

For the short term accelerated stability studies, samples of ranitidine hydrochloride and 1:1 w/w binary mixtures formed by ranitidine hydrochloride and the polymers were transferred to closed polytop vials and stored at 50˚C for three weeks in a Labcon laboratory oven (RSE projects, South Africa). The samples were examined for visual changes and were quantitatively analysed using UV-vis spectrophotometer to determine percentage degradation initially and at the end of the three weeks (Manikandan, Kannan & Manavalan, 2013).

## 3.7 FORMULATION METHOD OF MICROSPHERES

Ranitidine hydrochloride-loaded microspheres were prepared using the solvent evaporation method. A 20% ethyl cellulose solution was prepared by dissolving the required amount of ethyl cellulose in a 1:1 v/v mixture of acetone and methanol. The required amount of ranitidine hydrochloride and polymer were weighed and dissolved in 10 mL of methanol at different ratios at room temperature. Twelve and a half milliliters of the ethyl cellulose solution was added to the ranitidine/polymer/methanol mixture and agitated to form a homogenous mixture. This mixture was then dispersed in 100 mL liquid paraffin and stirred using a three-bladed propeller overhead stirrer at room temperature to form rigid spherical particles. The microspheres were filtered using a 250-micron sieve, washed thrice with petroleum ether and dried overnight in a laboratory oven at 40˚C (Saravanan & Anupama 2011). The dried microspheres were encapsulated into size 00 empty hard gelatin capsules. Figure 3.1 illustrates the process flow for manufacturing microspheres**.**

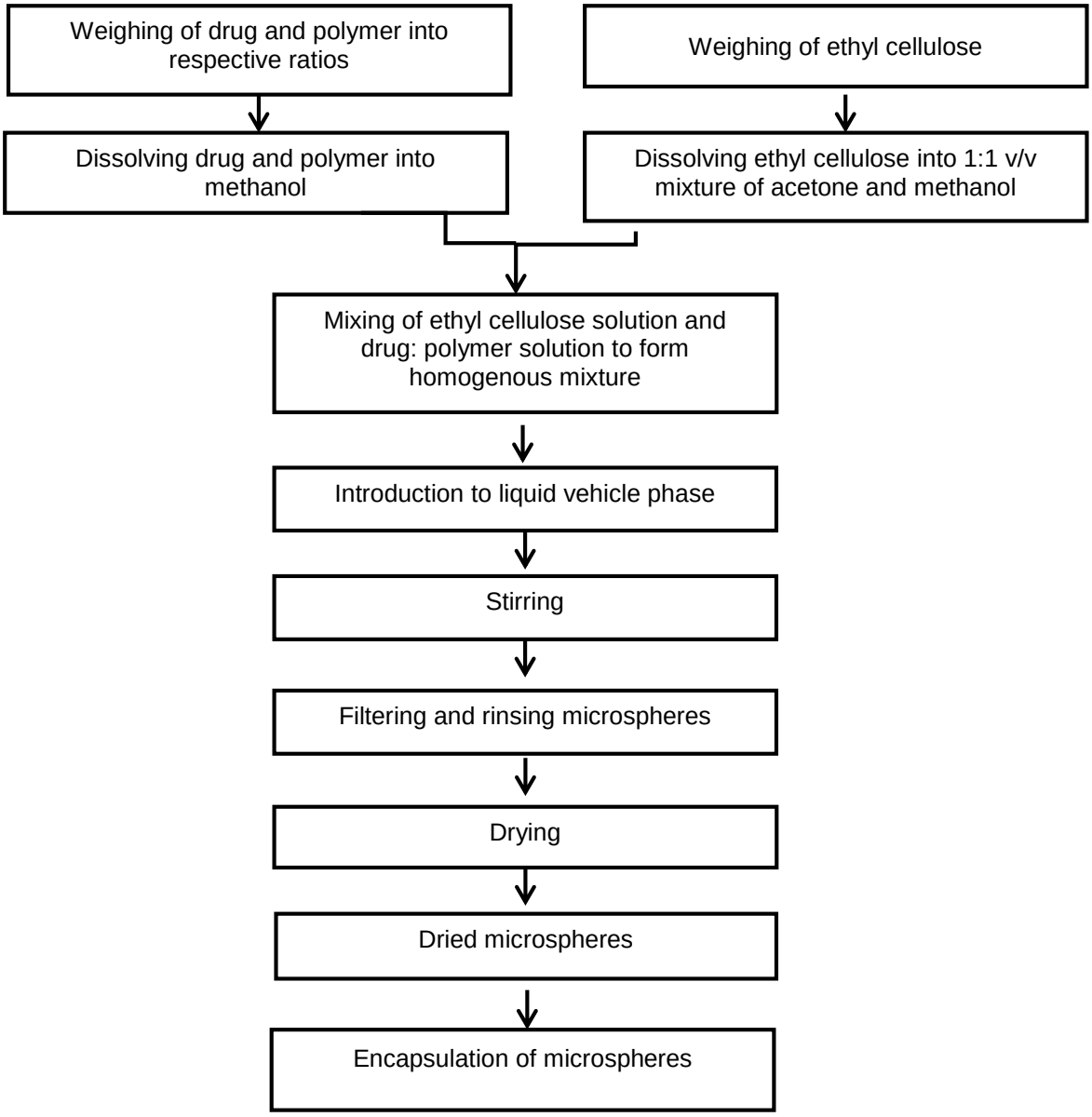



Figure 3.1: Process flow chart for manufacturing microspheres (Saravanan & Anupama, 2011).

## 3.8 POST FORMULATION STUDIES

All formulated microspheres were evaluated for parameters following methods in the United States Pharmacopoeia (USP). Post formulation studies included investigating the morphology and particle size analysis of the formulated microspheres, the flow properties of the microspheres using bulk and tapped densities, angle of repose, loss of drying and determining drug content.

### 3.8.1 Shape and surface morphology

The shape and surface morphology of the ranitidine hydrochloride loaded microspheres were investigated using a Sigma 500 VP (Zeiss, Germany) scanning electron microscopy (SEM). The samples for SEM study were prepared by lightly sprinkling the formulation on a double adhesive tape stuck on an aluminium stub. The samples were randomly scanned and microphotographs of the microspheres were taken using the SEM.

### 3.8.2 Particle size distribution

Particle size distribution was determined using Sieve Shaker (Endecott, United Kingdom) Six sieves were arranged in ascending order i.e. sieve no # 18 (1000 µm), # 20 (850 µm), # 25 (710 µm), # 30 (600 µm), # 35 (500 µm) and the collecting pan was placed at the bottom of sieve no # 35 to collect the microspheres that passed through it. The nest of sieves were shaken using a mechanical sieve shaker for 10 minutes and the retained microspheres were collected, weighed and used to calculate the frequency and the median particle diameter using Microsoft Excel™.

### 3.8.3 Bulk density

The bulk density of a powder is the mass of an untapped powder sample and its volume including the contribution of the inter-particulate void volume. Determining this characteristic of the microspheres predicts the processability and handling of the formulation (Thomas, 2005). It was measured by pouring the weighed powder into a measuring cylinder. It is expressed in grams per milliliter (g/ mL) and was calculated using Equation 3 (USP, 2012):

$$\text{Bulk density } (\text{g/mL}) = \frac{\text{M}}{V_o} \qquad \text{(Equation 3)}$$

Where;

M is the weight of microspheres

$V_o$ is the bulk volume of the microspheres

### 3.8.4 Tapped density

Tapped density is defined as the maximum packing density of microparticles. The tapped density of the microspheres is determined as an attempt to predict flow properties and compressibility which are vital characteristic to be considered for capsulation (Thomas, 2005). An accurately weighed sample of microspheres (M) was carefully added to a measuring cylinder and the initial powder volume was recorded then the cylinder was mechanically tapped 10 times and the volume ($V_{10}$) was measured. Tapping continued for an additional 500 taps and volume ($V_{500}$) measured. Tapping was repeated 1250 times and volume ($V_{1250}$) measured. The difference between the last two taps was determined and if the difference exceeds 2 mL, tapping was repeated with increments as 1250 intervals to obtain a difference between the succeeding measurements is less than or equal to 2 mL. The final volume ($V_f$) was noted and tapped density was then calculated using Equation 4 (USP, 2012):

$$\text{Tapped Density (g/mL)} = \frac{M}{V_f} \qquad \text{(Equation 4)}$$

Where;

M is the weight of the sample and

$V_f$ is the final tapped volume of the microspheres

### 3.8.5 Hausner's ratio and Carr's index

Hausner's ratio and Carr's index are methods commonly used to measure the flow properties of microparticles. Both Hausner's ratio and Carr's index were determined by using the bulk density and tapped density of the microspheres (Aulton, 2007). Table 3.2 shows the relationship between flow properties, Hausner's ratio and Carr's index. Hausner's ratio and Carr's index (also known as percentage compressibility) were calculated using Equation 5 and Equation 6 respectively:

$$\text{Hausner ratio } (\%) = \frac{V_o}{V_f} \quad \text{(Equation 5)}$$

$$\text{Carr's index } (\%) = (\frac{V_o - V_f}{V_o}) \text{ x } 100 \quad \text{(Equation 6)}$$

Where;

$V_o$ is the bulk volume of the microspheres

$V_f$ is the tapped volume of the microspheres

Table 3.2: Effect of Carr's index and Hausner's ratio flow properties (USP, 2012)

| Compressibility Index (%) | Flow Character | Hausner's Ratio |
|---|---|---|
| ≤10 | Excellent | 1.00-1.11 |
| 11-15 | Good | 1.12-1.18 |
| 16-20 | Fair | 1.19-1.25 |
| 21-25 | Passable | 1.26-1.34 |
| 26-31 | Poor | 1.35-1.45 |
| 32-37 | Very poor | 1.46-1.59 |
| >38. | Very, very poor | >1.60 |

### 3.8.6 Angle of repose

Angle of repose is a characteristic related to inter-particulate friction or resistance to movement between particles which has been used to characterize the flow properties of solids. The angle of repose of all the formulations was determined by the fixed funnel method. A funnel was fixed at a height approximately of 2 cm above a platform. The loose powder was then slowly passed through the funnel until a cone of powder is

formed. The general guidelines for evaluating flow properties based on the angle of repose are given in Table 3.3. The diameter and height of the powder cone were measured and the angle of repose determined using Equation 7:

$$\text{Angle of repose (degrees)} = \frac{h}{r} \qquad \text{(Equation 7)}$$

Where;

h is the height of the powder cone and

r is the radius of the heap of the cone

Table 3.3: Flow properties and the corresponding angle of repose (USP, 2012)

| Flow property | Angle of repose (degrees) |
|---|---|
| Excellent | 25-30 |
| Good | 31-35 |
| Fair- aid not needed | 36-40 |
| Passable- may hang up | 41-45 |
| Poor-must agitate, vibrate | 46-55 |
| Very poor | 56-65 |
| Very, very poor | >66 |

### 3.8.7 Loss on drying

Loss on drying is a procedure that determines the amount of water or volatile matter of any kind that is driven off from a sample when dried under specific conditions. Loss on drying was determined by accurately weighing one-gram of microspheres from each formulation transferring them into previously dried empty containers. The samples were spread out evenly and dried at 60°C in a laboratory oven for three hours.

After drying, the samples were cooled to room temperature in a desiccator and reweighed. Loss on drying was then calculated using the Equation 8 below (USP, 2014):

$$\text{LOD } (\%) = \frac{W_{start} - W_{dry}}{W_{start}} \text{ x } 100 \qquad \text{(Equation 8)}$$

Where;

$W_{start}$ is the amount of the microspheres weighed before drying and

$W_{dry}$ is the amount of the microspheres weighed after drying

### 3.8.8 Percentage yield

Percentage yield is the actual amount of a product that is available for use at the end of the manufacturing process. It was calculated by considering the total ingredients (drug and polymer) used for the formulation and the total weight of obtained microspheres (Darapu *et al.,* 2011). The percentage yield is calculated using Equation 9:

$$\text{Percentage yield } (\%) = \frac{\text{weight of collected microspheres}}{\text{weight of starting materials}} \text{ x } 100 \quad \text{(Equation 9)}$$

### 3.8.9 Drug content of microspheres

Drug content is the ratio of the weight of a drug entrapped into a carrier system to that of the total drug added. To determine drug content, 100mg of microspheres were weighed and kept overnight in 0.1 N HCl to extract ranitidine hydrochloride. This solution was then filtered through a 0.45-micron filter pore and suitable dilutions of the filtrate were prepared and the quantity of ranitidine was estimated at 314 nm using UV-vis spectrophotometer (Saravanan & Anupama, 2011). The percentage drug content was then calculated using Equation 10:

$$\text{Drug content } (\%) = \frac{\text{Abs sm}}{\text{Abs std}} \text{ x } \frac{\text{Std dil}}{\text{Sm}} \text{ x } \frac{\text{Potency}}{100} \text{ x } \frac{\text{Avg wt}}{\text{Label claim}} \text{ x } 100 \text{ (Equation 10)}$$

Where;

Abs sm is the absorbance of the sample

Abs std is the absorbance of the standard solution

Std dil is the dilution of the standard solution

Sm dil is the dilution of the sample

Avg wt is the average weight of the microspheres in milligrams

### 3.8.10 Percentage buoyancy

The buoyancy test was carried out to determine the lag time and total floating time of the prepared microspheres which are important for the sustained drug delivery in the gastric region (Awasthi & Kulkarni, 2013). Lag time is the time it takes for the floating dosage form to start to float and total floating time is the total time the dosage forms float on the surface of the medium. To determine lag time, 100 mg of microspheres were placed in beakers containing 100 mL of 0.1 N HCl and the time required for the microspheres to rise to the surface was measured (Akbari, Dholakiya, Shiyani, Lodhiya & Shastry, 2009).

To determine total floating time (percentage buoyancy), weighed amounts of the microspheres from each batch was placed in 900 mL of 0.1 N HCl and stirred at 100 rotations per minute in a Hanson SR8-Plus (Spectralab Scientific Inc, Canada) dissolution apparatus 2 (paddle apparatus) for 8 hours. At the end of the 8 hours, the layer of floating microspheres was pipetted and separated by filtration. The layer of the sunken microspheres was also collected and separated by filtration (Sanjivani, Swapnila, Shalaka, Uddhav & Jagdish, 2011). Both the collected particles were dried in a desiccator until constant weight. Both the fractions of the microspheres were weighed and the percentage buoyancy was determined using Equation 11:

$$\text{Buoyancy}\ (\%) = \frac{W_f}{W_f + W_s} \text{x}\ 100 \qquad \text{(Equation 11)}$$

Where;

$W_f$ is the weight of the floating microspheres and

$W_s$ the weight of the settled microspheres

## 3.9 CAPSULATION OF MICROSPHERES

The formulated microspheres were filled into size # 0 hard gelatin capsules using a semi-automated Zuma Milano® (Italy) capsuling machine and the caps were tightened using a Zuma Milano® 20127 (Italy) machine.

## 3.10 POST-CAPSULATION STUDIES

Post microsphere encapsulation, the obtained capsules were further evaluated for various parameters following USP guidelines. Post-capsulation studies included capsule dimensions' analysis, weight variation, buoyancy, *in vitro* drug release and dissolution kinetics.

### 3.10.1 Capsule dimensions

The length and width (diameter) of the dosage forms are important parameters for dosage form uniformity. The length and width of 20 randomly selected capsules was determined using a Vernier caliper (Fragram, South Africa). The average length and width of the capsules were then calculated and this was used to determine the standard deviation (USP, 2014).

### 3.10.2 Weight variation test

Weight variation test is a test to ensure the consistency of the dosage units; it ensures that each unit in a batch has a narrow range of the drug substance around the label claim. This test was performed as per USP (2014) guidelines. Twenty capsules were randomly selected from each batch; one capsule was weighed using a B154 electric weighing balance (Mettler Toledo, Switzerland) and recorded at a time and each was then opened to empty the contents of the capsule and the empty capsule shells were weighed and recorded. The net weight of each capsules' content was calculated by subtracting the weight of the empty shells from the weight of the intact capsule. The

average net weight of the capsules determined and the percentage deviation from the average net weight of each capsule was determined and checked to ensure that they do not exceed the limits shown in Table 3.4.

Table 3.4: Specification for capsule weight variation (USP, 2014)

| Average weight of capsule | Deviation (%) |
|---|---|
| Less than 300mg | ±10.0 |
| | ±20.0 |
| 300mg or more | ±7.5 |
| | ±15.0 |

### 3.10.3 *In vitro* drug release studies

The release rate of ranitidine hydrochloride from the encapsulated microspheres was examined as per USP (2014). A dissolution apparatus 2 (paddle apparatus) was used in 900 mL of dissolution medium containing 0.1 N hydrochloric (pH 1.2) at 37˚C ± 0.5 ˚C. The rotation speed of paddle used was 50 rotations per minute. Samples (5 mL) were taken from the dissolution apparatus at set time intervals: 30, 120, 240, 360, 480 and 720 minutes. The medium was replenished with an equal volume of the fresh medium during each time interval. The samples taken were immediately filtered using a 0.45 µm filter and introduced into a 100 mL volumetric flask. It was then filled up to 100 mL with 0.1 N HCl. The absorbance of these solutions was determined by employing a UV-vis spectrophotometer at a wavelength of 314 nm. The percentage drug release was calculated using Equation 12.

$$(\%)\text{Drug release} = \frac{\text{Abs sm}}{\text{Abs st}} \times \frac{\text{Std dil}}{\text{Sm dil}} \times \frac{\text{Potency}}{100} \times \frac{\text{Avg wt (mg)}}{\text{Label claim (100mg)}} \times 100 \quad \text{(Equation 12)}$$

Where;

Abs sm is the absorbance of the sample

Abs std is the absorbance of the standard solution

Std dil is the standard dilution

Sm dil is the sample dilution and

Avg wt is the average weight of the capsules

### 3.10.4 Model dissolution kinetics

When a new solid dosage form is produced, it is necessary to ensure that the dosage form shows dissolution in an appropriate manner. *In vitro* dissolution is recognised as an important aspect in drug development as it is used as a surrogate for assessment of bioequivalence. For this reason, mathematical formulas have been employed to express and analyse the dissolution results as a function of dosage form characteristics (Costa & Lobo, 2001; Shaikh, Kshirsagar & Patil, 2015). The mathematical modeling aids in optimizing the design of a therapeutic device to yield information on the efficacy of various release models (Ramteke, Dighe, Kharat & Patil, 2014). The kinetics of drug release from the formulated microspheres were determined using zero order, first order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas model. The determination of $R^2$ values was used as an indicator of the best model fit for release data (Costa & Lobo, 2001).

#### **3.10.4.1** Zero-order kinetic model

The zero-order kinetic model can represent dissolution of the drug from dosage forms that do not disaggregate and release the drug slowly. These dosage forms release the same amount of drug by unit time to achieve prolonged drug action. This relationship describes dissolution relationship present in drug delivery devices as oral osmotic tablets, transdermal systems and in matrix tablets with low-soluble drugs (Singhvi & Singh, 2011; Shaikh *et al.,* 2015). Equation 13 represents this model. To study the release kinetics, data obtained from the *in vitro* drug release studies was plotted as cumulative percentage drug released versus time.

$$C = C_o - K_o t \qquad \text{(Equation 13)}$$

Where;

C is the amount of drug released or dissolved

$C_o$ is the initial amount of drug in solution (usually zero)

$K_o$ is the zero order rate constant and

t is time

### **3.10.4.2** First order kinetic model

The first order kinetics describes a dosage form where the drug released at each time is proportional to the residual drug inside the dosage form. The application of this model was first proposed by Gibaldi and Feldman in 1967, and later by Wagner in 1969. This model has also been used to describe the absorption and/or the elimination of some drugs although it is theoretically difficult to conceptualise this mechanism. This relationship can be used to describe dissolution in dosage forms containing water-soluble drugs in porous matrices (Deshmukh, Soni, Jain & Amin, 2008; Ramteke *et al*., 2014). Equation 14 represents this model. To study the release kinetics, data obtained from the *in vitro* drug release studies was plotted as log cumulative percentage drug remaining versus time.

$$\mathrm{Log\ C} = \mathrm{LogC_O} - \mathrm{Kt}/2.303 \qquad \text{(Equation 14)}$$

Where;

Co is the initial concentration of drug

K is the first order constant and

t is time

### **3.10.4.3** Higuchi kinetic model

In 1961 and 1963, Higuchi developed models to study the release of water-soluble and low soluble drugs incorporated in semisolid and solid matrices. (Chime, Onunkwo & Onyishi, 2013). This model is based on various hypothesis, firstly: that the initial concentration of the drug in the matrix is much higher than the drugs' solubility. Secondly, that diffusion of drugs occurs only in one dimension. Thirdly, that drug particles are much smaller than system thickness. Fourthly, that the swelling of a matrix and dissolution is negligible. Fifthly, that drug diffusivity is constant and lastly that perfect sink conditions are maintained in the release environment (Shaikh *et al.,* 2015). This relationship can be used to describe the drug dissolution from several types of modified release dosage forms such as transdermal systems and matrix tablets with water-soluble drugs (Dash, Murthy, Nath & Chowdhury, 2010). Equation 15 represents this model and data obtained from the *in vitro* drug release studies was plotted as a cumulative percentage of drug release versus square root of time.

$$C = [D(2qt - C_S)C_S t \qquad \text{(Equation 15)}$$

Where;

C is the total amount of drug released per unit area of the matrix (mg/cm$^2$)

D is the diffusion coefficient for the drug in the matrix (cm$^2$ per unit time)

qt is the total amount of drug in a unit volume of the matrix (mg/cm$^3$)

Cs is the dimensional solubility of the drug in the polymer matrix (mg/cm$^3$) and

t is time

### **3.10.4.4** Hixson-Crowell kinetics model

Hixson and Crowell identified a model in 1931 that describes drug release from a dosage form that experiences a change in surface area and diameter. From this theory, an equation was derived for drug powders that contain uniformed size particles, which express the rate of dissolution based on the cube root of the particles (Costa & Lobo, 2001; Shaikh *et al*., 2015). This relationship describes dissolution from

dosage forms such as tablets in which dissolution occurs in planes that are parallel to the drug surface when the tablet dimensions diminish proportionally in such a way that the initial geometrical form keeps constant all the time (Dash *et al.,* 2010). Equation 16 represents this model. To study the release kinetics, data obtained *in vitro* drug release studies was plotted as the cube root of drug percentage remaining in matrix versus time.

$$C_O^{1/3} - C_t^{1/3} = K_{HC}t \qquad \text{(Equation 16)}$$

Where;

$C_t$ is the amount of drug released in time "t"

$C_o$ is the initial amount of drug in the dosage form and

$K_{HC}$ is the rate constant for Hixson-Crowell equation

### **3.10.4.5** Korsmeyer Peppas kinetic model

Korsmeyer, in 1983, developed a simple semi-empirical model that exponentially relates drug release to the elapsed time (Bagade, Dhole, Nemlekar, Pujari, Shete & Kharat, 2014). This relationship can be used to the linearization of drug release data from several formulations of microcapsules or microspheres (Ramteke *et al*., 2014). Equation 17 represents the relationship of this model. To study the release kinetics, data obtained *in vitro* drug release studies was plotted as log cumulative percentage drug release versus log time.

$$\frac{C_t}{C_\infty} = kt^n \qquad \text{(Equation 17)}$$

Where;

$C_t/C_\infty$ is the fraction of the drug released in time "t"

k is the rate constant and

n is the release exponent

## 3.11 SUMMARY

The materials and methods used to formulate ranitidine hydrochloride microspheres were discussed. The method of encapsulation of microspheres was also described. The method and equations used to validate the analytical method and to evaluate the microspheres and capsules were listed and discussed. The results of the data collected over the data collection period will be presented in Chapter Four.

# CHAPTER 4
# RESULTS AND DISCUSSION

## 4.1 INTRODUCTION

This chapter presents all results that were obtained during pre-formulation studies including the validation of the analytical method, preliminary formulation and compatibility studies as well as the post-formulation and post-capsulation studies. Results were collected in triplicate, analysed and discussed shortly after being presented.

## 4.2 VALIDATION OF ANALYTICAL METHOD

The UV-vis spectrophotometric method which was employed for the estimation of ranitidine hydrochloride from the microspheres was validated as per ICH guidelines. The diluted solutions of the drug were prepared as described in chapter 3.4.1.

### 4.2.1 Linearity studies

Linear studies were conducted as described in chapter 3.4.1.1 and the $R^2$ value was calculated using Microsoft Excel™. The constructed calibration curve is represented in Annexure A. The linear regression showed good linear relationships over the concentration range of 1 - 10 µg/mL for ranitidine hydrochloride and the $R^2$ was calculated to be 0.9977

### 4.2.2 Accuracy

The diluted solutions of the drug were analysed by the described method in chapter 3.4.1.2 and the results of the recovery studies are shown in Table 4.1. The three readings were the same or very closely related to the first UV-vis absorbance reading.

The calculated %RSD was less than 2% which indicates the accuracy of the used method (Zameeruddin, Molvi, Ahmed & Siraj, 2016).

Table 4.1: Accuracy recovery studies

| **Analysed sample concentration (µg/mL)** | **Triplicate UV-vis absorbance readings** | **% RSD** |
|---|---|---|
| 6 | 0.024 | 0 |
| | 0.024 | |
| | 0.024 | |
| 8 | 0.031 | 0 |
| | 0.031 | |
| | 0.031 | |
| 10 | 0.033 | 1.4 |
| | 0.034 | |
| | 0.034 | |

*__RSD__ = relative standard deviation

### 4.2.3 Precision

The intra-day precision of the developed method was expressed in terms of percentage relative standard deviation (% RSD). Table 4.2 shows the calculated % RSD values. The %RSD values found to be less than 2 indicated this method was precise for the determination of ranitidine hydrochloride (Wadulkar, Kalyankar, Pawar & Anitha, 2016).

Table 4.2: Intra-day precision studies

| Concentration (µg/mL) | Mean Absorbance ± Std dev (*n* = 3) | % RSD |
|---|---|---|
| 6 | 0.024 ± 0 | 0 |
| 8 | 0.031 ± 0 | 0 |
| 10 | 0.034 ± 0.001 | 1.7 |

### 4.2.4 Robustness

The robustness was determined as mentioned in chapter 3.4.1.4. Table 4.3 below shows the absorbance obtained by the two analysts and the calculated % RSD. The absorbance readings were closely and both % RSD values were found to be less than 2% indicating the reliability of the used method (Jain, Chaudhari, Patel, Patel, Patel, 2011).

Table 4.3: Robustness studies

| Component | Concentration of solution (*n* = 3) | UV-vis absorbance | |
|---|---|---|---|
| | | Analyst 1 (% RSD) | Analyst 2 (% RSD) |
| Ranitidine hydrochloride | 10 µg/mL | 0.034 (1.40) | 0.033 (1.41) |

### 4.2.5 Limit of detection and limit of quantification

Both LOQ and LOD were calculated as described in chapter 3.4.1.5. The linearity equation was found to be Y = 0.0035x – 0.0003. The LOQ and LOD for ranitidine hydrochloride in 0.1 N HCl was found to be 1.21 µg and 0.40 µg respectively indicating the sensitivity of the method.

## 4.3 PRELIMINARY FORMULATIONS

Various methods and method parameters were experimented with an attempt to yield microspheres as described in chapter 3.5. Table 4.4 summarizes the formulations manufactured including the drug: polymer (ratio w/w) used and the type and quantity of emulsifier/surfactant and the stirrer that was employed.

Table 4.4: Composition of microspheres in preliminary trials

| FORMULATION | RHCL | S100 | L100 | ETHOCEL | PEG4000 | HPMC | T80 | T20 | MG-ST | STIRRER |
|---|---|---|---|---|---|---|---|---|---|---|
| F1 | 1 | 1 | - | - | - | - | - | - | - | A |
| F2 | 1 | - | 1 | - | - | - | - | - | - | A |
| F3 | 1 | 1 | - | - | - | - | - | - | - | B |
| F4 | 1 | - | 1 | - | - | - | - | - | - | B |
| F5 | 1 | 1 | - | - | - | 0.5g | - | - | - | A |
| F6 | 1 | 2 | - | - | - | 0.5g | - | - | - | A |
| F7 | 1 | - | 1 | - | - | 0.5g | - | - | - | B |
| F8 | 1 | - | 2 | - | - | 0.5g | - | - | - | B |
| F9 | 1 | 1 | - | - | - | - | 0.5mL | - | - | B |
| F10 | 1 | 1 | - | - | - | - | 1mL | - | - | B |
| F11 | 1 | 1 | - | - | - | - | 2mL | - | - | B |
| F12 | 1 | - | 1 | - | - | - | 0.5mL | - | - | B |
| F13 | 1 | - | 1 | - | - | - | 1mL | - | - | B |
| F14 | 1 | - | 1 | - | - | - | 2mL | - | - | B |
| F15 | 1 | 1 | - | - | - | - | - | 1mL | - | B |
| F16 | 1 | 1 | - | - | - | - | - | 2mL | - | B |
| F17 | 1 | - | 1 | - | - | - | - | 1mL | - | B |
| F18 | 1 | - | 1 | - | - | - | - | 2mL | - | B |
| F19 | 1 | 1 | - | - | - | - | - | - | 0.3g | B |
| F20 | 1 | - | 1 | - | - | - | - | - | 0.3g | B |
| F21 | 1 | - | - | 10% | 1 | - | - | - | - | A |
| F22 | 1 | - | - | 10% | 1 | - | - | - | - | B |
| F23 | 1 | - | - | 20% | 1 | - | - | - | - | A |
| F24 | 1 | - | - | 20% | 1 | - | - | - | - | B |
| F25 | 1 | - | - | 20% | 0.5 | - | - | - | - | B |
| F26 | 1 | - | - | 20% | 2 | - | - | - | - | B |
| F27 | 1 | - | - | 20% | 2.5 | - | - | - | - | B |
| F28 | 1 | - | - | 20% | 3 | - | - | - | - | B |

*__RHCL__ = Ranitidine hydrochloride, __S100__ = Eugradit S100, __L100__ = Eugradit L100, __Ethocel__ =Ethyl cellulose, __HPMC__ = hydroxypropyl methylcellulose, __T80__ = Tween 80,__T20__ = Tween 20, __MG-ST__= magnesium stearate, __A__ = magnetic stirrer, __B__ = propeller type overhead stirrer

Preliminary formulations F1 to F4 were conducted in 1:1 drug: polymer ratio employing Eudragit S100 and L100 as polymers. No microspheres were formulated as, a solidified mass formation upon introduction of the drug/polymer/organic solvent mixture into the aqueous phase. This led to the experimentation with a suitable emulsifier (HPMC) in an attempt to stabilize the emulsions during the micro-emulsification process.

Formulations F5 to F8 consisted of the Eudragit polymers and HPMC. No microspheres were formed with the added HPMC as upon stirring, agglomerates of the polymer began to form which were inseparable and ultimately forming one ball mass.

Three surfactants were experimented with in while using the Eudragit polymers. Formulations F9 – F14 employed varying amounts of Tween 80; Tween 20 was added to formulations F15 to F18 and lastly magnesium stearate was incorporated to formulations F19 and F20. The addition of all three emulsifiers (magnesium stearate, Tween 20, Tween 80) made the aqueous solution oil-like thus preventing the coalescence of the polymer solution thus inhibiting the formation of microspheres. Stirring time was gradually increased from 1 hour to 9 hours and no microspheres formed.

Upon collection of the masses, the aqueous phase was yellow stained suggesting that ranitidine hydrochloride had leaked into the aqueous phase resulting in poor drug entrapment (Singh & Chaudhary, 2011) thus solvent evaporation method was used from Formulation F21 onwards employing ethyl cellulose and PEG 4000 as polymers and the vehicle phase was changed to liquid paraffin.

Formulations F21 and F22 had a constant amount of 10% ethyl cellulose solution. Formulation F21 did not yield microspheres as the magnetic stirrer created a vortex of the vehicle phase (liquid paraffin) and microsphere-forming particles adhered to the

wall of the beaker. Formulation F22 formed tiny agglomerations that disintegrated into powder after drying.

Formulations F23 to F28 consisted of a constant amount of 20% ethyl cellulose solution. No microspheres were formed with formulation F23 due to the vortex caused by the magnetic stirrer. Due to the material loss associated with the use of the magnetic stirrer, this equipment was not used further for preliminary formulations. Formulation F24 was the first successful method to yield microspheres with a 1:1 ratio w/w of ranitidine hydrochloride and PEG 4000.

Formulations F25 and F26 had drug: PEG 4000 ratios of 1:0.5 and 1:2 respectively and no microspheres were formed even after 9 hours of stirring. Throughout the stirring, drug particles segregated to the bottom of the beaker. Formulation F26, with a drug: PEG 4000 ratio of 1:2 w/w, successfully yielded microspheres. The increase of drug: PEG 4000 ratio beyond 1:2 w/w, as with formulation F27 and F28, resulted in mass formations of agglomerates shortly after the introduction of the drug/polymer solution to the vehicle phase.

Stirring at low speeds caused adherence of the microsphere forming solution to the walls of the beaker and to the propeller itself thus only a three-bladed overhead propeller could provide the required rotation speed without the formation of a vortex. For these reasons, ethyl cellulose and PEG 4000 were selected as the polymers and compatibility between them and ranitidine hydrochloride was determined before developing prototype formulations using the solvent evaporation method.

## 4.4 DRUG-EXCIPIENT COMPATIBILITY STUDIES

Compatibility between ranitidine hydrochloride and the selected excipient was determined as mentioned in chapter 3.6. The findings of differential scanning

calorimetry/ thermogravimetric analysis, Fourier-transformed infrared and short-term accelerated studies are presented and analysed in that respective order.

### 4.4.1 Differential scanning calorimetry/ thermogravimetric analysis

The TGA-DSC thermograms of ranitidine hydrochloride, ethyl cellulose, PEG 4000 and the two binary mixtures of ranitidine hydrochloride and each of the polymers are presented and analysed in this section and the findings discussed. The thermograms of ranitidine hydrochloride and the polymers were interpreted as follows: A slight change or broadening of the endothermic peak of ranitidine hydrochloride or the polymers was considered as a low degree of incompatibility; the disappearance of endothermic peaks or the appearance of new endothermic peaks was regarded as a high degree of incompatibility. A change in the peaks of at least 10˚C was considered significant (Heljo, 2007).

#### **4.4.1.1** Thermogram of pure ranitidine hydrochloride

The melting range of ranitidine hydrochloride to be between 143 – 145˚C (Ramachandran, Nandhakumar & Dhanaraju, 2011). The TGA-DSC thermogram of ranitidine hydrochloride, depicted in Figure 4.1 indicated a single melting endotherm at 145.9˚C, which illustrated the purity of ranitidine hydrochloride. The TGA curve revealed a one-step thermal degradation that occurred at 89˚C and ended at around 140˚C in which a mass loss of 2.10% was observed from the original sample weight due to the presence of moisture.

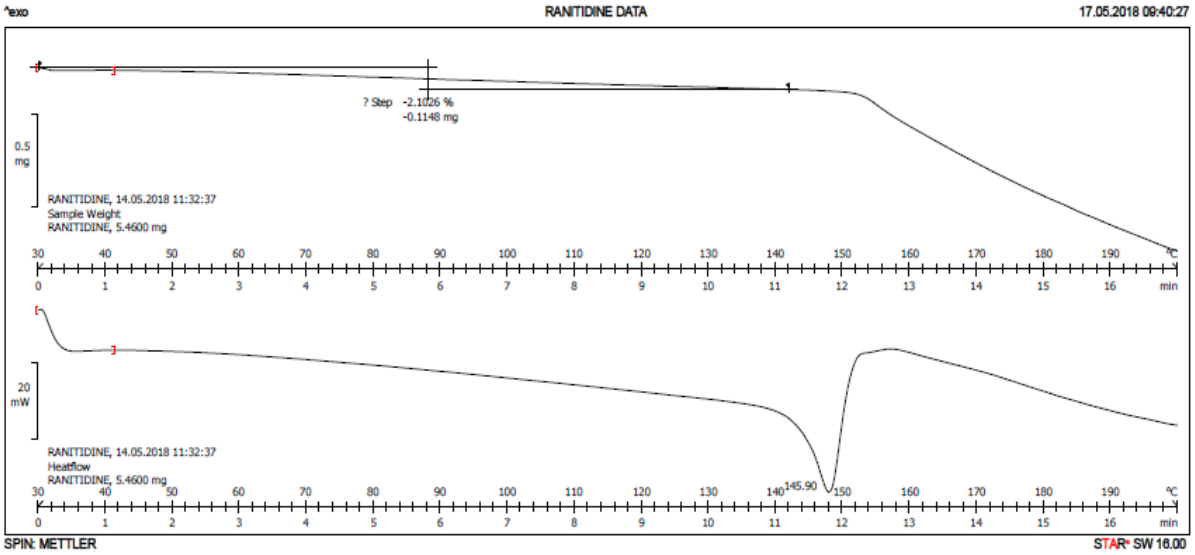



Figure 4.1: TGA-DSC thermogram of pure ranitidine hydrochloride.

### 4.4.1.2 Thermogram of ethyl cellulose

The TGA-DSC thermogram of ethyl cellulose is depicted in Figure 4.2. No endothermic peak was observed indicating the complete amorphous nature of ethyl cellulose (Govindasamy, Krishnamoorthy & Rajappan, 2013). The TGA curve indicates that no mass loss occurred with the sample.

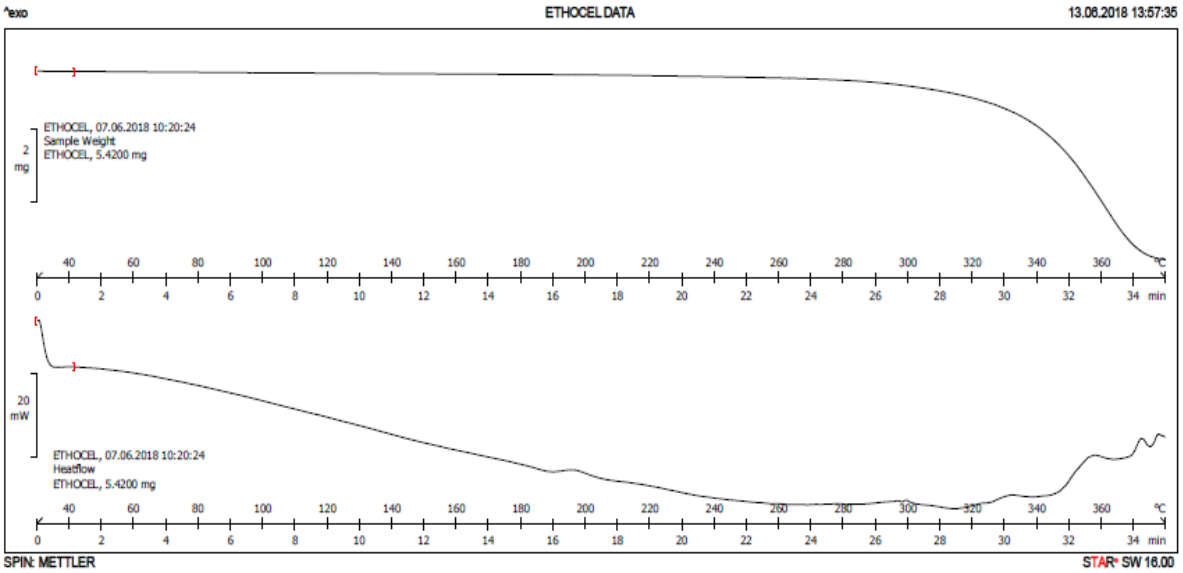



Figure 4.2: TGA-DSC thermogram of ethyl cellulose.

#### 4.4.1.3 Thermogram of PEG 4000

In literature, PEG 4000 are reported to have a melting point of 58 – 62°C (Ibolya, Gyeresi, Szabo-Revesz, & Aigner, 2011). In the TGA-DSC thermogram of PEG 4000 depicted in Figure 4.3 a single melting endotherm was observed at 60.77°C which indicated the purity of PEG 4000. The TGA curve indicates that no mass loss occurred.

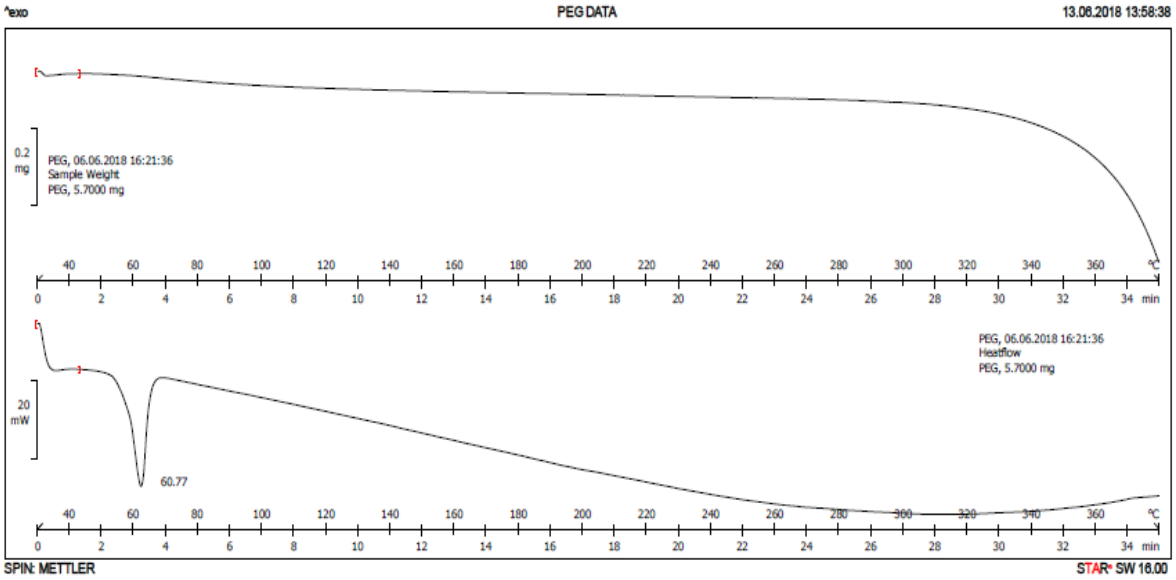



Figure 4.3: TGA-DSC thermogram of PEG 4000.

#### 4.4.1.4 Thermogram of ranitidine hydrochloride and ethyl cellulose binary mixture

Figure 4.4 depicts the DSC thermogram of the ranitidine hydrochloride and ethyl cellulose 1:1 w/w binary mixture. A slight change in the endothermic peak of ranitidine hydrochloride was observed from 145.9˚C to 146.9˚C. The TGA curve, depicted in Figure 4.5, showed a two-step thermal degradation; the mixture started to degrade thermally at 230˚C and it ended at 270˚C in which a 22.07% mass loss was observed. The second thermal degradation started at 290˚C and ended at around 380˚C. A mass loss of 73.92% was observed due to the decomposition of ranitidine hydrochloride (Perpetuo, Galico, Fugita, Castro, Eusebio, Treu-Filho, Silva, Bannach, 2013). The minor changes of endothermic peaks were considered insignificant which suggested that ranitidine hydrochloride and ethyl cellulose were compatible with one another.

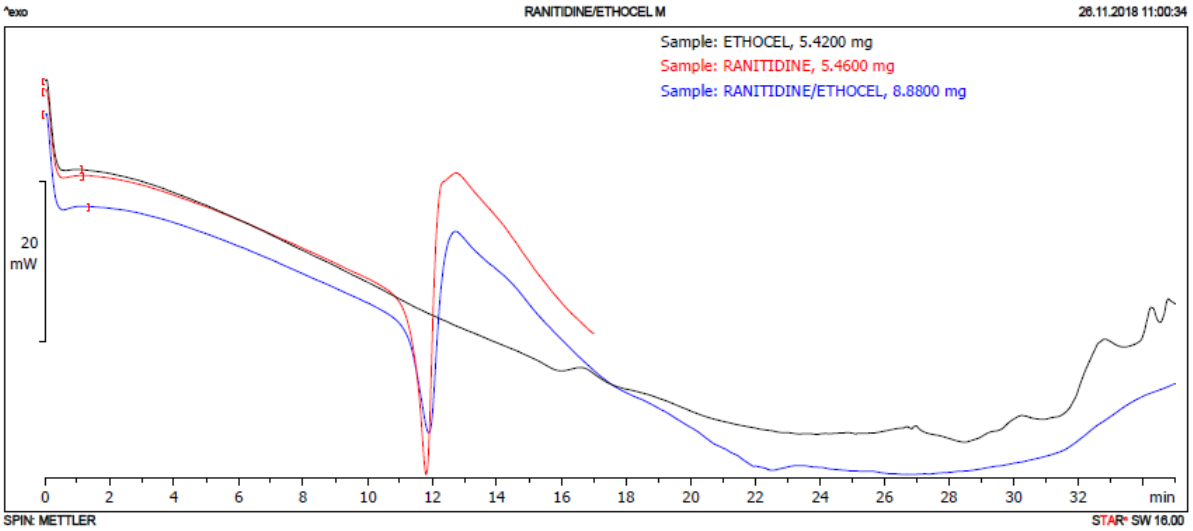



Figure 4.4: DSC Thermogram of the binary mixture of ranitidine hydrochloride and ethyl cellulose (1:1 w/w).

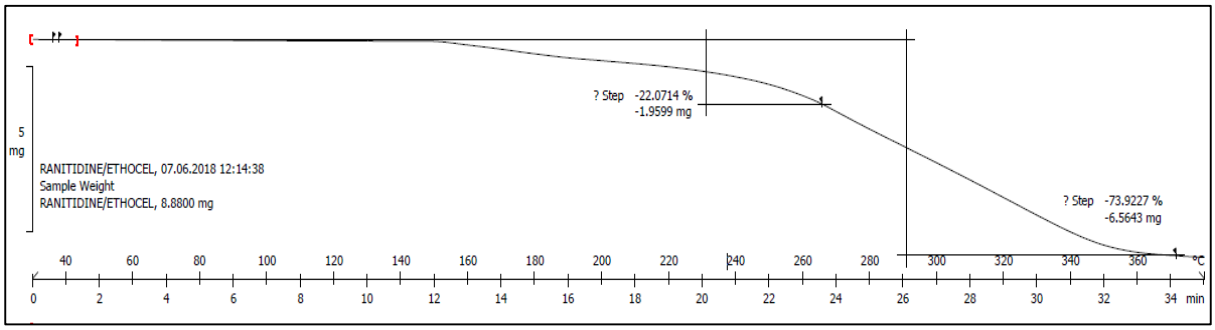



Figure 4.5: TGA curve of a binary mixture of ranitidine hydrochloride and ethyl cellulose (1:1 w/w).

### 4.4.1.5 Thermogram of ranitidine hydrochloride and PEG 4000 binary mixture

Figure 4.6 depicts the DSC thermogram of the ranitidine hydrochloride and PEG 1:1 w/w binary mixture. A slight shift in the melting point of both PEG 4000 and ranitidine hydrochloride is observed from 60.77 ˚C to 61.03 ˚C and from 145.90˚C to 145.14˚C respectively. The TGA curve, depicted in Figure 4.7, showed two-step thermal degradation events. The binary sample started to degrade thermally at 120 ˚C which ended at 160˚C with mass loss of 1.04%. The second thermal degradation occurred at 240˚C and ended around 300˚C with an increased mass loss of 31.05% due to the decomposition of ranitidine hydrochloride (Raut, Umekar, Kotagale, 2013). These

slight shifts are not significant which suggests that ranitidine hydrochloride and PEG 4000 are compatible with each other.

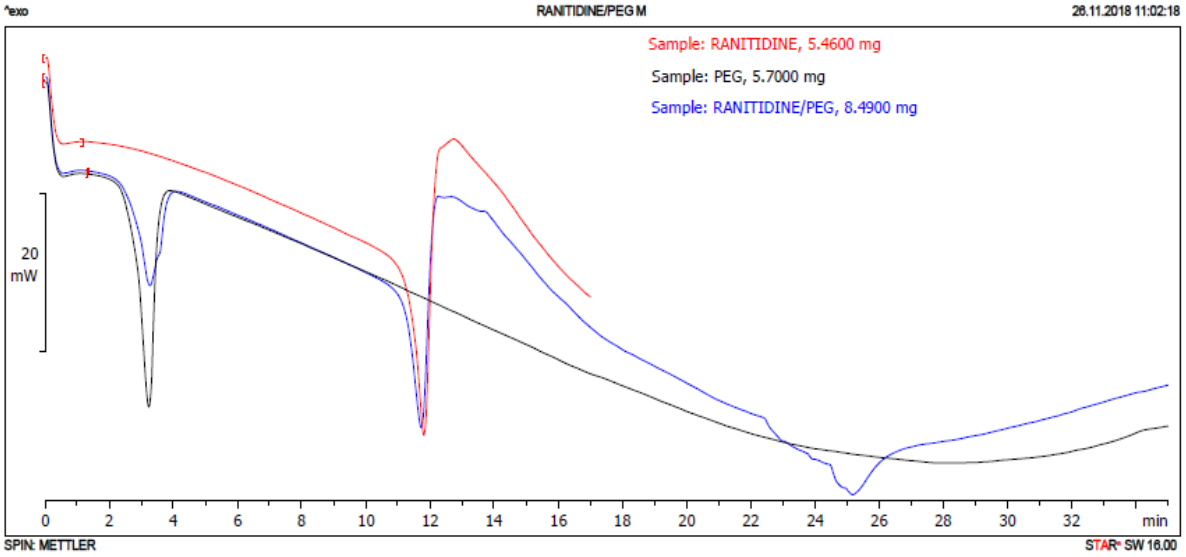



Figure 4.6: DSC Thermogram of the binary mixture of ranitidine hydrochloride and PEG 4000 (1:1 w/w).

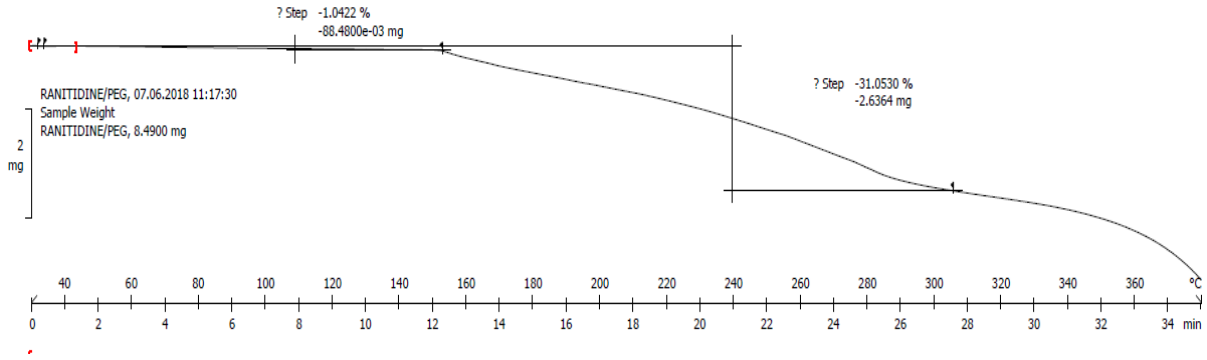



Figure 4.7: TGA curve of the binary mixture of ranitidine hydrochloride and PEG 4000 (1:1 w/w).

### 4.4.2 Fourier-transformed infrared spectroscopy

The IR spectra of ranitidine hydrochloride, ethyl cellulose and PEG 4000 and the binary mixtures of ranitidine hydrochloride and each of the polymers was analysed and compared to literature. The findings are presented in the respective order. The IR

spectrums were interpreted as follows: the appearance of the drugs characteristic peaks with no change or a slight change but still represents the same functional group was regarded as a low degree of incompatibility; the disappearance of any of the drugs/polymers characteristic peaks was regarded as a high degree of incompatibility (Manikandan *et al., 2013*).

### 4.4.2.1 Fourier-transformed infrared spectrum of ranitidine hydrochloride

The IR spectrum of ranitidine hydrochloride is shown in Figure 4.8 characteristic peaks were observed and compared with that of USP (2012) standards. Table 4.5 describes the characteristic peaks observed and their assigned functional groups which conform to the peaks of pure ranitidine in the USP.

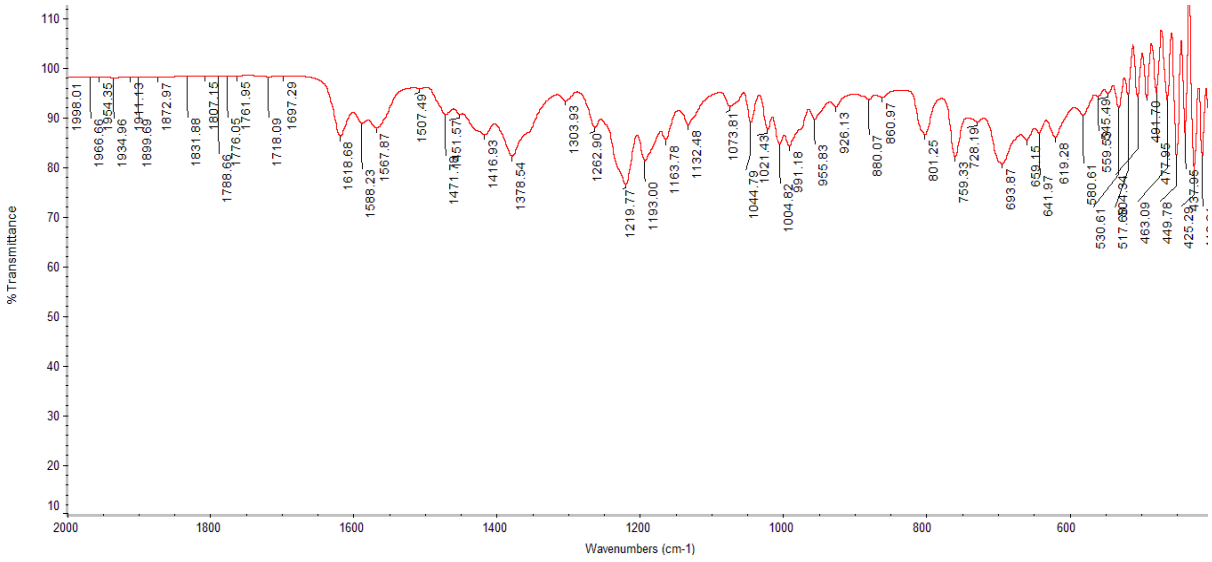



Figure 4.8: IR spectrum of ranitidine hydrochloride.

Table 4.5: Characteristic peaks and chemical groups observed in the IR spectrum of ranitidine hydrochloride

| Peak value ($cm^{-1}$) | Functional group assignment |
|---|---|
| 1618 | C=C stretching |
| 1588 | C−C stretching |
| 1417 | C−N symmetrical stretching |
| 1378 | NO2 symmetrical stretching |
| 1304 | C−N symmetrical stretching |
| 1263 | C−H in-plane stretching |
| 1220 | C−H out of plane stretching |
| 1074 | C−H in-plane deformation |
| 1045 | C−C stretching |
| 1021 | C−N stretching |
| 1005 | C−C−C stretching |
| 991 | C−O asymmetrical stretching |
| 956 | H−C−C stretching |
| 926, 880 | C−C stretching |
| 801 | C−H out of plane bending |
| 759 | C−C−C bending |
| 693 | H−C−C bending |

#### **4.4.2.2** Fourier-transformed infrared spectrum of ethyl cellulose

Lin (2008) identified the characteristic peaks of ethyl cellulose to be at 1052, 1369, 2880, 2970 and 3500 $cm^{-1}$ which are assigned to C-O-C stretching, C-H bending, C-H stretching, C-H stretching and O-H stretching respectively. The IR spectra of ethyl cellulose is shown in Figure 4.9 and Table 4.6 describes the characteristic peaks observed for ethyl cellulose and their assigned functional groups.

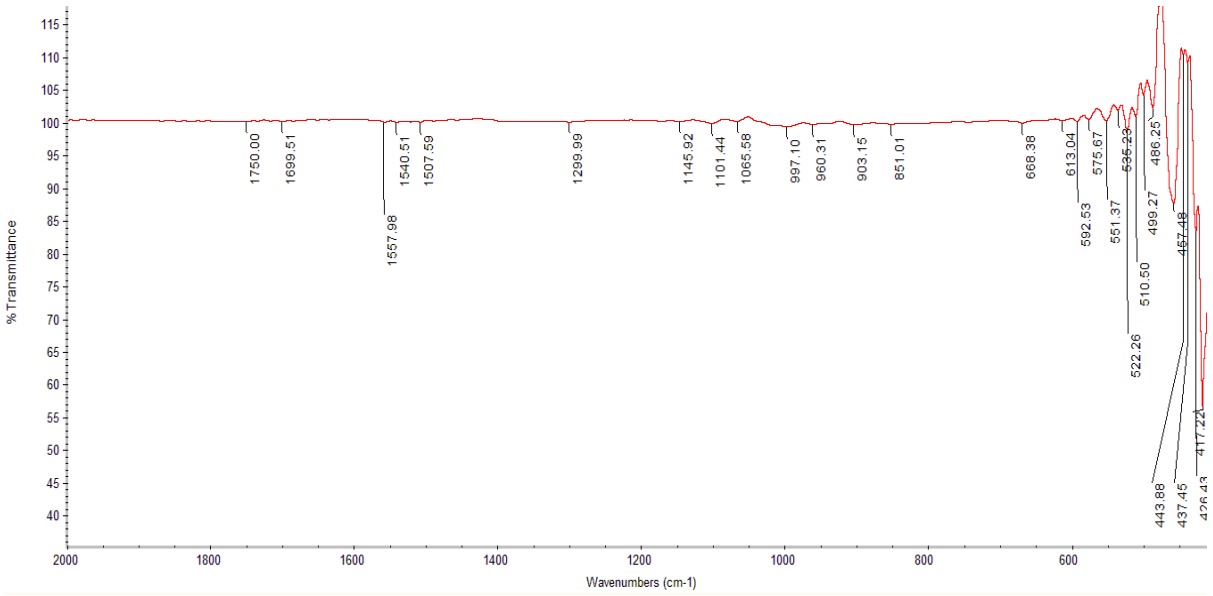



Figure 4.9: IR spectrum of ethyl cellulose.

Table 4.6: Characteristic peaks and chemical groups observed in the IR spectrum of ethyl cellulose

| **Peak value ($cm^{-1}$)** | **Functional group assignment** |
|---|---|
| 1065 | Ether C – O – C stretching |
| 1369 | C – H bending |

### 4.4.2.3 Fourier-transformed infrared spectrum of PEG 4000

The IR spectra of PEG 4000 is depicted in Figure 4.10. Shameli, Ahmad, Jazayeri, Sedaghat, Shabanzadeh, Jahangirian, Mahdavi & Abdollahi (2012) identified the characteristic peaks for PEG to be observed at 1464 and 1343 $cm^{-1}$ due to the bending of C – H bonds, peaks at 1279, 1240 and 2094 $cm^{-1}$ due to the stretching of O – H and C – O – H bonds and lastly, a peak at 525 $cm^{-1}$ due to the presence of an oxygen atom. Table 4.7 describes the characteristic peaks observed for PEG 4000 and their assigned functional groups.

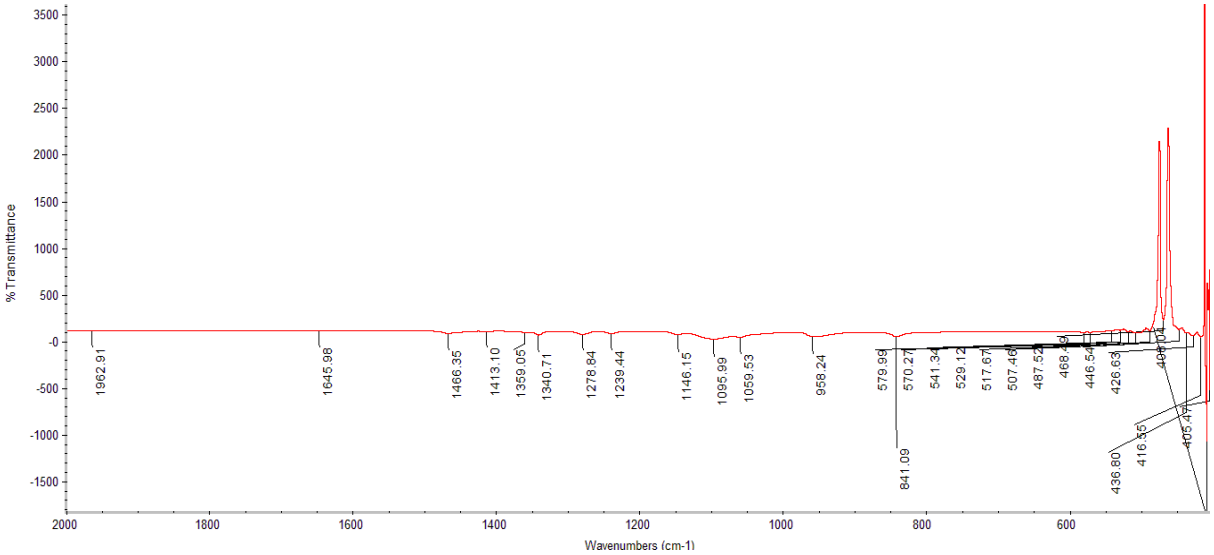



Figure 4.10: IR spectrum of polyethylene glycol 4000.

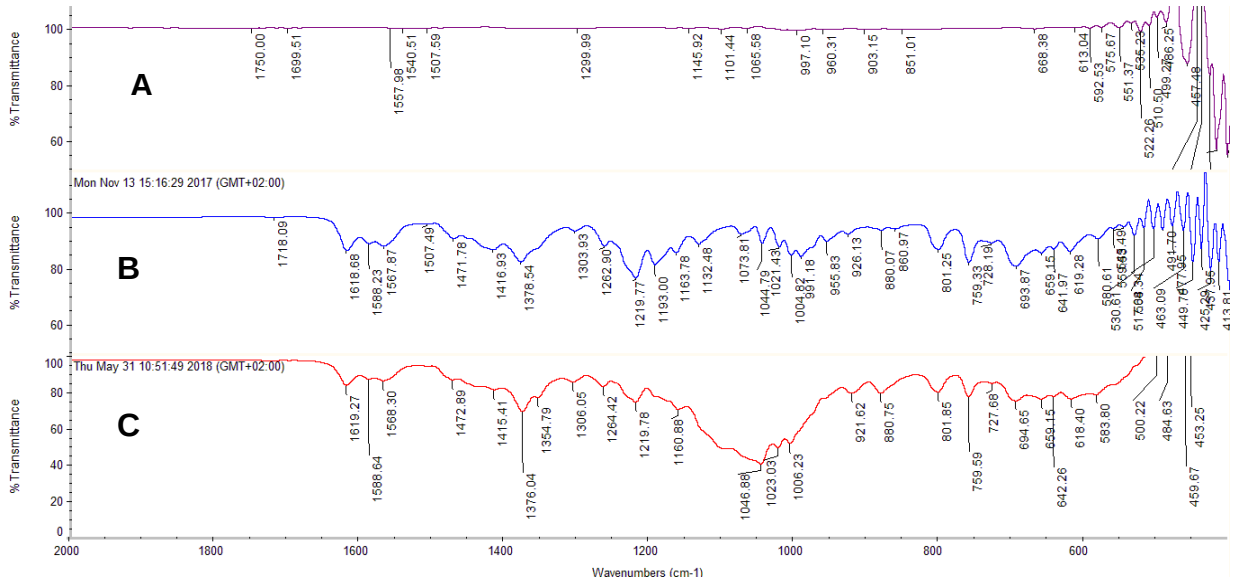
Table 4.7: Characteristic peaks and chemical groups observed in the IR spectrum of PEG 4000

| **Peak value ($cm^{-1}$)** | **Functional group assignment** |
|---|---|
| 1466 | C - H bending |
| 1340 | C - H bending |
| 1278, 1239 | O - H and C - O - H stretching |
| 529 | Oxygen atom |

### 4.4.2.4 Fourier-transformed infrared spectrum of ranitidine hydrochloride and ethyl cellulose

The IR spectrum of ranitidine hydrochloride and that of ethyl cellulose (were compared with the IR spectrum of the 1:1 (w/w) binary mixture of ranitidine hydrochloride and ethyl cellulose depicted in Figure 4.11. Eight minor peaks of ranitidine were observed in the binary mixture, namely: from 1618 $cm^{-1}$ to 1619 $cm^{-1}$, 1417 $cm^{-1}$ to 1415 $cm^{-1}$, 1378 $cm^{-1}$ to 1376 $cm^{-1}$, 1304 $cm^{-1}$ to 1306 $cm^{-1}$, 1263 $cm^{-1}$ to 1264 $cm^{-1}$, 1005 $cm^{-1}$ to 1006 $cm^{-1}$, 926 $cm^{-1}$ to 922 $cm^{-1}$ and lastly from 693 $cm^{-1}$ to 695 $cm^{-1}$. One shift peak

is observed for ethyl cellulose which is from 1369 $cm^{-1}$ to 1354 $cm^{-1}$. Considering the shifting of the peaks, a low degree of incompatibility was expected between ranitidine hydrochloride and ethyl cellulose.

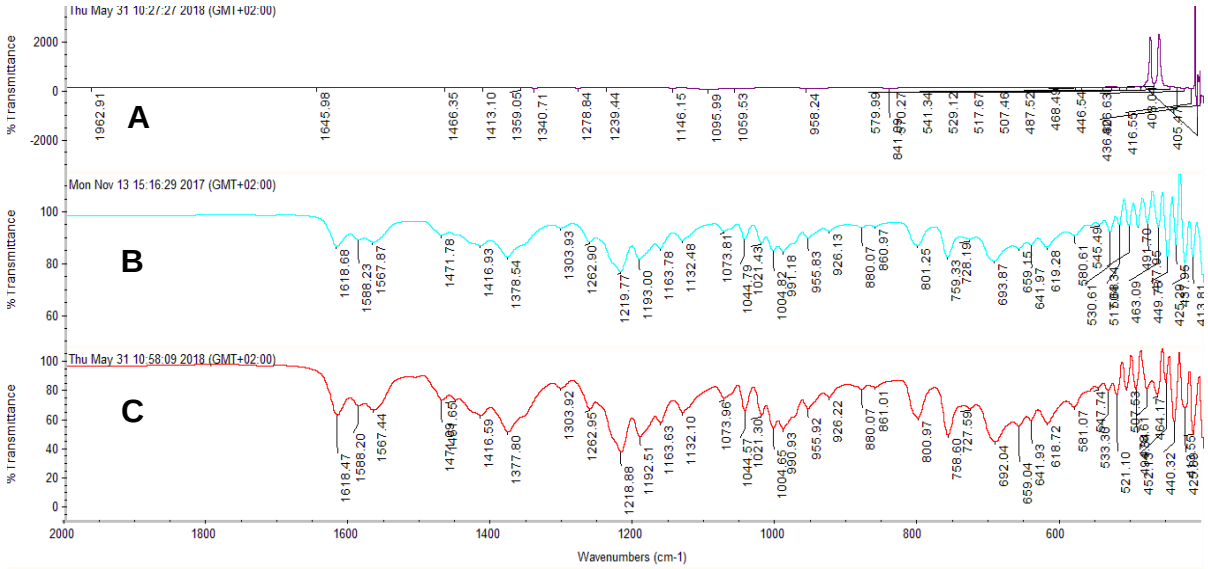



***A** = IR spectrum of ethyl cellulose, **B** = IR spectrum of a binary mixture of ranitidine hydrochloride and ethyl cellulose & **C** = IR spectrum of ranitidine hydrochloride.

Figure 4.11: IR spectrum of binary mixture of ranitidine hydrochloride and ethyl cellulose (1:1 w/w)

#### 4.4.2.5 Fourier-transformed infrared spectrum of ranitidine hydrochloride and PEG 4000

The IR spectrum of ranitidine hydrochloride and that of PEG 4000 were compared with the IR spectrum of the 1:1 (w/w) binary mixture of ranitidine hydrochloride and PEG 4000 depicted in Figure 4.12. Only two slight peak shifts of ranitidine were observed in the spectrum of the binary mixture, which was a peak shift from 1220 $cm^{-1}$ to 1219 $cm^{-1}$ and a peak shift 693 $cm^{-1}$ to 692 $cm^{-1}$. With regards to PEG 4000, only two characteristic peaks are observed and they have slightly shifted from 1466 $cm^{-1}$ to 1471 $cm^{-1}$ and from 529 $cm^{-1}$ to 533 $cm^{-1}$. Considering the minor shifting of the peaks, a low degree of incompatibility was expected between ranitidine hydrochloride and PEG 4000.




*__A__ = IR spectrum of PEG 4000, __B__ = IR spectrum of a binary mixture of ranitidine hydrochloride and PEG 4000 & __C__ = IR spectrum of ranitidine hydrochloride.

Figure 4.12: IR spectrum of binary mixture of ranitidine hydrochloride and PEG 4000 (1:1 w/w)

### 4.4.3 Short term accelerated studies

Short term accelerated studies were conducted as described in chapter 3.6.3 and the collected samples were assayed as described in chapter 3.8.9. According to WHO (2018) guidelines, a significant change is defined as “a change from the initial content of API of 5% or more detected by assay”. The assay, calculated percentage degradation and observed physical changes of ranitidine hydrochloride and the binary mixtures of ranitidine hydrochloride: polymers are summarized in Table 4.8.

Table 4.8: Results of UV-vis analysis data for short-term accelerated studies at time 0 and 3 weeks

| Sample | Drug: polymer ratio (w/w) | Initial results (mg) | After 3 weeks (mg) | % degradation | Physical change |
|---|---|---|---|---|---|
| **RHCL** | - | 100.81 | 99.74 | 1.06 | None |
| **RHCL + E** | 1:1 | 101.42 | 100.27 | 1.13 | None |
| **RHCL + P** | 1:1 | 101.21 | 99.78 | 1.41 | None |

*__RHCL__= ranitidine hydrochloride, **E** = ethyl cellulose & **P** = PEG 4000

From the results in Table 4.7, the percentage degradation for ranitidine hydrochloride and the binary mixtures were found to be less than 5% and no physical change was observed after the accelerated stability study. This falls within the specifications thus indicating compatibility between ranitidine hydrochloride and the selected polymers.

## 4.5 FORMULATION STUDIES

Two batches of ranitidine hydrochloride loaded microspheres were successfully manufactured with ethyl cellulose and PEG 4000 using the method described in chapter 3.7. The two batches consisted of a constant 20% of ethyl cellulose and a varying ratio of ranitidine hydrochloride to PEG 4000. The first batch, henceforth referred to as formulation F24, is the product of a 1:1 w/w ratio of ranitidine hydrochloride: PEG 4000 and; the second batch, from now on referred to as formulation F26, is the product of a 1:2 w/w ratio of ranitidine hydrochloride: PEG 4000. All post-formulation studies were conducted on both formulations F24 and F26.

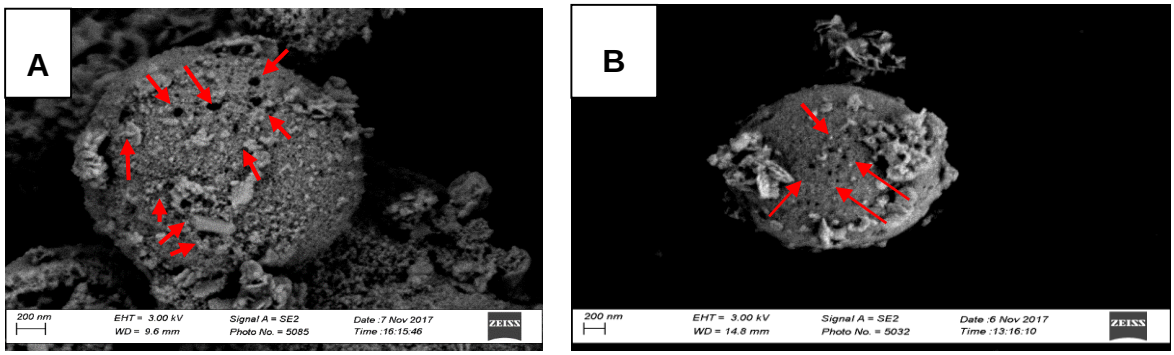
## 4.6 POST-FORMULATION STUDIES

The formulated microspheres were evaluated for various aspects such as shape and morphology, micrometric properties, percentage yield, drug content and percentage

buoyancy as described in chapter 3.8. The results for those aspects are presented and discussed in that order.

### 4.6.1 Shape and surface morphology

Microphotographs acquired (Figure 4.13) using scanning electron microscope that revealed that the microspheres formulated were spherical in shape with a porous surface (as highlighted in the microphotographs). The porosity was due to diffusion and evaporation of solvents during the agitation and overnight drying.




**A** = microsphere from formulation F26; **B**= microsphere from formulation F24

Figure 4.13: Microphotographs of microspheres

### 4.6.2 Particle size distribution

The particle size distribution of the formulation F24 and F26 microspheres was determined as described in chapter 3.8.2. Majority of formulation F24 microspheres were retained on the 600 µm and 710 µm sieve (25% each). A considerably high fraction of microspheres was retained at the 850 µm and 1000 µm sieves (15 and 18 % respectively). A total of 17% of the microspheres retained between the collecting pan and the 500 µm pan. The particle size distribution plot for formulation F24 is shown in Figure 4.14. The median particle diameter (MPD) of formulation F24 microspheres was found to be 596.45 µm.

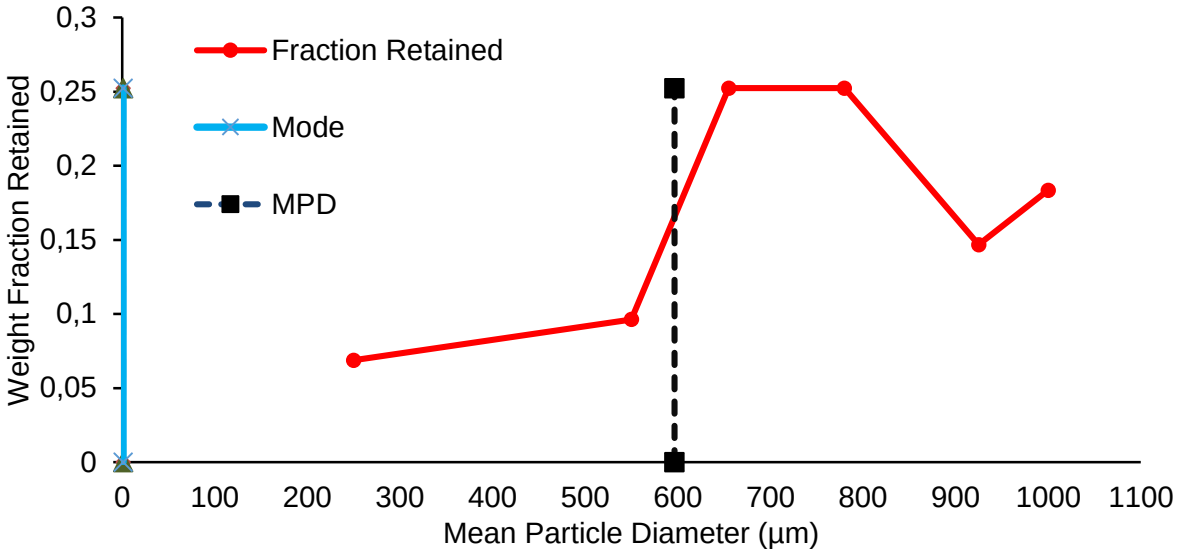



Figure 4.14: Particle size distribution of formulation F24 microspheres

A great portion of formulation F26 microspheres were unable to pass through the 1000 µm sieve (34%). A total of 26, 14 and 12% of the microspheres were retained on the 710 µm, 600 µm and 850 µm respectively. The collecting pan had the lowest number of microspheres (7%). It was evident through formulation F26 that the amount of the polymer used to directly proportional to the size of the microspheres. The particle size distribution plot for formulation F26 is shown in Figure 4.15. The MPD of the formulation F26 was found to be 660.52 µm.

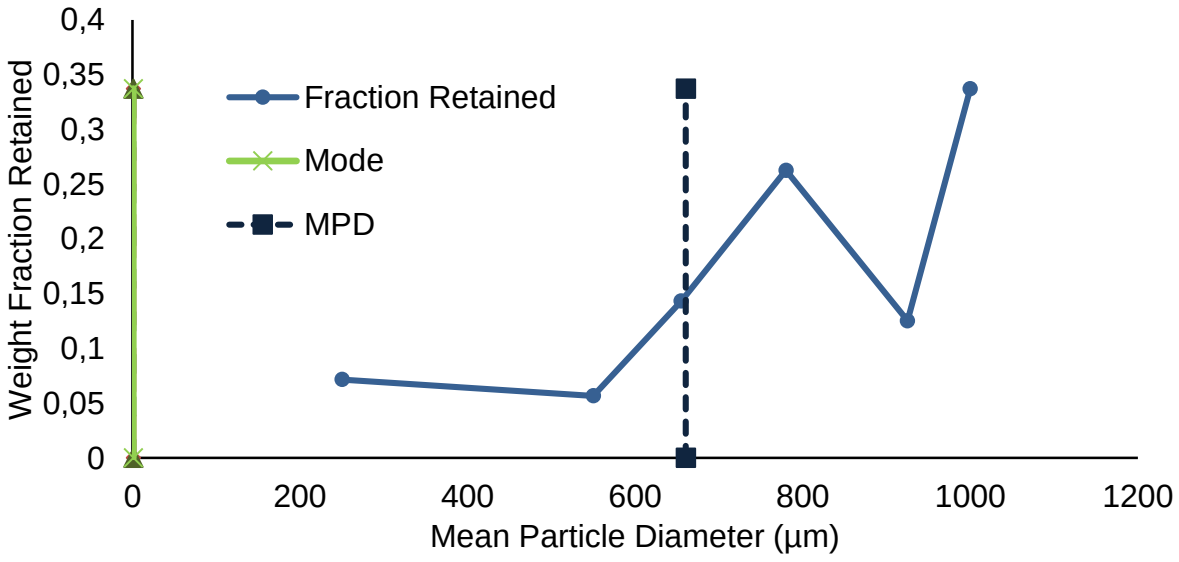



Figure 4.15: Particle size distribution of the microspheres

### 4.6.3 Bulk density and Tapped density

Bulk and tapped density were performed on the formulated microspheres as described in chapter 3.8.3 and 3.8.4. Bulk densities of 0.48 g/mL and 0.5 g/mL were observed for formulation F24 and F26 respectively. Tapped densities of 0.56 g/mL and 0.59 g/mL were observed for formulation F24 and F26 respectively.

### 4.6.4 Hausner's ratio and Carr's index

Hausner' ratio was calculated to be 1.17 and 1.18 for formulation F24 and F26 respectively. Carr's index was calculated to be 14.29% and 15% for formulation F24 and F26 respectively. Both formulations were between the 1.12-1.18 Hausner's ratio range and were both within the 11 – 15% range for compressibility index meaning that both formulations are categorized as having a good flow character.

### 4.6.5 Angle of repose

The angle of repose was determined on the formulated microspheres as described in chapter 3.8.6. The angle of repose for both formulation F24 and F26 was calculated to be 26 degrees. This put the microspheres in the 25 – 30 range for of the angle of repose meaning both the formulations have excellent flow properties which facilitated the capsulation of the microspheres.

### 4.6.6 Loss on drying

The loss on drying for formulation F24 and F26 was 4.08% and 1.01% respectively. In literature, when ranitidine hydrochloride is tested for loss on drying it is recorded not to lose more than 0.75% of its initial weight (USP, 2014). Both the microsphere formulations fall outside this criterion, which is attributed to the loss of moisture due to the incorporation of ethyl cellulose that has a loss on drying range of 44 - 51% (USP, 2018).

### 4.6.7 Percentage yield

The percentage yield for the formulated microspheres was determined as described in chapter 3.8.8. The calculated percentage yields for formulation F24 and F26 were 99.5% and 98.85% respectively. Material loss is attributed to PEG 4000 which would stick onto the walls of the glass beaker and get trapped into the edges of the propeller blades when stirring.

### 4.6.8 Drug content of microspheres

The calculated theoretical drug content of 100mg of formulation F24 and F26 microspheres are 31 mg and 24 mg respectively. Good percentages of drug content were observed in both formulations in which formulation F24 had a drug content of 80% (approximately 25 mg per 100 mg of microspheres) and formulation F26 had a drug content of 88% (approximately 23 mg per 100 mg of microspheres).

### 4.6.9 Percentage buoyancy

Buoyancy studies revealed a lag time of zero was required for both formulations. The percentage buoyancy of formulation F24 and F26 was calculated to be 14,5 and 52% at the end of the 8 hours respectively. It is evident that formulation F26 had better floating properties which is attributed to cavities in the microspheres and the presence of pores on the surface of the microspheres (Singh & Chaudhary, 2011). When introduced into the dissolution media, PEG dissolves forming pores on the microspheres due to matrix erosion; this phenomenon makes the microspheres to float (Saravanan & Anupama, 2011). The floating ability of the microspheres for eight hours

may be considered satisfactory, particularly for formulation F26. Figure 4.16 shows floating microspheres being assessed for buoyancy capabilities with the microspheres of formulation F26.

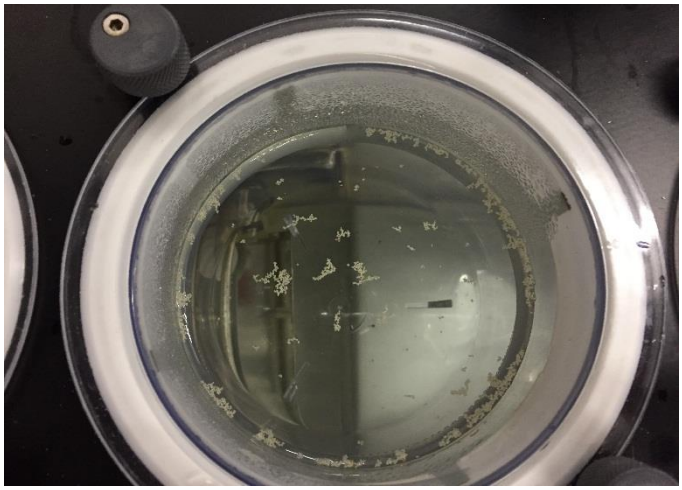


Figure 4.16: Floating microspheres during buoyancy testing.

## 4.7 POST-CAPSULATION STUDIES

The microspheres were capsulated as described in chapter 3.9. Therefore, post-capsulation tests were conducted as mentioned in chapter 3.10 and are represented and discussed in that order.

### 4.7.1 Capsule dimensions

Table 4.9 and Table 4.10 below indicates the statistical length and diameters dimensions of twenty capsules filled with formulation F24 and F26 microspheres respectively which were measured as described in chapter 3.10.1. The capsules were found to comply with the USP specifications for filled capsules.

Table 4.9: Dimensions of 20 encapsulated microspheres (Formulation F24)

| Capsule no = 20 | Diameter (mm) | Length (mm) |
|---|---|---|
| Total for 20 caps | 146.72 | 425.34 |
| Average for 20 caps | 7.336 | 21.267 |
| Std. dev | 0.0567914 | 0.110553631 |

*Std dev= standard deviation

Table 4.10: Dimensions of 20 encapsulated microspheres (Formulation F26)

| Capsule no = 20 | Diameter (mm) | Length (mm) |
|---|---|---|
| Total for 20 caps | 145.72 | 420.68 |
| Average for 20 caps | 7.286 | 21.034 |
| Std dev | 0.068702256 | 0.113527182 |

*Std dev = standard deviation

### 4.7.2 Weight variation test

Weight variation test was performed as described in chapter 3.10.2. Table 4.11 and Table 4.12 represents the weight variation statistics for weight obtained when weighing intact capsules, weighing the empty capsule shells and the calculated fill content of 20 formulation F24 and F26 capsules respectively.

Table 4.11: Weight variation stats for 20 encapsulated microspheres (Formulation F24)

| Capsule no = 20 | Total mass (g) | Empty shell (g) | Fill mass (g) |
|---|---|---|---|
| Total for 20 capsules | 8.3552 | 1.821 | 6.5342 |
| Average for 20 capsules | 0.41776 | 0.09105 | 0.32671 |
| Std dev | 0.011709708 | 0.001384691 | 0.011898956 |

*Std dev = standard deviation

Table 4.12: Weight variation stats for 20 encapsulated microspheres (Formulation 27)

| Capsule no = 20 | Total mass (g) | Empty shell (g) | Fill mass (g) |
|---|---|---|---|
| Total for 20 capsules | 8.8515 | 1.7731 | 7.0784 |
| Average for 20 capsules | 0.442575 | 0.088655 | 0.35392 |
| Std dev | 0.013242033 | 0.00214929 | 0.01335994 |

*Std dev = standard deviation

Both formulation F24 and F26 capsules weighed above 300 mg and as per USP, the first deviation limit applicable is ± 7.5% and the second being ± 15% in. For each of the batches to pass this test, a minimum of 18 capsules must comply with the first deviation limit and no more than 2 capsules must go beyond the second deviation.

For formulation F24 capsules, one capsule did not comply with the first deviation limit but it did not exceed the second deviation limit which means this batch has passed the weight variation test.

For the formulation F26 capsules, one capsule did not comply with the first deviation limit but it did however, comply with the second deviation limit which means this batch also passes the weight variation test.

### 4.7.3 *In vitro* drug release

The drug release of ranitidine hydrochloride from the microspheres was determined as mentioned in the previous chapter 3.10.3. The percentage cumulative drug release was calculated for all the intervals (30, 120, 240, 360, 480 and 720 minutes) and is shown in Table 4.13. The dissolution data was also used to plot *in vitro* dissolution profile for both formulations as depicted in Figure 4.17.

Table 4.13: Percentage *in vitro* drug release

| Time (minutes) | % *in vitro* drug release ± SD | |
|---|---|---|
| | **Formulation F24 (*n*=6)** | **Formulation F26 (*n*=6)** |
| 30 | 35 ± 0.52 | 30 ± 0.75 |
| 120 | 54 ± 0.75 | 56 ± 1.94 |
| 240 | 57 ± 0.75 | 73 ± 1.37 |
| 360 | 62 ± 0.52 | 89 ± 1.22 |
| 480 | 67 ± 1.03 | 107 ± 1.33 |
| 720 | 69 ± 1.09 | 108 ± 1.04 |

***SD** = standard deviation

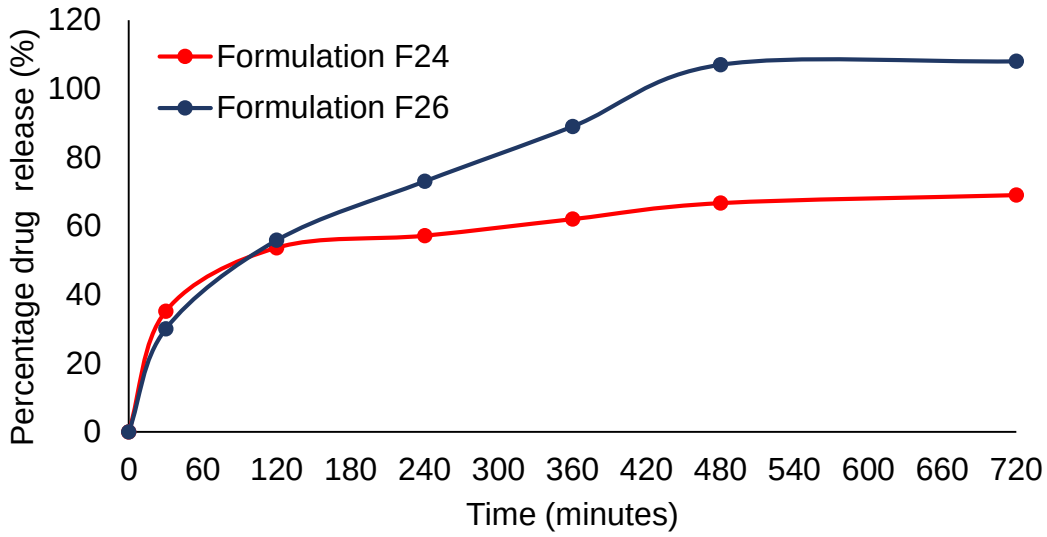



Figure 4.17: Dissolution profiles of the prototype formulations

At the first 30 minutes, the drug release was 35 and 30% for the formulation F24 and F26 respectively. This initial burst release occurred because of the drug that was present on the surface of the microspheres (Rahman, Ahmed, Hasan & Reza, 2016). Formulation F24 has a delayed yet incomplete drug release of 69% seen at 720 minutes. Formulation F26 showed delayed and complete drug release at 480 minutes.

Ethyl cellulose is a hydrophobic water-impermeable matrix that retards drug release while PEG is water soluble polymer commonly used to increase the solubility of water-insoluble products. During the formulation of the ranitidine loaded microspheres, PEG 4000 was incorporated to enhance drug release so that when the microspheres come into contact with the dissolution media, the fluid permeates into the particles, solubilizes the PEG 4000 and the drug becomes soluble and passes through the channels made by the dissolution of PEG (Malipeddi *et al.*, 2016). Formulation F24 had retarded drug release that was attributed to the slower rate of diffusion of the dissolution medium into the microspheres (Rama, Senapati & Das, 2005). Increasing the amount of PEG 4000 enhanced permeation of the dissolution fluid into the microsphere, allowing for complete and controlled drug release as seen in formulation F26 (Zanetti-Ramos, Soldi, Soldi & Lemos-Senna, 2006).

### 4.7.4 Dissolution kinetics

All the data that was obtained from *in vitro* release studies were fitted to various kinetics equations as described in chapter 3.10.4. Figures 4.18, 4.19, 4.20, 4.21 and 4.22 depicts the curves of the mathematical models and the calculated $R^2$ values for both microsphere formulations.

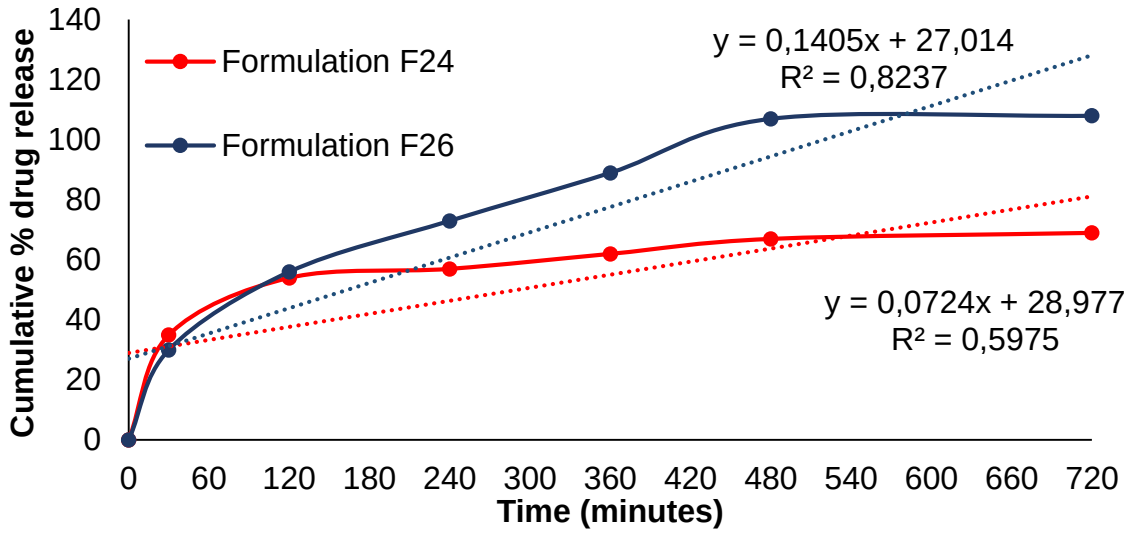



Figure 4.18: Zero-order kinetics model

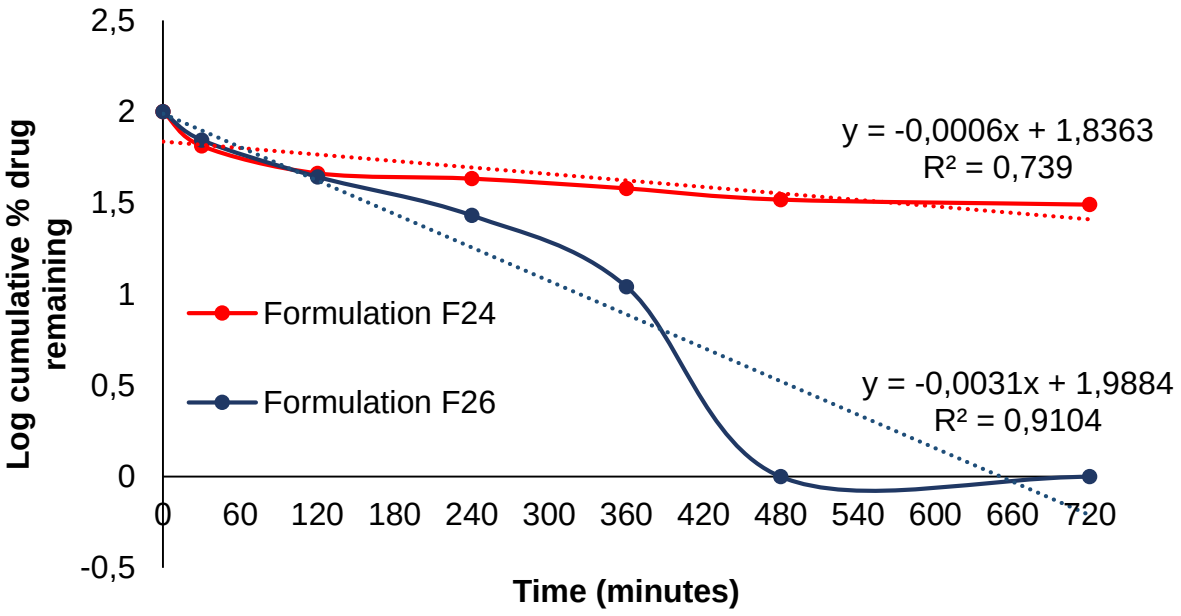



Figure 4.19: First-order kinetics model

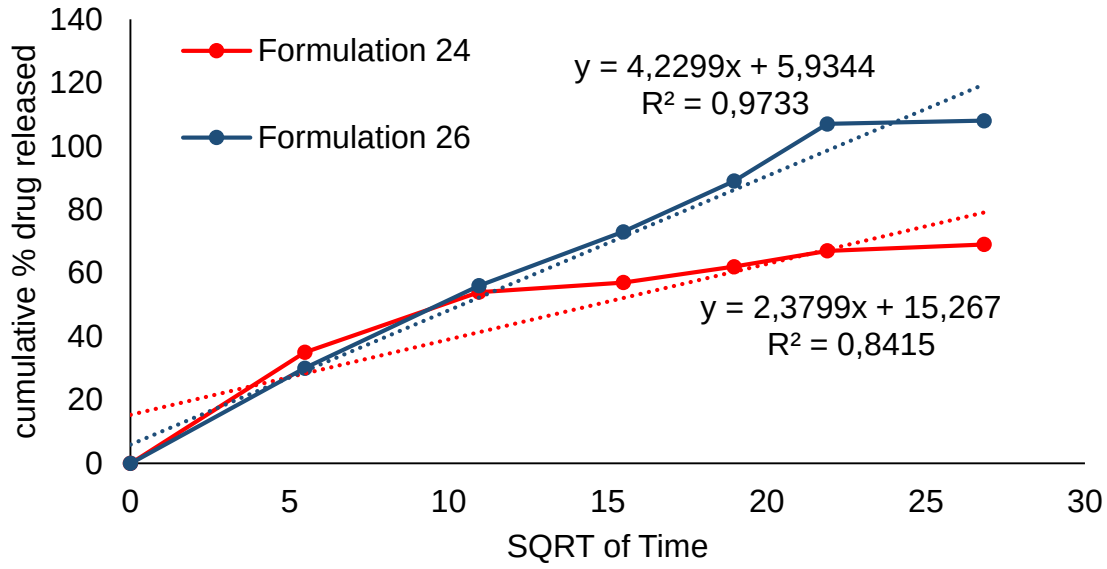



Figure 4.20: Higuchi kinetics model

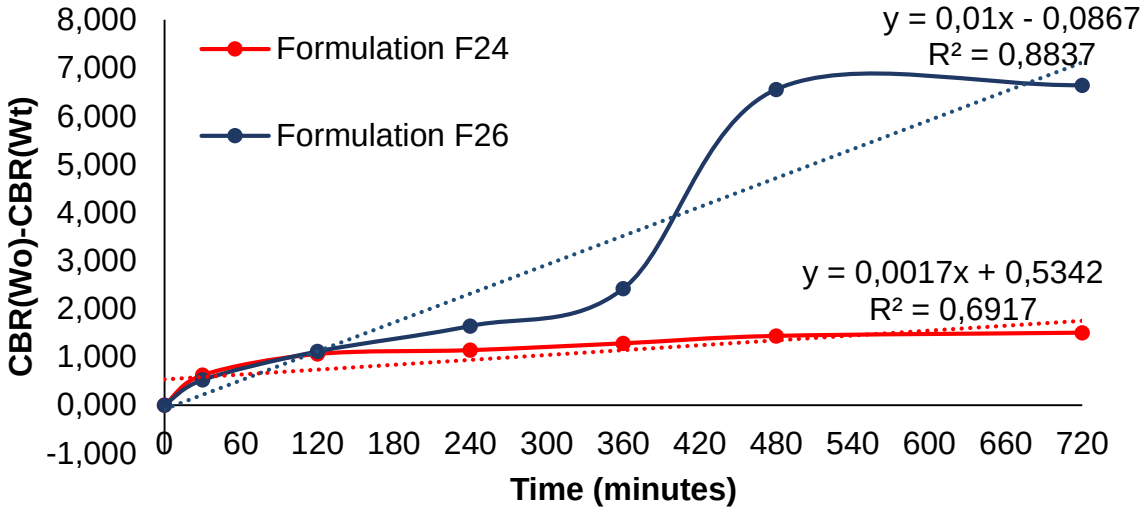



Figure 4.21: Hixson-Crowell kinetics model

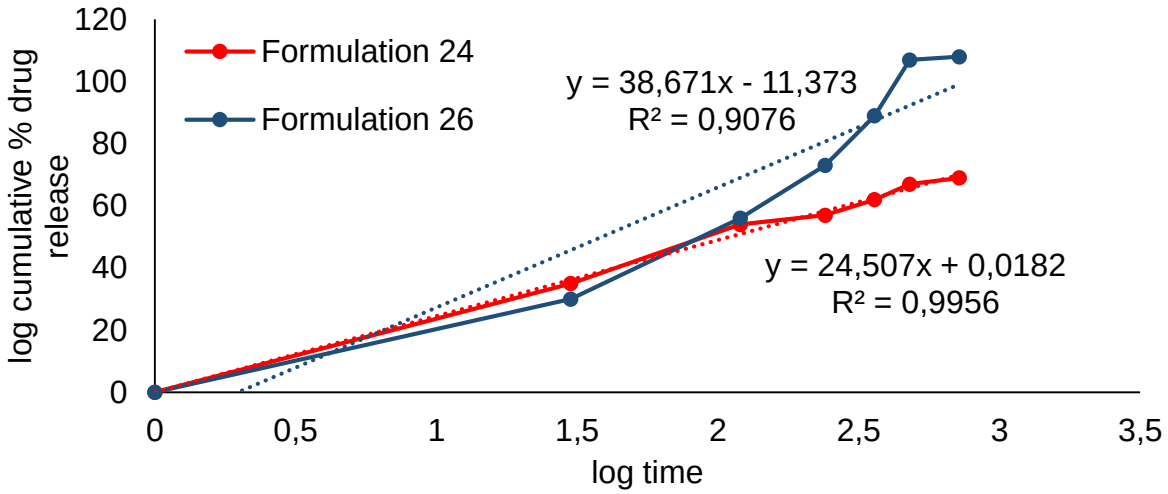



Figure 4.22: Korsmeyer-Peppas kinetics model

A summary of the calculated $R^2$ is presented in Table 4.14. The highest $R^2$ for formulation F24 was for the Korsmeyer-Peppas kinetics model with an $R^2$ value of 0.9956 followed by the Higuchi kinetics model with $R^2$ values of 0.8415. The highest $R^2$ for formulation F26 was for the Higuchi kinetics model with an $R^2$ value of 0.9733 followed by the Korsmeyer-Peppas kinetics model with an $R^2$ value of 0.9076. With respect to the $R^2$ value, it was concluded that the F24 and F26 formulations are best described by the Korsmeyer-Peppas and Higuchi kinetic models respectively (Marabathuni, Deveswaran, Bharath, Basavaraj & Madhavan, 2012). As described in chapter 3.10.4.3 and 3.10.4.5, the Higuchi kinetics model is best fitted for the dissolution profile of water-soluble drugs entrapped in a matrix and the Korsmeyer-Peppas kinetics model best describes the dissolution profile of microparticles and microspheres.

Table 4.14: Summary of dissolution release kinetics

| Mathematical model | Regression value ($R^2$) | |
|---|---|---|
| | Formulation F24 | Formulation F26 |
| Zero Order | 0.5975 | 0.8237 |
| First Order | 0.739 | 0.9104 |
| Higuchi | 0.8415 | 0.9733 |
| Hixson-Crowell | 0.6917 | 0.8837 |
| Korsmeyer-Peppas | 0.9956 | 0.9076 |

## 4.8 SUMMARY

The selected analytical method was validated; polymers were selected through the preliminary and their compatibility with ranitidine hydrochloride was confirmed. The microspheres were successfully manufactured. The data collected during post-formulation studies were graphically presented and discussed. The next chapter will provide a summary of the findings and present the conclusion for the overall study. It will also present suggested recommendations that the researcher has for further development of the study.

# CHAPTER 5

# SUMMARY, CONCLUSION AND RECOMMENDATIONS

## 5.1 INTRODUCTION

The oral route is the most preferred route for drug delivery to the systemic circulation. Although it is acceptable to patients, it has a major constraint for drugs that are not absorbed uniformly along the gastrointestinal tract. Several gastroretentive drug delivery systems have been developed and proved advantageous in enhancing drug absorption, improving bioavailability and reducing inconsistencies in plasma drug concentration. Thus, as with other drug candidates that have altered absorption, the formulation of ranitidine hydrochloride into a gastroretentive drug delivery system may well reduce morbidity and mortality due to peptic ulcers by ensuring the availability of a cost-effective dosage form that has increased residence time hence encapsulated ranitidine-loaded microspheres were manufactured for this purpose.

This study explored the *in vitro* drug release of ranitidine hydrochloride from the encapsulated microspheres. Pre-formulation studies included validating the UV-vis spectrophotometric analytical method, preliminary and compatibility studies. Preliminary studies were conducted to aid selection of polymers and manufacturing method. After the polymers were selected, any possible interactions between the polymers and ranitidine hydrochloride were investigated through compatibility studies using DSC/TGA, FTIR and short-term accelerated stability studies.

Ranitidine hydrochloride loaded microspheres were formulated, capsulated and their physical characteristics, buoyant compatibilities and *in vitro* drug release profile and release kinetics were accessed. The obtained results are summarized in this chapter and the conclusions made and suggested recommendations are presented.

## 5.2 SUMMARY OF RESULTS

The UV-vis spectrophotometric method was developed and validated in accordance with ICH guidelines for the identification and quantitation of ranitidine hydrochloride. The method was found be sensitive, accurate, precise and linear over the concentration range 1 – 10 μg/mL.

Ethyl cellulose and PEG 4000 were selected as polymers through preliminary formulation studies and compatibility with ranitidine hydrochloride was investigated. The thermal behaviour of ranitidine hydrochloride investigated using DSC/TGA in conjunction with characteristic peaks identified by FTIR indicated that the raw material compound was pure ranitidine hydrochloride. DSC analysis, FTIR and short-term accelerated studies revealed that ranitidine hydrochloride was compatible with ethyl cellulose and PEG 4000. Manufacturing of microspheres using ethyl cellulose and PEG 4000 as polymer could then commence.

Two batches of microspheres were prepared using a solvent evaporation method adapted from literature. The formulations had varying ratios of 1:1 and 1:2 w/w of ranitidine hydrochloride and PEG 4000 which were referred to as Formulation F24 and F26 respectively. SEM images of the images revealed that the formulations were spherical in nature and had a porous surface indicating solvent evaporation. The manufacturing method had good percentage yields of 99.5% and 98.85% for formulation F24 and F26 respectively. Drug content was determined to be 80% and 88% for formulation F24 and F26 respectively.

Particle size analysis suggested that increasing the polymer weight increased the size of the microspheres. The median particle diameter for formulation F24 microspheres was 596.45 μm and that for formulation F26 microspheres was 660.52 μm which supports the effect of increasing polymer weight. Evaluation of the physical characteristics of the microspheres revealed that microspheres had good flow properties which facilitated the capsulation of spheres. The dimensions of the capsules

were assessed and a weight variation test was conducted in which both formulations compiled with the USP specifications for weight variation.

The buoyancy test revealed zero lag time was required for both formulations. Formulation F26 exhibited good buoyancy capabilities in which 52% of the microspheres were buoyant after 8 hours and only 14.5% of the formulation F26 microspheres were buoyant at the end of the 8 hours. *In vitro* drug release studies revealed that increasing PEG 4000 facilitated drug release in which a complete drug release was seen at 480 minutes. Dissolution kinetics studies revealed that the drug release of formulation F24 and F26 were best described by the Higuchi and Korsmeyer-Peppas kinetics models respectively.

## 5.3 CONCLUSION

Ranitidine hydrochloride loaded microspheres were successfully formulated with ethyl cellulose and PEG4000 using a solvent evaporation method. The microspheres were able to float for 8 hours with sustained drug release over a period of 4 – 8 hours. Formulation F27, which was considered the best formulation, had greater drug encapsulation, better floating abilities and resulted in complete drug release. This success reveals the possibility of developing a gastroretentive drug delivery system of ranitidine hydrochloride which can improve its bioavailability and provide a sustained release of the drug.

## 5.4 RECOMMENDATIONS

The microspheres have the potential to be adapted to commercial manufacture however the formulation requires further testing and investigation prior to being commercialized. The following recommendations are essential for optimizing the formulation:

- Investigate the solvent residuals (methanol) with the use of gas chromatography.

- Performing stability studies on the microspheres to determine the shelf-life of the formulations.
- Using another analytical method e.g. high-performance liquid chromatography (HPLC) to supplement the UV-vis spectrophotometric method for identifying and quantifying ranitidine hydrochloride.
- Investigating the drug release and buoyancy behaviour with *in vivo* studies and determining the bioavailability or ranitidine hydrochloride.
- Conduct toxicology studies to assess the effect the new drug release system.

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# ANNEXURE

**Annexure: Standard calibration curve of ranitidine hydrochloride in 0.1 HCl**

| Sr. No. | Concentration (µg/100mL) | Absorbance | | | Average absorbance |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | |
| 1 | 0 | 0 | 0 | 0 | 0 |
| 2 | 1 | 0.001 | 0.002 | 0.002 | 0.002 |
| 3 | 2 | 0.007 | 0.007 | 0.008 | 0.007 |
| 4 | 4 | 0.014 | 0.014 | 0.015 | 0.014 |
| 5 | 6 | 0.021 | 0.021 | 0.024 | 0.022 |
| 6 | 8 | 0.028 | 0.028 | 0.028 | 0.028 |
| 7 | 10 | 0.033 | 0.034 | 0.034 | 0.034 |