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Design, Optimization, and Characterization of Novel Polymer-based Formulations for Controlled Release of Drugs

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DESIGN, CHARACTERIZATION, AND OPTIMIZATION OF NOVEL POLYMER-BASED
FORMULATIONS FOR CONTROLLED RELEASE OF DRUGS

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Mario Alberto Cano Vega

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ABSTRACT

Cano Vega, Mario A., M.S.A.B.E., Purdue University, August 2016. Design, Optimization, and Characterization of Novel Polymer-based Formulations for Controlled Release of Drugs. Major Professor: Meng Deng.

Pharmaceutical products are a key aspect of treatment and prevention of disease. For example, dibenzazepine (DBZ) is a drug that has proved to be useful for the treatment of obesity while progesterone is a common drug for hormonal replacement therapy in women. However, administration of these drugs by conventional dosage forms offers little control over the drug distribution and concentration in the body and often result in unintended adverse consequences on other cells/tissues.

Recent advances in nanotechnology and polymer science have enabled the design and development of controlled release systems that would allow spatiotemporal delivery of drugs with improved efficacy. In this work, DBZ-loaded polyester nanoparticles and progesterone-loaded cellulose composite films were synthesized and optimized as two novel systems for controlled drug delivery.

Encapsulation of DBZ in polyester nanoparticles was accomplished using an optimized nanoprecipitation method. The DBZ-loaded nanoparticles were characterized with an average particle size of ~210 nm, low polydispersity index, and high encapsulation

efficiency. *In vitro* release test demonstrated the ability of the nanoparticles to support the DBZ release in a controlled manner.

Progesterone-loaded cellulose composite films were produced from ethyl cellulose/hydroxypropylmethylcellulose (EC/HPMC) using the solvent casting method. Release profiles of progesterone were tunable by simply changing the ratio of EC and HPMC.

CHAPTER 1. INTRODUCTION

Development of new drug molecules is an expensive and time-consuming process that requires a deep understanding of all physical, chemical, pharmacological and biopharmaceutical properties of drugs that only a few companies can afford. For this reason, new drugs are first analyzed and formulated to be released rapidly from the dosage forms which are widely used in pharmaceutical industry. However, immediate release (IR) dosage forms may lead to uncontrolled drug concentration in blood that promotes side effects and even reach toxic levels and multiple administrations are needed which also promotes undesirable effects and decreases patient compliance (Ting, 2006).

Pharmaceutical development is a continuous process that does not end once the drug product is approved and released to the market but evolves and becomes a study with specific objectives that focuses on establishing a better safety profile and understanding the needs of patients (Y. Qiu & Zhang, 2009). After post marketing research, the existing knowledge about a particular drug growths and permits scientists to design delivery devices that can control the release of a drug improving its efficacy, safety and achieving financial benefits by extending patent life (Uhrich, Cannizzaro, Langer, & Shakesheff, 1999).

Drug products exist in a variety of forms that can be used for different routes of administration such as oral, transdermal, nasal, intravenous, intramuscular, pulmonary, etc., aiming either a systemic or a local effect. Selection of either route of administration or local/systemic effect depend on the pharmacological, toxicological, biopharmaceutical, and intended purpose of the formulation (Bouwman, Fenton-May, & Brun, 2015).

The controlled drug delivery systems enable to achieve temporal and distribution control over drug release. The temporally controlled release system permits to deliver the drug over an extended period of time or at specific time points that guarantee the maximum possible benefits by maintaining constant concentration at optimal level across the time (Uhrich et al., 1999).

On the other hand, controlling the distribution of drug by using local application of controlled drug delivery systems enables drug administration to the target tissue and provides pharmacologic therapy with elevated drug levels at the target tissue and minimal peripheral side effects, which is a useful approach when high drug concentrations are needed but these concentrations produce severe side effects in other tissues where the drug is not needed (Bouwman et al., 2015).

For the purpose of this work dibenzazepine and progesterone, currently available as IR dosage forms, were used as model drugs for the development of these two types of drug delivery systems. Dibenzazepine was included in a polymeric device to achieve distribution control, while progesterone was formulated into a delivery system for temporal control

release. A brief description of the clinical use of these drugs, as well as the drawbacks of current administration techniques, will be presented as an introduction.

The N-[(1S)-2-[[(7S)-6,7-Dihydro-5-methyl-6-oxo-5H-dibenz[b,d]azepin-7-yl]amino]-1-methyl-2-oxoethyl]-3,5-difluorobenzeneacetamide also known as Dibenzazepine (DBZ) (Figure 1) is a drug that inhibits the activity of γ-secretase, which is well known due to its importance in the Notch signaling pathway (Jiang et al., 2015). Current research has revealed the importance of this signaling pathway on the cell fate and cellular metabolism in almost all cells and tissues (Bi et al., 2014).
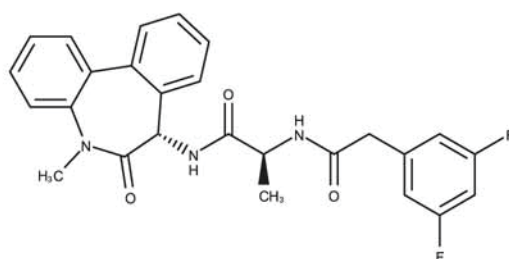
Figure 1 Structural formula of DBZ.

Notch signaling has been demonstrated to have effects on the metabolism of glucose and lipids, cell proliferation, and most recently on the homeostasis of adipose tissue (Bi & Kuang, 2015). Bi and coworkers (Bi et al., 2014) showed that pharmacological inhibition of Notch signaling promotes browning of white adipocytes increasing energy consumption through degradation of fat and improves insulin sensitivity. As a consequence, Notch inhibitors have risen as promising alternatives for treatment of obesity.

DBZ and other γ-secretase inhibitors are administered to treat different diseases such as Alzheimer and cancers using systemic injection or ingestion of capsules or compacts.

However, these techniques generate multiple side effects or gastrointestinal toxicity which is a limiting factor for its application. Due to the multiple interactions and the negative effects of DBZ in most tissues, it is needed to develop a drug delivery system for local injection that can be uptaken by cells and releases the drug inside of cells where is needed to prevent negative side effects (Doody et al., 2013).

The (8S,9S,10R,13S,14S,17S)-17-acetyl-10,13-dimethyl-1,2,6,7,8,9,11,12,14,15,16,17-dodecahydrocyclopenta[a]phenanthren-3-one or progesterone (Figure 2) is a steroid hormone that is important for reproductive function and it has been used as a part of hormone replacement therapy in women (Hsia, Ho, Tan, & Weihmuller, 2005).

The therapeutically effective blood levels range from about 0.1 ng/mL to about 400 ng/mL that depend on the therapeutic benefit desired, as well as other variables such as age, weight, metabolism, and physiological conditions (Hsia et al., 2005). Uncontrolled blood levels of progesterone lead to severe side effects that include abdominal cramps, depression, dizziness, headache, diarrhea, musculoskeletal pain, nausea, anxiety, fatigue, cough, irritability, emotional lability, and bloating (Aronson, 2009).
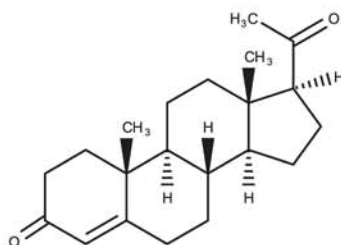
Figure 2 Structural formula of progesterone.

Administration of progesterone is done by conventional drug delivery systems such as intramuscular solution, vaginal gel, topical cream, vaginal suppository, and oral capsule (Cerner Multum, 2015). The therapeutically effective blood level may be achieved in one or more administrations but precise dose may be impossible due to the limitations of dosage forms. If lower or higher doses are required for specific patients, the lack of alternatives may lead to complications during treatment (Hsia et al., 2005). Conventional oral drug delivery systems need to be fully redesigned in order to obtain patient-centered (individualized) dosage forms that meet the required characteristics (Aronson, 2009).

Dibenzazepine and progesterone have proved to be potential candidates for the treatment of different conditions, but uncontrollable dosing, release and/or systemic administration lead to severe side effects that dramatically decrease their usability. In order to fully exploit the beneficial effects of these drugs, it is necessary to redesign its delivery vehicles so that we can obtain the maximum benefit with the minimum or even null undesirable effects increasing the efficacy, safety, and patient compliance.

The current work aims to show the process for the design, optimization, and characterization of an injectable system that contains DBZ-loaded nanoparticles for spatiotemporally controlled intracellular delivery of DBZ and progesterone-loaded composite films for oral administration and temporal controlled release of progesterone. Next chapter briefly describes some of the major aspects and theoretical considerations for the design, development, and characterization of these dosage forms.

CHAPTER 2. LITERATURE REVIEW

2.1 Novel/Special dosage forms for controlled release of drugs

Controlled release systems identify to a specific group of drug delivery devices that are designed to achieve a sustained therapeutic effect by continuously releasing a drug over a period of time and when necessary in a specific site after a single dose administration. The use of controlled release dosage forms increases patient adherence by reducing the need for frequent administration, decreases the incidence of severe side effects because drug concentration is maintained within the therapeutic window, and offer the possibility of customizing drug delivery profiles (Ratila, Priti, Vidyadhar, & Sunil, 2011).

Controlled release dosage forms have been developed in different forms such as polymeric matrixes (tablets), gels, patches, injectable systems, micro and nanoparticles, films, etc. Selecting and designing a proper delivery device is a challenging task and factors such as safety, efficacy, compatibility, stability, manufacturability, transport, and storage need to be considered (Y. Qiu & Zhang, 2009).

Different routes of administration require specific design characteristics that permit formulators to explore either conventional or non-conventional formulation approaches to enhance the efficacy and safety of the drug and the utility of the delivery device.

Conventional drug administration methods are widely exploited in the pharmaceutical industry but have many problems that can be solved by using new approaches (Ali & Kolter, 2012). Last but not least, the design of the new dosage form needs to consider patient compliance since the administration of the delivery system has to be done with minimum impact to the patient life (Sam, Ernest, Walsh, & Williams, 2012).

Novel strategies in the pharmaceutical field take advantage of the recent progress in the area of nanotechnology to improve the biopharmaceutical properties of the new delivery systems, deliver the drug in a controlled manner and minimize undesirable side effects. The aims of pharmaceutical nanotechnology can be described as follows(Kumar, 2010):

- Protect the drug from environmental and/or enzymatic degradation.

- Improve the absorption of drugs by increasing permeation through the epithelium.

- Modify the pharmacokinetic profile of drugs.

- Improve cellular uptake and distribution across the cell increasing the drug efficacy.

Application of nanotechnology tools in pharmaceutical research has resulted in drug delivery systems with improved chemical stability, ease of administration, capability of transporting and releasing the drug to the target site at a controlled rate, biodegradable and non-toxic (Kumar, 2010).

Current strategies in pharmaceutical research also permit to offer personalized medicine which is becoming more important in contemporary therapeutics. Nowadays, multiple

attempts are being made to develop dosage forms that are adapted to the specific needs and even preferences of patients (Laine & Davidoff, 1996).

The use of extemporaneous preparations is a promising option for patient-centered medicine, but it lacks of ability for ensuring the product quality, stability, and performance (FDA, 2013). Polymeric films have arisen as an interesting strategy to obtain personalized medicines for patients since they can be fabricated in large scale assuring the performance, stability, and quality of the drug product. These films can be further manipulated and/or combined under standardized conditions to fulfill specific needs of patients (Visser, Woerdenbag, Hanff, & Frijlink, 2016). The following section will be used to describe those approaches and its application in drug delivery.

2.1.1 Nanoparticles

Nanoparticles are solid systems, in the sub-micrometric range (1 – 1000 nm) made of polymeric materials that can be either biodegradable or non-degradable in which the drug can be dissolved, entrapped, encapsulated or adsorbed throughout the matrix or confined into an aqueous or oily core (Letchford & Burt, 2007; Mora-Huertas, Fessi, & Elaissari, 2010).

In the pharmaceutical field, the term nanoparticle has been used to identify two types of dosage forms that have big differences, nanospheres commonly called nanoparticles and nanocapsules (Mora-Huertas et al., 2010). The nanocapsules are reservoir-like systems with a liquid core, aqueous or lipidic, surrounded by a polymeric layer (Letchford & Burt, 2007). The physical properties and performance of these systems depend on the

manufacturing method (Christine Vauthier & Bouchemal, 2009) and the polymeric materials employed in their fabrication (L. Y. Qiu & Bae, 2006; C. Vauthier, Fattal, & Labarre, 2004).
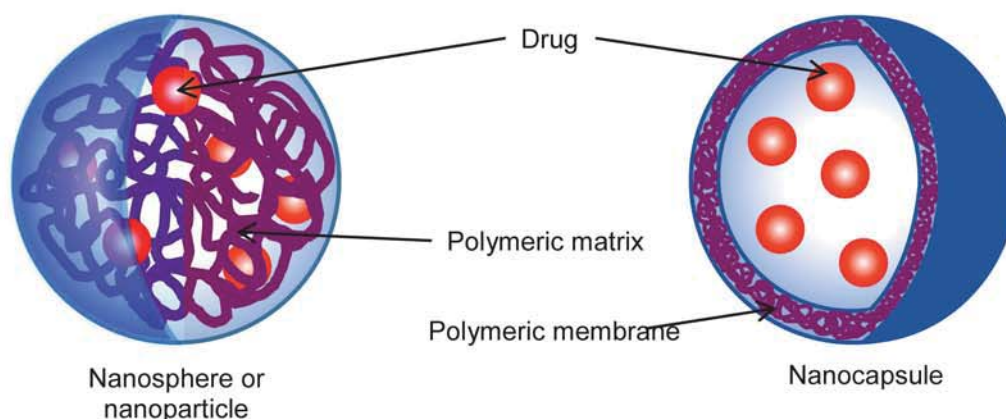
Figure 3 Graphical representation of the structure of the polymeric nanoparticles. Adapted from: (Bei, Meng, & Youan, 2010)

Nanospheres are solid polymeric matrices where the drug is either entrapped inside the nanoparticles in the form of molecular clusters bonded by different types of forces or adsorbed on their surface. These systems provide multiple advantages for the formulation of drug products such as protection from enzymatic degradation and adverse environmental conditions that result in improved drug stability, feasibility of incorporating both hydrophilic and hydrophobic drugs, possibility of varying routes of administration and the opportunity of controlling the release rate from nanoparticles (Letchford & Burt, 2007; Christine Vauthier & Bouchemal, 2009).

2.1.2 Thin-film delivery systems

The term thin-film in drug delivery refers to a system made of drug, polymer, plasticizer, and filler(s) in a film geometry that depending on the combination of parameters such as

drug concentration, film-forming polymers, plasticizer and its concentration, and film thickness can promote either immediate or controlled release of a drug through difussion, swelling or erosion (Holowka & Bhatia, 2014).

In the biomedical field, thin films are valuable due to its large surface area, adhesion capacity to different surfaces, and absorption of liquids becoming a useful device for the prevention of loss of body fluid, protect wounds from contamination and release bactericidal drugs to inhibit infection during tissue repair and closure of wounds (Pereira et al., 2014).

In the pharmaceutical field, films are considered as solid dosage forms that can be administered using different routes. For oral drug delivery, films can be as large as a postage stamp and can be placed on the tongue for immediate release or on the inside of the cheek for sustained release and due to its ease of administration and patient compliance over conventional solid dosage forms, films have attracted great interest from pharmaceutical companies (Visser et al., 2016).

Other strategies in the pharmaceutical field take advantage of thin films versatility for controlling drug release. Also known as 3D Integrated Pharmaceuticals, this approach provides the possibility of having different drugs, adjusting the dose and tuning the release kinetics by combining predesigned polymer films to generate an integrated system that meets predefined characteristics becoming into an interesting approach for patient-centered medicine (Pinal, Zhou, & Otte, 2014).

The majority of the pharmaceutical films available on the market are designed for immediate release of hydrophilic drugs and they are made of hydrophilic polymers. However, it has been shown that thin-films can be a suitable system for immediate and controlled release of hydrophobic drugs as well (Krull, Li, Davé, & Bilgili, 2015).

2.2 Formulation and manufacturing methods

The formulation of a drug product is an important step during pharmaceutical development that consists of the selection of excipients and its proportions to produce an effective, safe and stable drug product. The effect of formulation excipients, pharmaceutical processing and its interactions is critical, and complete understanding of the impact of each variable involved should be based on scientific knowledge since even negligible variations may either result in loss of efficacy or toxicity (Sam et al., 2012).

Depending on the complexity of the formulation and the unit operations involved, mechanistic and empirical modeling can be developed to explain the relevant parameters that affect the performance of the dosage form. Integrated product formulation and process design leads to robust products with optimal quality features, minimizing composition-related processing issues or process-related performance problems (Wurth, Demeule, Mahler, & Adler, 2016).

2.2.1 Nanoparticles formulation and manufacturing

The design and development of a nanoparticles-based system is a task that should take into account the expected purpose of the formulation (Mora-Huertas et al., 2010). Particle size and surface charge are considered as critical quality attributes and understanding of

the relationship between these parameters, formulation, and manufacturing process is important since particle size greatly affect final physicochemical properties (D. Sharma et al., 2014), the efficiency and pathway of cellular uptake (Hu, Chiang, Hong, & Yeh, 2012).

Research about this issue has demonstrated that particle size has significant impact on the distribution through the body and particles larger than 200 nm display accumulation in the liver and increased clearance (Blanco, Shen, & Ferrari, 2015). Furthermore, during the internalization process, larger particles need stronger driving force and additional energy to get into the cell limiting its efficacy (Oh & Park, 2014).

Surface charge plays a key role in the internalization of nanoparticles and on cell viability and compatibility. Nanoparticles with neutral charge and negative charge have much lower cellular uptake rate while positively charged nanoparticles are taken up more efficiently improving the efficacy of drug delivery (Hu et al., 2012), but careful optimization is needed since positively charged particles generally display more toxicity associated with cell wall disruption (Fröhlich, 2012).

Surface charge is also important for the physical stability of the nanoparticles. Zeta potential values between −10 and +10 mV are considered neutral and the physical stability can be compromised, while nanoparticles with zeta potentials values greater than +30 mV or less than −30 mV are considered cationic or anionic and stable due to electrostatic effects (Clogston & Patri, 2011).

Due to the importance of these parameters in the performance of the nanoparticle-based drug delivery systems, comprehensive understanding should be achieved in order to successfully apply nanoparticles in drug delivery.

The manufacturing methods for nanoparticles includes the emulsion-solvent evaporation method, double emulsion and evaporation method, emulsion-diffusion method and solvent displacement (Rao & Geckeler, 2011). Selection of an appropriate method for the preparation of nanoparticles depends on the physicochemical properties of the polymer, the drug to be loaded and the intended properties of the new drug delivery system (Rao & Geckeler, 2011).

The solvent displacement method (Figure 4) also known as nanoprecipitation has proved to be a suitable technique for encapsulation of poorly soluble drugs, and it is widely used since it contains few basic components and its reproducibility. The first component is the organic solution that contains the drug and a polymer that can be either natural or synthetic dissolved in a semipolar solvent such as acetone, ethanol or acetonitrile (Rao & Geckeler, 2011). Acetone is the preferred solvent due to its beneficial properties such as low boiling point and high miscibility with water (Fessi, Puisieux, Devissaguet, Ammoury, & Benita, 1989; Mishra, Patel, & Tiwari, 2010).
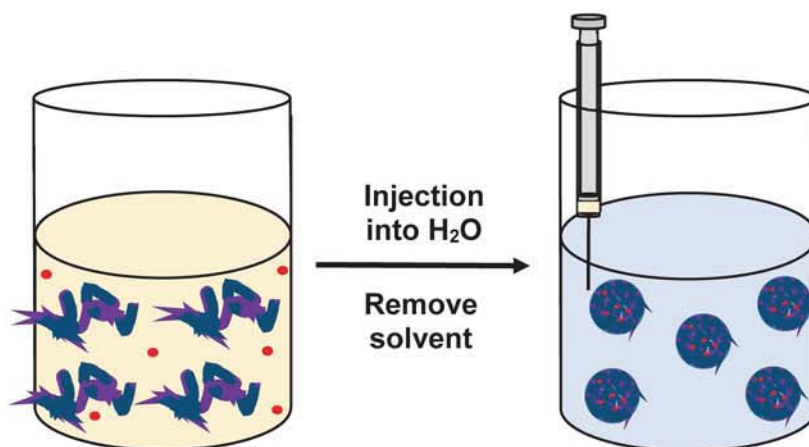
Figure 4 Solvent displacement or nanoprecipitation technique for preparation of nanoparticles.

The organic solvent is well known to play a significant role on the average particle size. In this sense, Chang and co-workers (Song et al., 2006) demonstrated the effect of three different type of solvents in the average particle size of nanoparticles. The water miscibility/solubility of solvent is an important parameter, and they found that small particles (~70 nm) can be produced using partially water soluble solvents, medium size particles (~200 nm) when using fully water-soluble solvents and large particles (~400 nm) with water-immiscible solvents.

They concluded that when using partially miscible solvents, it is possible to produce stable emulsion droplets after organic solvent diffusion which were stabilized by the surfactant leading to stable particles with small average particle size. On the other hand, when using an immiscible-water solvent, larger droplets were formed and aggregation is significant due to solvent hydrophobicity which led to larger particle size. Finally, when using completely miscible solvents, stable emulsions are not formed and the polymer immediately precipitates leading to larger particles (Song et al., 2006).

The second component is the non-solvent phase which is an aqueous solution containing a surfactant that reduces the surface tension and stabilizes the dispersion (Rao & Geckeler, 2011). The nanoprecipitation takes place when the solution that contains the drug and polymer is poured or injected into the non-solvent phase and the nanoparticles precipitate by the rapid solvent diffusion which is then allowed to evaporate (Fessi et al., 1989; Pal, Jjana, Manna, Mohanta, & Manavalan, 2011).

Nanoprecipitation technique is based on the interfacial deposition of a polymer after displacement of an organic solvent miscible with water. Rapid diffusion of the solvent into non-solvent phase result in a decrease of interfacial tension between two phases, which increases the surface area and leads to the formation of small droplets of organic solvent (Fessi et al., 1989).

As detailed before, formulation scientists should bear in mind that each excipient and the level selected for the formulation of nanoparticles may have a significant effect on physical properties of nanoparticles that consequently affect their performance. For example, chemical properties of the polymer and its interaction with the drug determine the localization of the latter, which can be adsorbed to the surface or encapsulated in the polymer matrix (Singh & Lillard Jr, 2009). Higher polymer concentration increases the particle size presumably due to a higher viscosity of the organic phase that leads to the formation of droplets with a larger size at the interface. Also, factors such as the concentration of stabilizer in the aqueous phase modify the surface properties and impart stability to the nanoparticles preventing coalescence and aggregation of nanoparticles (D.

Sharma et al., 2014). Case by case considerations need to be done in order to fully understand and optimize the parameters that govern the physicochemical properties of a system.

2.2.2 Thin-films formulation and manufacturing

The manufacturing of thin films can be achieved by different methods such as hot-melt extrusion, semisolid casting, solid-dispersion extrusion, rolling and solvent casting (Krull et al., 2015). This last technique is widely used since it does not need highly specialized equipment or unit operations. Solvent casting (Figure 5) involves mixing of a polymer, drug, and fillers dissolved in a suitable solvent that is then poured into a mold followed by solvent evaporation or drying steps resulting in thin films with a thickness of a millimeter fraction (Salit, Jawaid, Yusoff, & Hoque, 2015).

Polymer solution

Solvent casting

Polymer solution + mold

Drying
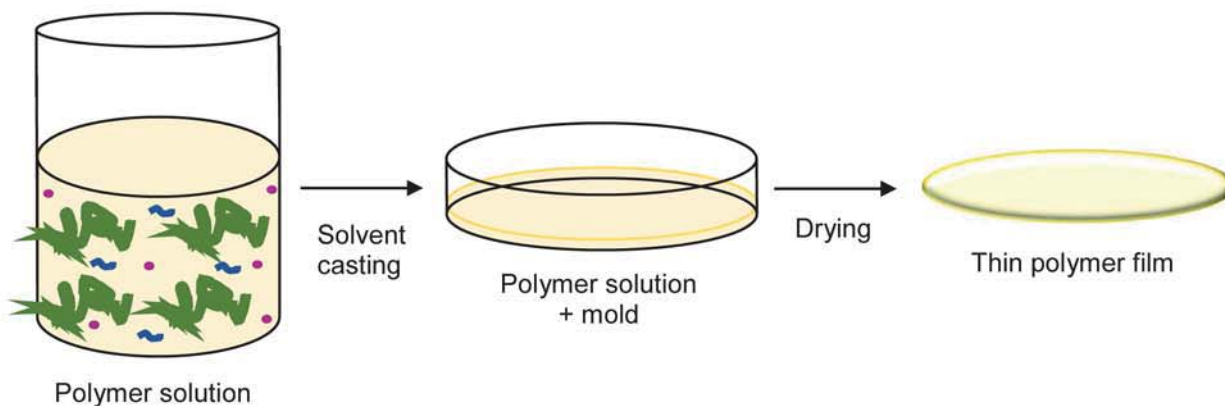
Thin polymer film

Figure 5 Solvent casting technique for preparation of thin polymer films.

During the solvent casting process it is necessary that the polymer and other components such as the drug, plasticizers and filler(s) must be soluble in water, solvent or a mixture of both and form a clear and stable solution with a reasonable solid content and viscosity

which then leads to the formation of a homogeneous film that can be easily removed from its casting substrate (Siemann, 2005).

According to Holowka and coworkers (Holowka & Bhatia, 2014), the film formation has two stages. During the first stage, the non-uniform solvent evaporation promotes the distribution of solutes between the solvent interface and the surface, while the second stage includes the lateral tension sensed by the solution relative to the surface inducing a compression that promotes the alignment of the solution constituents and formation of a film.

The films obtained by this process are formed by two phases: a particle phase which is made of a hydrophobic drug or polymer and provides hardness and toughness to the film, and the matrix phase that stabilizes the particles and is responsible or the elasticity of the film (Holowka & Bhatia, 2014).

During formulation process, drug loading and release profiles are parameters usually considered as critical quality attributes. Evaluation of the physical and chemical properties of the raw materials, plasticizer and fillers must be completed to meet specific drug-loading needs and release rates requirements (Steele, Loo, & Venkatraman, 2016).

Another critical factor that influences polymer physicochemical properties and the attributes of the final product is the glass transition temperature (Tg) that is closely related to the flexibility of the polymer chain. Polymers at a temperature below the Tg are in a glassy state and have limited molecular mobility and low diffusion rates. In contrast, polymer at a temperature above the Tg are in a rubbery state with higher mass transfer

rates of water and drug molecules throughout the matrix (Kamaly, Yameen, Wu, & Farokhzad, 2016).

Films made of pure polymer are brittle and stiff due to extensive interactions between polymer molecules. The addition of plasticizers, which are non-volatile compounds with high boiling point and low molecular weight, increases the free volume between the polymer chains which allows polymer chains to move more easily and improves processability without altering the original chemical properties of the material (H. Lim & Hoag, 2013; Ramos, Fernandes, Silva, Pintado, & Malcata, 2012).

Due to the diversity of requirements and the current advances in chemical synthesis, a wide range of polymeric materials are available for the design and development of delivery devices. Multiple factors can affect the performance of the delivery forms and various considerations are needed in order to find a suitable material.

2.3 Polymeric materials

The success of a delivery system relies on the correct selection of its components and its intrinsic physical and chemical properties. Polymeric materials have acquired importance in pharmaceutical development due to its wide range of physical and chemical properties and processability that allow formulation scientists to transform such adaptable resources into diverse devices with tunable properties (Uhrich et al., 1999). In the field of controlled release of drugs, these materials have proved to be usable since polymeric devices delay the release of drug molecules, inhibiting the dissolution of the drug out of the device or controlling the flow of drug solutions(L. Y. Qiu & Bae, 2006).

Polymers can be classified depending on their source (natural, synthetic or semi-synthetic), structure (linear, branched, crosslinked) or degradability (biodegradable or non-biodegradable), but classification of polymeric materials can be a challenging task due to the wide diversity of sources and chemical compounds used for its synthesis (Kadajji & Betageri, 2011).

Biodegradable polymers are materials that can be naturally excreted in its unaltered form or be degraded to its monomeric components that are then excreted. Biodegradable polymers can be suitable for most biomedical and pharmaceutical applications although it is also determined by the administration route (Makadia & Siegel, 2011). Alternatively, non-biodegradable polymers remain unaltered indefinitely when implanted in the body and cannot be removed without external intervention (Kamaly et al., 2016). For example, for oral drug delivery, non-degradable polymers are acceptable for administration because the delivery system can be excreted after the drug has been released (Uhrich et al., 1999).

Natural polymers are extracted from natural resources which make them suitable for implantation, injection or ingestion. Polymers such as starch, cellulose, chitosan, etc.; are widely investigated due to their advantageous physical and chemical properties. In addition, these materials have the advantage of being approved for pharmaceutical applications by the Food and Drug Administration (FDA) in the USA (Pillai & Panchagnula, 2001).

Depending on the purpose of the device that is being developed, synthetic polymers are an important alternative due to their vast diversity in chemistry that provides a wide range of options for the development of controlled release dosage forms. Poly (esters) are the best characterized and most widely used synthetic polymers and have been approved by FDA for its use in pharmaceutical and biomedical applications (Burg, 2014).

2.3.1 Poly (esters): Poly D,L-lactic-co-glycolic acid (PLGA)

Polyesters are polymers with ester bond linkages in the carbon backbone that, because of their advantageous properties such as biodegradation and processability, have been extensively investigated. Poly (lactic acid) (PLA) and poly (glycolic acid) (PGA) were the first poly (esters) investigated and showed interesting properties for their use in pharmaceutical applications. PLA and PGA can be obtained by condensation of their natural precursors, lactic acid or glycolic acid, respectively, or by ring opening polymerization and the final product is a chain formed by successive monomeric units linked together by ester linkages (Burg, 2014; Christine Vauthier & Bouchemal, 2009).

Poly D,L-lactic-co-glycolic acid (PLGA) (Figure 6) is a group of copolymers formed by glycolic acid and lactic acid at different ratios that combine both hydrophilicity and hydrophobicity from their precursors. In order to design a better controlled drug delivery device, it is indispensable to understand the physical, chemical and biological properties of PLGA that depend on multiple factors including the molecular weight, the ratio of glycolic acid and lactic acid, the size of the dosage form, exposure of water, environmental pH and temperature (Makadia & Siegel, 2011).

Different degradation rates can be achieved by increasing the PLA/PGA comonomer ratio, which decreases hydrolysis rates by reducing the hydrophilicity of the PLGA and vice versa, with an exception of 50:50 ratio of PLA/PGA, which exhibits the fastest degradation rate (Houchin & Topp, 2009; Keles, Naylor, Clegg, & Sammon, 2015). In aqueous solution, PLGA degrades due to hydrolysis of its ester linkages giving rise to its monomeric components that can be removed by natural metabolic pathways (Gentile, Chiono, Carmagnola, & Hatton, 2014).
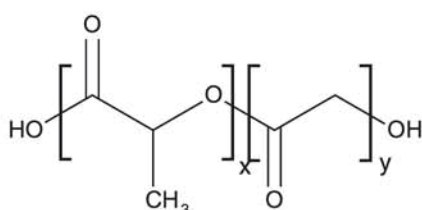
Figure 6 Structural formula of Poly D,L-lactic-co-glycolic acid.

PLGA is soluble in a wide range of common solvents including chlorinated solvents, tetrahydrofuran, acetone or ethyl acetate. This aspect has to be considered when developing a suitable manufacturing process. PLGA can be processed to obtain different shapes and sizes and it can encapsulate molecules with different chemical properties and sizes which make this polymer very versatile for pharmaceutical applications (Makadia & Siegel, 2011).

There are different commercially available options and each company identifies its products by using a special nomenclature. The commercial brand RESOMER® has different polymer grades that include different lactic: glycolic acid ratios with different end groups. RESOMER RG 504® is a poly (D,L-lactide-co-glycolide) 50:50 (Mw 38,000-54,000) with ester

as end group (by using different end groups it is possible to control degradation and water uptake). In general, the increase of carboxylic end groups (acid groups) result in autocatalysis increasing the degradation rate, while the presence of ester as an end group decreases the degradation rate (Keles et al., 2015; "Resomer "; "RESOMER(R) Reaching the target without leaving traces," 2015).

2.3.2 Cellulose derivatives: Ethylcellulose and hydroxypropyl methyl cellulose

Cellulose is a polymer widely used in the food, cosmetic and pharmaceutical industries. Cellulose (Figure 7) consist of linear chains of β(1-4)-linked – D-glucopyranosyl units with suitable mechanical properties for film formation and strong inter-molecular hydrogen bonds between polymer chains that make them suitable for controlled release applications (J. Li & Mei, 2006).
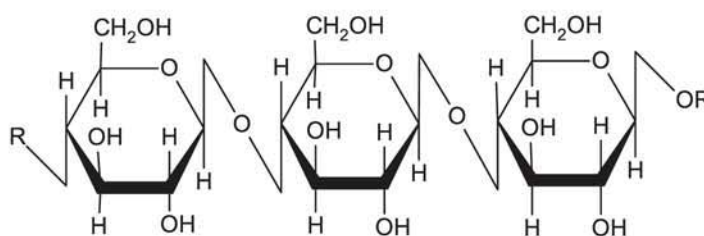
Figure 7 Structural formula of cellulose.

Cellulose has semi-synthetic derivatives which can be classified into two groups, cellulose ethers, and cellulose esters. For the purpose of this paper, we will focus on the ether derivatives which are compounds produced by replacing the hydrogen atoms of hydroxyl groups in the units of cellulose with alkyl groups. Examples of the most widely used

cellulose ethers are methyl cellulose (MC), ethyl cellulose (EC), hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC), hydroxypropylmethylcellulose (HPMC) and carboxymethyl cellulose (CMC). All these polymers present unique physical and chemical properties primarily determined by their chemical structure, molecular weight and degree of substitution (Shokri & Adibkia, 2013).

Ethylcellulose (Figure 8) is an inert polymer 'generally recognized as safe' (GRAS) by the U.S. FDA (Dow, 2016). Production of EC includes the conversion of cellulose into alkali cellulose by treatment with a strong aqueous solution of sodium hydroxide and then alkylated with ethyl chloride or sulfate. The excess reagents are removed by washing and distillation (Koch, 1937).
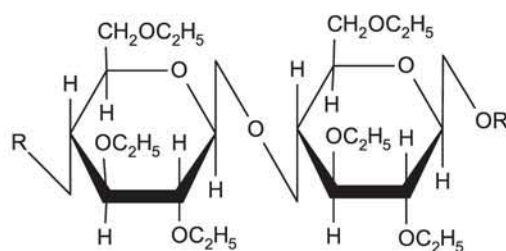
Figure 8 Structural formula of ethylcellulose.

The physical and chemical properties of ethyl cellulose depend on the degree of etherification. Commercial products usually contain 47 to 48 percent ethoxy content which dramatically reduces its aqueous solubility, but EC is still soluble in polar and non-polar solvents. Other properties such as viscosity, which is known as an important factor in the release of drugs, can be controlled by decreasing or increasing the polymer chain (Koch, 1937).

HPMC is used as an additive that dissolves in water and creates a pore structure that releases drug faster. Understanding the interactions of EC and HPMC in the blend are of major importance to the drug release profile (Lua, Cao, Rohrs, & Aldrich, 2007; Sakellariou & Rowe, 1995).

HPMC (Figure 9) is also recognized as GRAS ingredient by the FDA and has been used in the pharmaceutical industry as an important component in the formulation of swellable-soluble matrices. HPMC involves the transformation of cellulose into alkali cellulose and reaction with methylene chloride and propylene oxide (Chan, Wong, Chua, York, & Heng, 2003; L. Wang, Dong, & Xu, 2007). The ratio of hydroxypropyl and methyl substitution provides a specific HPMC its particular characteristics and, as a consequence there are many commercial options which are identified by different codes.

The Dow Chemical Company identifies its products by using a letter that relates to the degree of substitution followed by an indication of the viscosity of their aqueous 2% w/w solutions (in centiPoises) at 20 °C and a final suffix that identifies the grade of the material such as premium (P), low viscosity (LV), controlled release (CR) or food grade (FG). HPMC E6, which is the object of this study, have a methoxy substitution of 28 -30 % and hydroxypropyl substitution of 7-12% (C. L. Li, Martini, Ford, & Roberts, 2005).
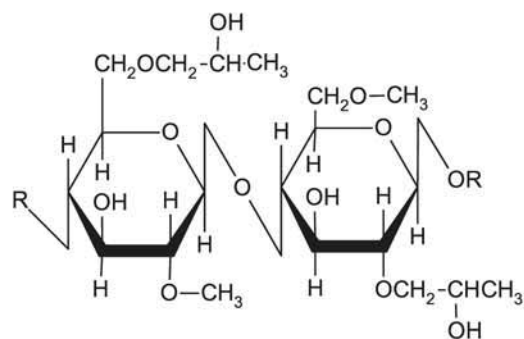
Figure 9 Structural formula of hydroxypropylmethylcellulose.

Next section briefly describes some of the characterization techniques for nanoparticles and polymer thin films that acquire importance for the understanding of the effect of formulation parameters on the final performance of drug products.

2.4 Considerations for the characterization of drug delivery systems

2.4.1 Physical properties of nanoparticles and polymer thin films

Physical and chemical characterization of novel dosage forms is necessary in order to provide a complete description and guarantee the performance of the produced dosage form. Nanoparticles and thin film characterization is usually an intricate task due to the complex composition and the number of factors that modify its performance such as the chemical properties of the drug and polymer, the chemical structure and amount of stabilizers, the amount of plasticizers, the water and solvent ratio and the diffusion rate of the organic phase into the aqueous phase, and its physical properties such as the small size, in the case of nanoparticles (Couvreur, Barratt, Fattal, & Vauthier, 2002).

Appropriate measurement of critical quality attributes of nanoparticles such as particle size, particle size distribution, and surface charge is needed during the development stage

as well as in the regular manufacturing process and consequently, different techniques have been employed for this purpose. The average particle size and the polydispersity index can be measured by Dynamic Light Scattering (DLS), which is based on the dispersion of the light caused by the Brownian motion of the particles (J. Lim, Yeap, Che, & Low, 2013). Imaging techniques such as Transmission Electron Microscopy (TEM) provides information on the morphology and size of the nanoparticles (Couvreur et al., 2002). Additionally, the surface properties can be measured through determination of zeta potential of the nanoparticles via the mobility of the charged particles monitored by an electrical potential (Clogston & Patri, 2011).

For polymer thin films, the thickness is measured by micrometer screw gauge or calibrated digital Vernier Calipers at different locations (corners and center) and should be in a range of 5-300 µm (Bala, Pawar, Khanna, & Arora, 2013).

Determination of drug content is a requirement for all dosage forms and different methods have been developed. For solid dosage forms such as thin films, this is determined by the standard assay method described for the particular drug in the pharmacopeia (Convention & Revision, 2010), or by more sensitive methods when available. Content uniformity is determined by estimating the drug content in individual films which are dissolved in a suitable solvent for polymer and drug of interest and then quantified by Reverse Phase High Performance Chromatography (RP-HPLC) with UV spectrometry as detector (Bala et al., 2013).

For colloidal systems such as nanoparticles, precise determination of the drug content is not easy due to the small size and because the particles are suspended in aqueous media. Methods for quantification include centrifugation and quantification of the drug in the supernatant by using RP-HPLC with UV spectrometry as detector (Pal et al., 2011).

2.4.2 In Vitro release test for nanoparticles and polymer thin films

Performance and consistent product quality of controlled-release dosage forms are usually evaluated using the *in vitro* release test in which amount of drug dissolved is quantified as a function of time (Brown et al., 2011), and when possible the release test serves as a prediction of the *in vivo* performance of drug delivery systems (Shen & Burgess, 2013).

Different procedures and techniques are employed on a case-by-case basis, and the method may be specific for a dosage form class, formulation type or even to a particular product. There is no standard release test available for nanoparticles but multiple strategies such as membrane diffusion methods (dialysis, reverse dialysis and glass basket dialysis), sample and separation methods and continuous flow methods have been employed for this purpose (Shen & Burgess, 2013).

On the other hand, drug release from polymer thin films is evaluated using standard methods such as basket apparatus (USP I) (Sievens-Figueroa et al., 2012) and the flow-through cell dissolution apparatus (USP IV) (Krull et al., 2015) and when necessary other non-conventional methods have been developed in order to fulfill specific necessities during the development stage (Brown et al., 2011). Due to the different characteristics of

the novel /special dosage forms and their sites and modes of administration, it is essential to consider the composition of the dissolution medium, apparatus selection, agitation (flow rate) and temperature (Brown et al., 2011; Shen & Burgess, 2013).

Optimization of release test aims to achieve a discriminating method able to differentiate formulation and manufacturing variables that may affect product performance. Cautious evaluation is needed to recognize whether the procedure is too sensitive or appropriately discriminating. Optimization can be done by assessing the results from multiple batches that exemplify probable variations in formulation and manufacturing process or by intentionally vary formulation or fabrication parameters to further characterize the discriminating power of the procedure (FDA, 1997).

In some cases, it is necessary to imitate the physiological conditions and typical media for release include the use of buffers with pH values within the physiological range. For example, nanoparticles intended to be released and subsequently trapped by cells, need to be characterized under different pH conditions, including physiological (pH 7.4) and lysosomal (pH 5.0) conditions (Baltazar et al., 2012; Shen & Burgess, 2013; Utembe, Potgieter, Stefaniak, & Gulumian, 2015).

Other dosage forms that include poorly soluble compounds or hydrophobic polymers, may require a dissolution media containing a surfactant (e.g. sodium lauryl sulfate, polysorbate, etc.) or an aqueous organic solvent mixture as dissolution medium, but in all cases, a proper justification for this type of medium is needed (FDA, 2014).

It is necessary to highlight that any method used in the early phase of formulation development should be critically evaluated and, if possible, simplified based on the accumulated experience. The final method may not necessarily closely imitate the *in vivo* environment, but should still test the key performance indicators of the formulation (Brown et al., 2011).

Analysis of release profiles can be done in different ways depending on the purpose and the information that is needed. The comparison of a single-point of the release profile may be suitable to distinguish the differences in the overall performance but a comparison of the complete release profile acquired under identical conditions for different formulations is recommended. For this purpose model dependent and model independent approaches can be used (FDA, 1997).

The model independent approach takes advantage of difference factor (f_1), similarity factor (f_2) and other statistical tools such as ANOVA-based methods and multivariate analysis (Principal Component Analysis or PCA) to distinguish even small differences in the release behavior of the different dosage forms. The improved statistical analysis approach enables the scientist to better distinguish significant modifications and help to make more unbiased decisions during the development stage (Y. Wang, Snee, Keyvan, & Muzzio, 2016; Yuksel, Kanık, & Baykara, 2000).

Model dependent approaches take advantage of empirical or theoretical mathematical models to describe the release profiles and make a quantitative interpretation of the information obtained from the release experiments. The use of specific parameters in the

equation helps the formulator scientist to explain the release curve which is closely related to the performance of the dosage form (Costa & Sousa Lobo, 2001). Some of the most relevant and commonly used mathematical models describing the release curves are shown in Table 1.

Table 1 Mathematical models used to describe drug release curves (Costa & Sousa Lobo, 2001; Y. Wang et al., 2016).

Model	Equation
Zero order	$Q_t = Q_0 + K_0 t$
First order	$\ln Q_t = \ln Q_0 + K_1 t$
Second order	$Q_t/Q_\infty = (Q_\infty - Q_t)K_2 t$
Higuchi	$Q_t = K_H(t)^{1/2}$
Korsmeyer-Peppas	$Q_t/Q_\infty = K_k t^n$

CHAPTER 3. MATERIALS AND METHODS

3.1 Nanoparticle preparation

The preparation of PLGA nanoparticles was accomplished by using the nanoprecipitation method. For this purpose, 6 factors at 3 different levels were evaluated (Table 2).

Table 2 Experimental factors for the optimization of formulation and manufacturing method of PLGA nanoparticles.

Factor	Low	Medium	High	Units
Concentration of PLGA	0.005	0.01	0.02	g/mL
Volume of organic phase	5	7.5	10	mL
Concentration of surfactant	0.05	0.275	0.5	%
Stirring speed	200	400	600	rpm
Temperature	20	40	60	°C
Volume of aqueous phase	25	62.5	100	mL

Table 3 shows the different combinations tested during the optimization stage.

Table 3 Experimental conditions for the optimization of PLGA nanoparticles.

Polymer concentration in the organic phase	Volume of organic phase	Amount of surfactant	Stirring speed	Temperature	Volume of aqueous phase
g/mL	mL	%	rpm	°C	mL
0.0100	5	0.5	200	20	100
0.0100	7.5	0.275	400	40	62.5
0.0200	5	0.05	200	60	100
0.0050	10	0.05	600	60	25
0.0050	10	0.5	600	20	100

Table 3 Continued above table.

0.0100	7.5	0.275	400	40	62.5
0.0100	5	0.05	200	20	25
0.0200	5	0.5	600	60	25
0.0100	10	0.05	600	20	100
0.0200	5	0.5	600	20	25
0.0100	7.5	0.275	400	40	62.5
0.0100	5	0.05	600	60	100
0.0100	10	0.05	200	20	25
0.0100	10	0.5	200	60	100
0.0050	10	0.5	200	60	25

The PLGA nanoparticles and DBZ-loaded PLGA nanoparticles were prepared from PLGA 50:50 (Boehringer Ingelheim, Resomer 504, Mw 38,000-54,000) by the solvent displacement (nanoprecipitation) method. An organic phase, consisting of a predefined amount of PLGA and a suitable amount of DBZ (Tocris bioscience) dissolved in acetone, was injected into the aqueous stabilizer solution. The injection procedure was carried out using a needle under magnetic stirring. The stabilizer solution consisted of a PVA (Sigma-Aldrich, 87-90% hydrolyzed, Mw 30,000-70,000) aqueous solution. The nanoparticles were stirred 6 hr to allow solvent evaporation. The particle suspension was rinsed three times by centrifugation (12,000 rpm, 4 °C, and 45 min) and dispersed in water by stirring 15 min.

3.2 Transmission electronic microscopy for PLGA nanoparticles

Nanoparticles were characterized for morphology using a transmission electron microscope (FEI Tecnai G2 20) operating at 200 kV. For TEM observations, PLGA

nanoparticles suspension were diluted in water. Nanoparticles were carefully placed on 400 mesh formvar-coated copper TEM grid followed by staining with 2% uranyl acetate solution for 5 min. Water was removed until partially dried and the sample was allowed to dry at room temperature.

3.3 Particle size, particle size distribution and Z potential for DBZ-loaded PLGA nanoparticles

Average particle size and polydispersity index (PDI) of the developed nanoparticles were determined 24 hr after preparation by dynamic light scattering using Malvern Zetasizer. Particle size and particle size distribution investigation was performed in triplicate by diluting the nanoparticle suspension in deionized water. The final concentration was approximately 0.15 mg of nanoparticles per 1 mL.

Zeta potential was measured using Malvern Zetasizer. The nanoparticles suspension was diluted with deionized water and measurements were performed in triplicate.

3.4 Stability of DBZ-loaded nanoparticles under different pH values

DBZ-loaded nanoparticles were prepared as described above. Saline solutions with pH 3, 4, 5, 6, and 7.5 were prepared as described in the U.S. Pharmacopeia (Convention & Revision, 2010). All solutions were filtered using Whatman filter papers (diameter 90 mm) to remove any suspended solid. DBZ-loaded nanoparticles suspension was diluted using the saline solutions. The particle size of the resulting suspension was measured using DLS right after dilution of the nanoparticles, and then the samples were stored at room

temperature for 7 days. The samples were sonicated and their particle size was measured again using DLS.

3.5 Encapsulation efficiency for nanoparticles

The amount of drug present in the nanoparticles was determined as the difference between the total amount of drug in the nanoparticles suspension and the amount of drug present in the supernatant after centrifugation. Nanoparticles were separated from the aqueous medium by ultracentrifugation at 14,000 rpm for 45 min. Supernatant sample (1 mL) was extracted with 1 mL chloroform. The organic layer was then separated and allowed to evaporate. The dried sample containing DBZ was then reconstituted with 1 mL of a 40:60 % v/v solution of acetonitrile in water. The total mass of drug was quantified by dissolving 1 mL of nanoparticles suspension before centrifugation into 24 mL of acetonitrile. The quantitative determination of DBZ was performed using HPLC (Thermo High Performance Liquid Chromatograph). A UV detector at 232 nm was used for spectrophotometric analysis. Separation was achieved by using a reverse phase column (150 mm x 4.6 mm, Pentafluorophenylpropil, 5 µm) with a flow rate of 1 mL/min. The mobile phase consisted of a mixture of acetonitrile and 0.1% phosphoric acid solution in a ratio of 50:50. The encapsulation efficiency was calculated according to the following formula:

$$\text{Encapsulation efficiency} = \frac{\left(\text{Mass of the total drug - Mass of free drug}\right)}{\text{Mass of total drug}} \times 100$$

3.6 Release studies for nanoparticles

The release of DBZ from PLGA nanoparticles was performed by the dialysis bag diffusion technique in phosphate buffer saline (PBS, pH 7.4) and acetate buffer (pH 5) at 37 °C in an orbital shaker at 100 rpm. Specifically, 1 mg of DBZ loaded PLGA nanoparticles dispersed in 1 mL of water was transferred to a dialysis bag (SpectrumLab, 10-12 KDa) and immersed into a 50 mL falcon tube containing 40 mL of PBS or acetate buffer. At predetermined intervals, 12 mL of buffer solution in the falcon tube was removed and replaced with pre-warmed fresh buffer solution. The sample was extracted with chloroform. The organic layer was then separated and allowed to evaporate. The dried sample containing DBZ was then reconstituted with 400 µL of a 2:1 solution of acetonitrile in water. The DBZ concentrations in the released samples were determined using HPLC with a UV–Vis spectrophotometer as described above.

3.7 Experimental design for selection of dissolution media for characterization of progesterone-loaded EC-HPMC composite films

Selection of dissolution media was carried out employing 140 mL of 0.1 N hydrochloric acid and a predefined amount of ethanol as dissolution media per vessel at room temperature. Three small circles (1 cm diameter) were obtained from model films and processed in each release experiment. The effect of the amount of ethanol in the dissolution medium was studied at different levels (20, 30 and 45% v/v). Aliquots of 5.0 mL were withdrawn at 30, 60, 90, 120, 180, 240, and 300 min, filtered with Nylon filters (25 mm) and placed into glass vials. Concentration of progesterone in the dissolution

media was monitored using HPLC (Shimadzu Liquid Cromatograph) using a Zorbax C_{18} column (4.6 mm ID x 250 mm) with a mobile phase consisting of water: acetonitrile (25:75 % v/v) at a flow rate of 1 mL/min with UV-VIS detection at 254 nm.

The two types of model films were prepared by solvent casting technique. Briefly, dibutyl phthalate, progesterone, HPMC E6 (Dow Chemical Company) and EC Standard 7 (Dow Chemical Company) were dissolved in ethanol at 60 °C and stirred with a stir bar until complete homogenization. The resulting solution was poured into Petri dishes and allowed to dry at room temperature. Dibutyl phthalate was used as a model plasticizer. Table 4 shows the composition of the model films.

Table 4 Formulation of model films for selecting the dissolution media.

Sample	Formulation 1	Formulation 2
Excipient	% in formulation	% in formulation
Progesterone	16.5	13.7
EC Standard 7	61.9	51.3
Dibuthyl phthalate	21.6	17.9
HPMC E6	0.0	17.1

3.8 Experimental design for selection of manufacturing process of progesterone-loaded EC-HPMC composite films

Comparison of two manufacturing procedures was carried out using a model formulation. Table 5 shows the composition of the model formulation.

Table 5 Formulation of the model film for selection of manufacturing process.

Sample	Model formulation
Component	% in formulation
Progesterone	13.9
Dibutyl phthalate	52.2
EC Standard 7	16.4
HPMC E6	17.4

Process 1 was carried out by dissolving the components in ethanol at 60 °C using the following order of addition: 1) dibutyl phthalate, 2) progesterone, 3) HPMC E6, and 4) EC Standard 7. Each component was added and stirred with a stir bar until completely dissolved before addition of the next component. After the addition of EC, the solution was stirred for 5 hr at 600 rpm and then poured into a petri dish and allowed to dry at room temperature for 24 hr.

Process 2 involved two steps. First, dibutyl phthalate was dissolved in ethanol. Then, all solid components (EC Standard 7, HPMC E6 and progesterone) were blended in its solid state. The blend of solid components was carefully added to the ethanol solution at 60 °C and stirred for 5 hr. The resulting solution was poured into a petri dish and allowed to dry at room temperature for 24 hr.

Comparison of these two films was carried out by using release test. Thickness was measured using a Vernier Caliper (Mitutoyo) at different positions in the film (corners and center). Individual films (1 cm diameter) were weighted. Low concentration of ethanol was used in order to increase discriminative properties of dissolution media. For this purpose 140 mL of 0.1 N hydrochloric acid: ethanol (10% v/v) was used as dissolution

media. Aliquots of 5.0 mL were withdrawn at 30, 60, 90, 120, 180, 240, and 300 min, filtered with nylon filters and placed into glass vials. Dissolution media was replaced with fresh media at room temperature. The concentration of progesterone in the dissolution media was monitored using HPLC.

3.9 Experimental design for evaluation of the effect of EC and HPMC on the release of progesterone from films

Different combinations of EC Standard 7 and HPMC E6 were employed for constructing a model that consider the effect of these two excipients on the release of progesterone from thin films. The composition of the films is shown in Table 6.

Table 6 Composition of films used for evaluation of EC and HPMC effect on progesterone release.

Formulation	Progesterone	EC Standard 7	HPMC E6
1	10	41	11
2	10	45	8
3	10	49	4
4	10	53	0
5	10	30	9
6	10	33	6
7	10	36	3
8	10	39	0
9	10	45	0
10	10	33	0

Evaluation of these formulations was performed by using release test. The optimized release method comprised a beaker as a dissolution vessel with 140 mL of 0.1 N hydrochloric acid:ethanol (85:15% v/v) with magnetic stirring at room temperature.

Aliquots of 5.0 mL were withdrawn at 30, 60, 90, 120, 180, 240, and 300 min, filtered with nylon filters (25 mm) and placed into glass vials. Fresh media was added to replenish the liquid removed. The concentration of progesterone in the dissolution media was monitored using HPLC.

3.10 Statistical analysis

All data are reported as mean ± SD (n=3) and the difference between the groups was tested by Origin Pro (Origin Labs) or SAS (SAS Institute) using a t-test, ANOVA test or Tuckey test for mean comparison. Principal Component Analisis (PCA) for release test comparison was carried out without previous data treatment using Origin Pro. Mechanistic analysis of release data was performed using DDsolver (Zhang et al., 2010). In all cases, the factors were found significant as P-value < 0.05.

CHAPTER 4. RESULTS AND DISCUSSION

4.1 Optimization of PLGA nanoparticles formulation and manufacturing process

The aim of the initial stage was to investigate the factors that have a significant impact on the particle size and particle size distribution of PLGA nanoparticles. Screening of different factors at 3 different levels was performed in order to find the most suitable conditions to produce PLGA nanoparticles of ~200 nm. The different factors and levels were established based on literature information and preliminary experiments. Manufacturing of PLGA nanoparticles was accomplished by using nanoprecipitation method. The effect of these parameters on the particle size was evaluated by using DLS (Table 7).

Table 7 Particle size and PDI value of PLGA nanoparticles prepared under different experimental conditions.

Polymer conc. in the organic phase	Vol of organic phase	Amount of surfactant	Stirring speed	Temp	Vol of aqueous phase	Z-average	PDI
g/mL	mL	%	rpm	°C	mL	nm	
0.0100	5	0.5	200	20	100	129	0.143
0.0100	7.5	0.275	400	40	62.5	108	0.135
0.0200	5	0.05	200	60	100	165	0.131
0.0050	10	0.05	600	60	25	58	0.145
0.0050	10	0.5	600	20	100	88	0.172
0.0100	7.5	0.275	400	40	62.5	106	0.124
0.0100	5	0.05	200	20	25	130	0.128
0.0200	5	0.5	600	60	25	145	0.169

Table 7 continued above table.

0.0100	10	0.05	600	20	100	123	0.102
0.0200	5	0.5	600	20	25	183	0.146
0.0100	7.5	0.275	400	40	62.5	102	0.121
0.0100	5	0.05	600	60	100	114	0.076
0.0100	10	0.05	200	20	25	107	0.127
0.0100	10	0.5	200	60	100	89	0.108
0.0050	10	0.5	200	60	25	71	0.103

The analysis of the particle size showed that under the tested conditions it is possible to obtain PLGA nanoparticles with a Z-average from 58 nm and up to 183 nm. Analysis of data was carried out using multiple linear regression in SAS. Figure 10 shows the magnitude and the importance of each variable in the particle size distribution of the PLGA nanoparticles.

The chart displays the absolute value of the standardized effects to identify important effects. The standardized effects are the t-statistics which are calculated by dividing each coefficient by its standard error (coefficient/standard error of the coefficient). The reference line corresponds to t, where t is the $(1 - \alpha/2)$ quantile of a t-distribution with degrees of freedom equal to the degrees of freedom for the error term. Any effect that extends beyond this reference line is potentially important in the final output (particle size) (Minitab, 2016).
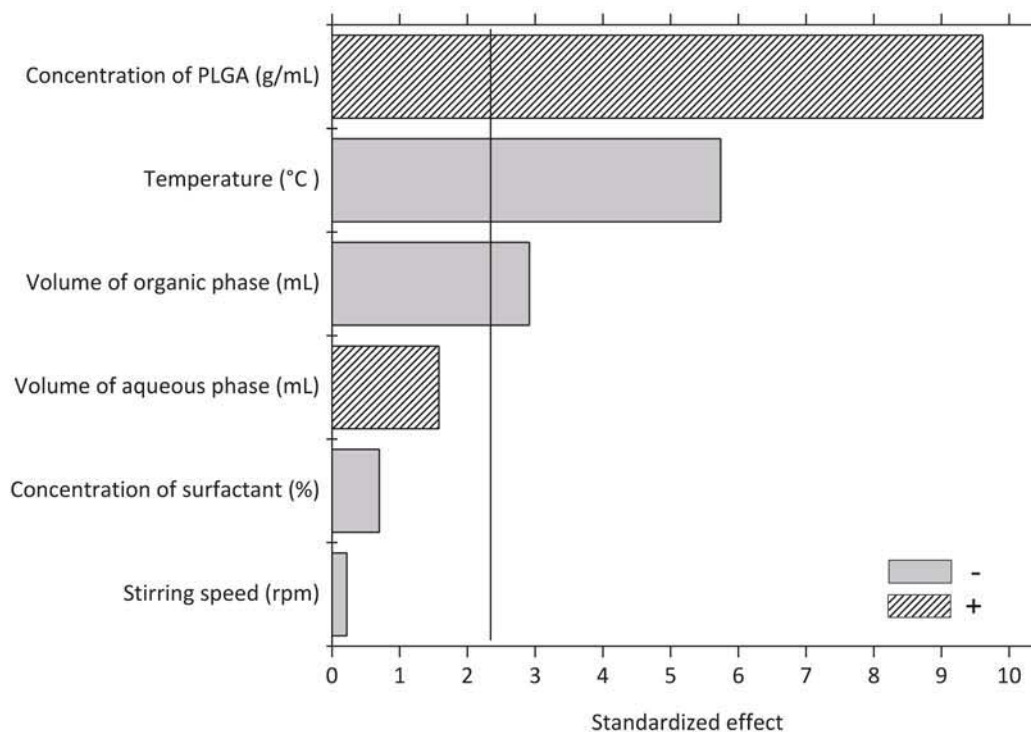
Figure 10 Pareto chart of standardized effects for particle size distribution (α=0.05, d.f. = 8).

Analysis of these results showed that three variables have a statistical significant effect on particle size. Other three variables have some effect but it is considered not to be significant. From the chart, we can see that higher concentrations of PLGA in the organic phase led to larger particles. This outcome is in good agreement with the results published by other authors who argued that increasing the polymer concentration in the organic phase leads to a higher viscosity which results in increase of forces resisting droplet breakdown that leads to the formation of nanodroplets with a larger size (D. Sharma et al., 2014; N. Sharma, Madan, & Lin, 2016).

On the other hand, both temperature and volume of the organic phase produced smaller particles when used at high levels (60 °C and 10 mL respectively). In this sense, Sharma

and coworkers (D. Sharma et al., 2014) found that decreasing the volume of the organic phase increases particle size which could be due to the organic phase volume available at the time of formation of nanodroplets. Temperature also decreases particle size of the nanoparticles presumably due to a reduction of viscosity of PVA solution as a consequence of an increase of temperature (Briscoe, Luckham, & Zhu, 2000). Alteration of the viscosity of the emulsion either by the change of aqueous/organic phase ratio or temperature resulted in lower viscous resistance against the shear force during the formation of nanodroplets leading to smaller droplets and as a consequence to smaller nanoparticles (N. Sharma et al., 2016).

The effect on the average particle size can be explained by the following reduced model:

Particle size (nm) = 110.4 + 4962.1*Concentration of PLGA - 3.6*Volume of organic

phase - 0.6*Temperature

Further analysis by ANOVA indicated a significant effect of independent factors (P-value < *0.05*) on response particle size with an adjusted R-square value of 0.9533. All factors considered in the model were statistically significant (P-value < 0.05). A positive value in the model for a response represents a direct relationship and negative value indicates an inverse relationship between response and a factor.

The polydispersity index (PDI) is a measurement of the homogeneity of particle size and PDI values greater than 0.3 indicate the aggregation of particles (nanoComposix, 2015). The analysis of the results showed that under the tested conditions the PDI values ranged

from 0.076 to 0.172. ANOVA analysis showed that there is no relationship between the independent factors (P-value = 0.4707 > 0.05) and the response.

Optimization of final formulation was carried out taking into account all information obtained from screening experiments showed above. The aim of this step was to select formulation components and levels as well as manufacturing conditions for the production of nanoparticles with a particle size of ~200 nm. As stated before, higher polymer concentration (0.02 mg/mL), low temperature (20 °C) and smaller volume of the organic phase (5 mL) led to larger particles.

Other factors such as stirring speed, the volume of the aqueous phase and surfactant concentration, although non-significant, still seemed to have influence on the particle size. The volume of aqueous phase (100 mL) was selected to increase the organic/aqueous phase ratio which appeared to increase particle size. The concentration of surfactant was kept at the higher level to increase the interfacial stability of nanoparticles and prevent coalescence and aggregation of nanoparticles during solvent evaporation stage. Table 8 shows the optimized levels of the formulation components and manufacturing process parameters for production of PLGA nanoparticles.

Table 8 Optimized parameters for the production of PLGA nanoparticles.

Factor	Optimized values	Units
Concentration of PLGA	0.02	g/mL
Volume of organic phase	5	mL
Concentration of surfactant	0.5	%
Stirring speed	600	rpm
Temperature	20	°C
Volume of aqueous phase	100	mL

4.2 Particle size, particle size distribution and zeta potential of DBZ-loaded nanoparticles

PLGA nanoparticles were prepared using the experimental conditions stated above. Next step included characterization of this system by using TEM, and nanosizer to measure particle size, particle size distribution, and surface charge properties. Also, based on the dosage necessities, a suitable amount of DBZ was added to the organic phase to prepare the DBZ-loaded PLGA nanoparticles. TEM images give a better understanding of the real geometric size of the particles and further confirmed the nearly spherical shape of DBZ-loaded PLGA nanoparticles (Figure 11).
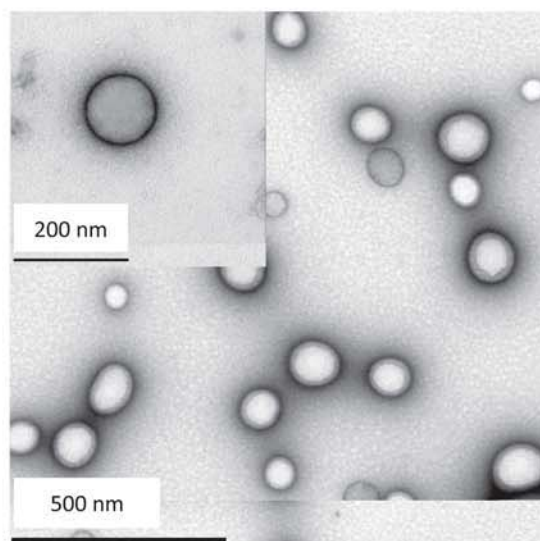
Figure 11 TEM images of the optimized PLGA nanoparticles formulation.

Several batches of blank PLGA nanoparticles and DBZ-loaded nanoparticles were prepared for measurement of particle size and particle size distribution. Blank nanoparticles were found to have a Z-average of 212 ± 9.0 nm and DBZ-loaded PLGA nanoparticles showed a

Z-average of 221 ± 12.0 nm. Figure 12 shows the particle size distribution for DBZ-loaded

PLGA nanoparticles.
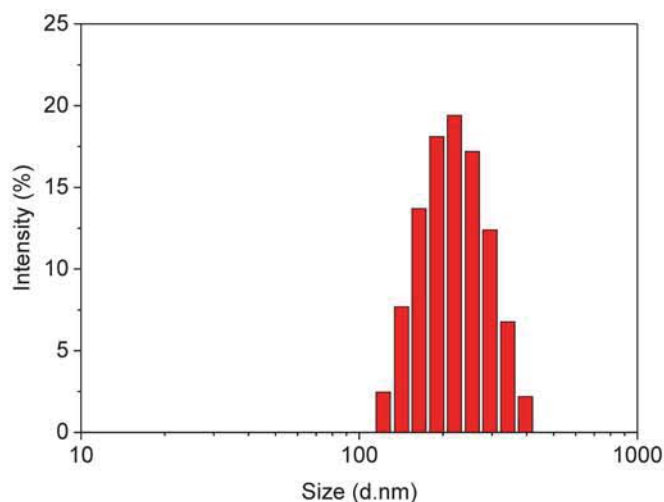
Figure 12 Intensity-based particle size distribution of DBZ-loaded nanoparticles.

On the other hand, the PDI values for blank PLGA nanoparticles is 0.104 ± 0.054 and for

the DBZ-loaded nanoparticles is 0.13 ± 0.075. Particle size slightly increased after addition

of the DBZ (P-value < 0.05). In this sense, Govender and coworkers (Govender, Stolnik,

Garnett, Illum, & Davis, 1999) found that higher amount of drug loaded into the

nanoparticles increased the particle size of the nanoparticles and concluded that high drug

loading affected the process of PLGA precipitation and formation of spherical particles.

The average zeta-potential (n= 3) of the PLGA nanoparticles and DBZ-loaded PLGA

nanoparticles was determined to be -22.8 ± 6.0 mV and -20.2 ± 3.0 mV, respectively. This

is in full agreement with previous reports of PLGA nanoparticles prepared with PVA as a

stabilizer that has been reported to have zeta potential values ranging from -10.0 to -20.0

mV (Mura et al., 2011).

4.3 Stability of DBZ-loaded PLGA nanoparticles at different pH

Stability of the nanoparticles was tested at different pH values. The aim of this part of the study was to investigate the effect of pH in the DBZ-loaded PLGA nanoparticles. Nanoparticles were diluted and incubated at room temperature for 7 days in buffer solutions with different pH values.

Figure 13 Particle size of DBZ-loaded PLGA nanoparticles under different pH values
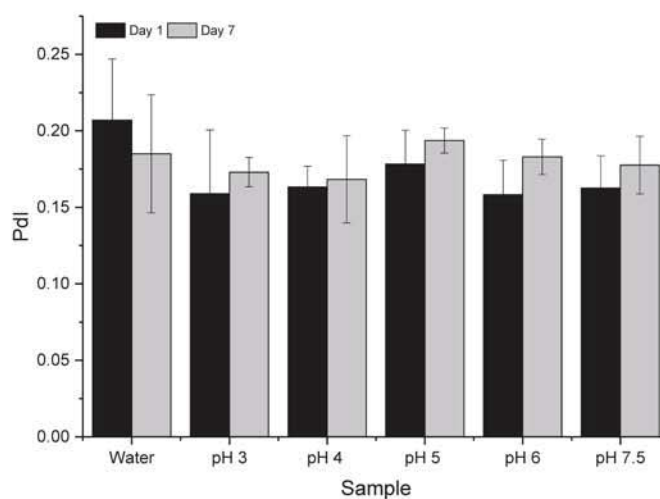
Figure 14 Polydispersity index of DBZ-loaded nanoparticles under different pH values.

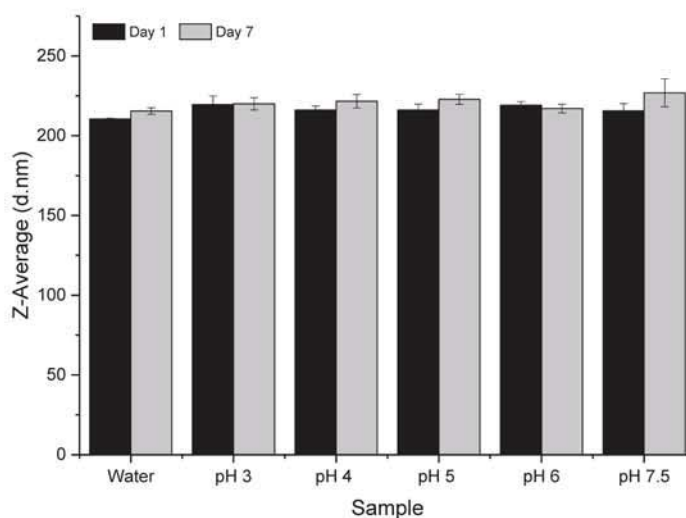Results from these experiments showed nanoparticles diluted in water with a particle size of 210 nm ± 6 nm and acceptable PDI value of 0.2. Figure 13 shows that nanoparticles dispersed in different buffer solutions, ranging from pH 3 to pH 7.5, were stable right after dilution and up to seven days. The particle size ranged from 210 to 220 nm and there was not a significant difference between groups. Polydispersity index was lower than 0.2 in all cases (Figure 14).

4.4 Encapsulation efficiency of DBZ-loaded PLGA nanoparticles

It has been reported that encapsulation efficiency depends on a great number of factors such as the preparation method, drug/polymer ratio, hydrophobicity/hydrophilicity of drug, concentration of surfactant in aqueous phase, pH of aqueous phase, etc. (Alshamsan, 2014; Govender et al., 1999; Sah & Sah, 2015; D. Sharma et al., 2014).

The encapsulation efficiency for this system was 94.3 ± 4.0 %. This high encapsulation efficiency was expected since other authors have reported the utility of nanoprecipitation method for the encapsulation of hydrophobic drugs. High encapsulation efficiency is due to the low solubility of the drug in the aqueous phase resulting in a higher amount of drug remaining on the hydrophobic polymeric matrix (Barichello, Morishita, Takayama, & Nagai, 1999).

4.5 Release studies of DBZ-loaded PLGA nanoparticles

The characterization of the drug release rate from nanoparticles is very important and it depends on different factors such as desorption of the drug from nanoparticles surface, diffusion of the drug through the polymeric matrix, erosion of the nanoparticle and

combination of erosion/diffusion processes (Soppimath, Aminabhavi, Kulkarni, & Rudzinski, 2001).

Membrane diffusion method is widely used for the characterization of drug release for nanoparticles. Drug release studies were performed in buffered saline solutions at pH 7.4 (physiological pH) and pH 5 (lysosomal pH) to observe the effect of pH on the release rates in the conditions that most closely mimic the environment that the nanoparticles encounter *in vivo*. Figure 15 shows the observed release profiles of DBZ from PLGA nanoparticles.
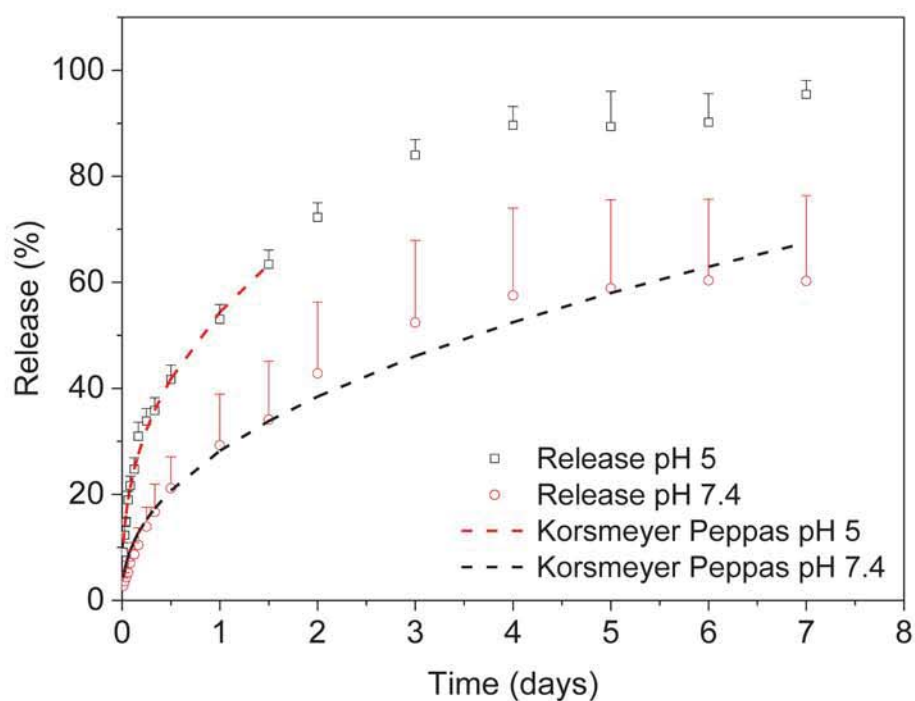

Figure 15 In Vitro release of DBZ from poly (lactide-co-glycolide) nanoparticle in buffered solution, pH 5 and 7.4

The release rate of DBZ from PLGA nanoparticles at pH 5.0 is significantly faster than at pH 7.4 (P-value < 0.05). At the acidic pH, more than 50% of the drug had been released during

in 24 hr, and up to 90% after 4 days. Release at pH 7.4 indicates that the formulation is able to deliver the drug in a controlled manner over an extended period of time since it was observed that at the same time points, 24 hours and 4 days, PLGA nanoparticles released 30% and 58% of the drug respectively.

It has been established that the differences in the release rates from PLGA nanoparticles under different pH conditions is influenced by an accelerated degradation of PLGA under acidic conditions that is subsequently enhanced by an increasing concentration of the degradation products which are also acidic. As a consequence, there is a decrease in the molecular weight of the initial polymer leading to easier access of dissolution media into the nanoparticles and faster release of the drug (Betancourt, Brown, & Brannon-Peppas, 2007).

In order to establish the mathematical model to describe the release of DBZ from the PLGA nanoparticles, the release profiles obtained using the dialysis method were adjusted to zero order, first order, Higuchi, and Korsmeyer-Peppas models, using the add-in DDsolver (Zhang et al., 2010).

 Selection of an appropriate model is essential to evaluating the release characteristics and for this purpose, the R-squared adjusted Akaike Information Criterion (AIC) and the Model Selection Criterion (MSC) were employed (Table 9). The better model possesses the highest value of R-squared adjusted, the lowest AIC value and the largest MSC (Zhang et al., 2010).

Table 9 Statistical criteria for selection of the best mathematical model for DBZ-loaded PLGA nanoparticles.

Model		pH 5	pH 7.4
Zero-Order	K (%*min^{-1})	0.01	0.01
	Rsqr_adj	0.28	0.67
	AIC	170.34	141.43
	MSC	0.23	1.04
First-Order	K (%*min^{-1})	0.00	0.00
	Rsqr_adj	0.86	0.85
	AIC	140.11	118.76
	MSC	1.91	2.30
Higuchi model	K (%*min$^{-1/2}$)	1.52	0.69
	Rsqr_adj	0.91	0.95
	AIC	67.79	108.74
	MSC	2.37	2.86
Korsmeyer-Peppas Model	K (%*min^{-n})	3.51	1.18
	n	0.38	0.46
	Rsqr_adj	0.99	0.96
	AIC	44.56	105.30
	MSC	4.30	3.05

* All calculations were performed using DDsolver (Zhang et al., 2010).

The model that better fits the data obtained from the release experiments of DBZ-loaded PLGA nanoparticles is Korsmeyer-Peppas model. It is important to highlight that this equation can be used to analyze the first 60% of the release curve, regardless of the geometric shape. For this reason, data analysis was carried out until about 60% of the total release of DBZ from nanoparticles at pH 5.0 (Figure 15). The calculated n value is n = 0.38 at pH=5.0 and n= 0.46 at pH=7.4. In both cases, n value is lower than 0.5, indicating that the drug release mechanism from polymeric nanoparticles is Fickian diffusion (Peppas & Sahlin, 1989; Juergen Siepmann & Peppas, 2011). Fickian difussion release occurs due to

a chemical potential gradient in which solute moves from a region of high concentration to one of low concentration (Peppas & Sahlin, 1989).

The release of drugs from PLGA nanoparticles was similar to the behavior of PLGA matrices described by other authors. Release from PLGA matrices is a complex process that involves various routes that take place at parallel time scales. The initial step involves the penetration of the dissolution media and a rapid release of the DBZ which is called burst release and has been identified as a common characteristic during the release of drug from PLGA matrices, followed by deeper penetration of the media to the center of the nanoparticles leading to hydrolytic reactions that cause erosion and diffusion of degradation products of PLGA and constant diffusion of hydrophobic drugs with low molecular weight through the hydrophobic polymeric matrix (Ford Versypt, Pack, & Braatz, 2013; Makadia & Siegel, 2011).

As polymer degradation continues, more degradation products diffuse from the nanoparticle leading to an accelerated diffusion rate of the releasing drug which is a common characteristic of bulk-eroding polymers such as PLGA (Ford Versypt et al., 2013; Makadia & Siegel, 2011). In general, small-molecule drugs will diffuse through the PLGA matrix much faster and complete release of the drug is expected to occur before the total degradation and erosion of the PLGA polymer matrix (Hines & Kaplan, 2013).

4.6 Selection of dissolution media for characterization of progesterone-loaded EC and EC/HPMC composite films

For a drug to be effective it is necessary for it to reach the blood stream at a suitable concentration which is governed by the release of the drug from the dosage form, the dissolution of the drug into the surrounding media and pharmacokinetics of the drug itself (Y. Qiu & Zhang, 2009). Because of the critical nature of these steps, *in vitro* release test is relevant during the development stage of a new dosage form.

Release tests can be designed with different purposes that include simulation of *in vivo* conditions, assessing the lot-to-lot quality of a drug product or guide the development of new formulations. The approaches for setting the experimental conditions for release test depend on the intended purpose of the method that is being developed (Siewert et al., 2003).

The aim of this first set of experiments was to find a standardized test method to characterize the release of different formulations of progesterone-loaded EC/HPMC composite films. Hydrochloric acid 0.1 N/ethanol mixtures were employed for this purpose.

A volume of 140 mL of dissolution media was used for the release studies. This was determined to be the minimum volume needed to dissolve the progesterone and obtain an acceptable analytical response when using HPLC. Figure 16 shows the release profiles obtained under the experimental conditions described above.
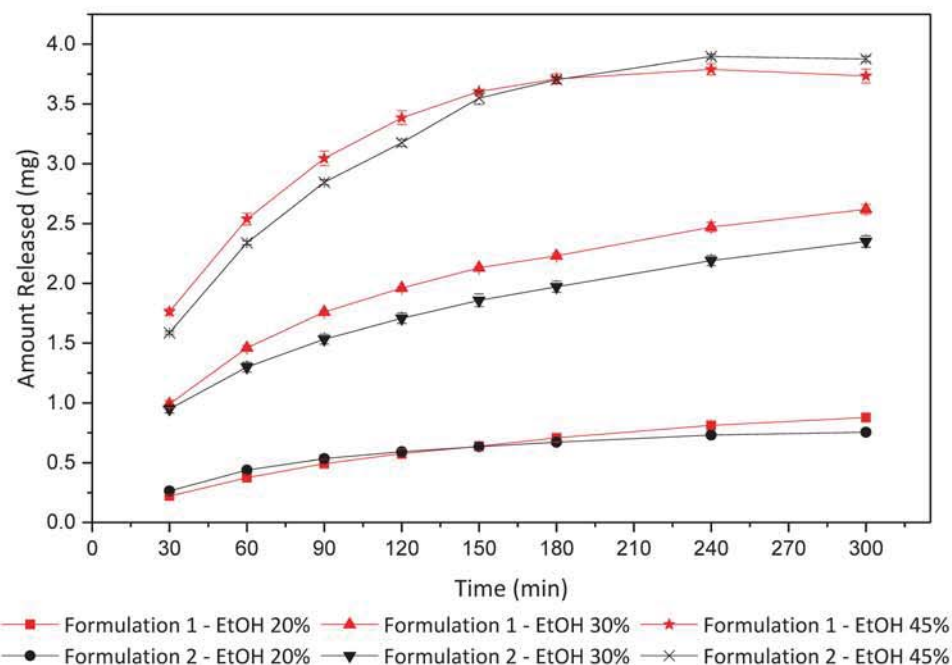
Figure 16 Release profiles of progesterone in three different dissolution media, Hydrochloric acid 0.1 with 20, 30 and 45% Ethanol (v/v).

The release profiles from the three media showed different levels. Higher concentrations of ethanol on the dissolution media led to higher amounts of progesterone released from the EC and EC/HPMC composite films. Table 10 shows the amount of progesterone released (mg) at 300 minutes.

Table 10 Average values of the amount of progesterone dissolved from model formulations using different dissolution media.

Vol 140 mL	t = 300 min	Amount Released (mg) (Mean ± S.D.)		P-value
		F 1	F 2	
Dissolution media composition	HCl 0.1 N/EtOH (80:20 v/v)	0.88 ± 0.02	0.75 ± 0.02	0.0027
	HCl 0.1 N/EtOH (70:30 v/v)	2.62 ± 0.04	2.35 ± 0.05	0.0016
	HCl 0.1 N/EtOH (55:45 v/v)	3.74 ± 0.06	3.88 ± 0.02	0.0176

Further analysis using ANOVA test and t-test confirmed that all means are significantly different (P-value < 0.05) and any concentration of ethanol could be used as an alternative

dissolution media. Additionally, a more detailed analysis was carried out using Principal

Component Analysis (PCA) to describe the release curves in a model-independent manner

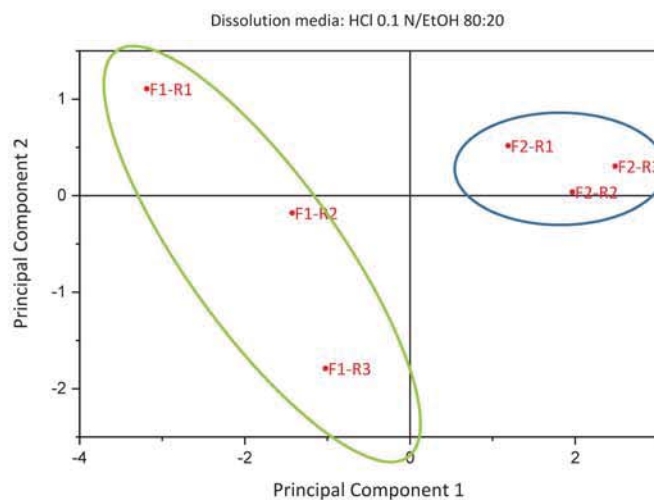(Siegel & Rathbone, 2012; Y. Wang et al., 2016).

Figure 17 PCA for release profiles in HCl 0.1 N: Ethanol (80:20 % v/v).
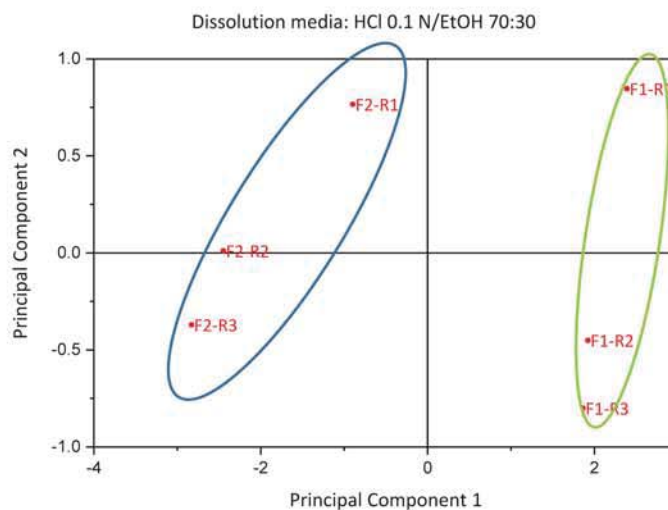
Figure 18 PCA for release profiles in HCl 0.1 N: Ethanol (70:30 % v/v).
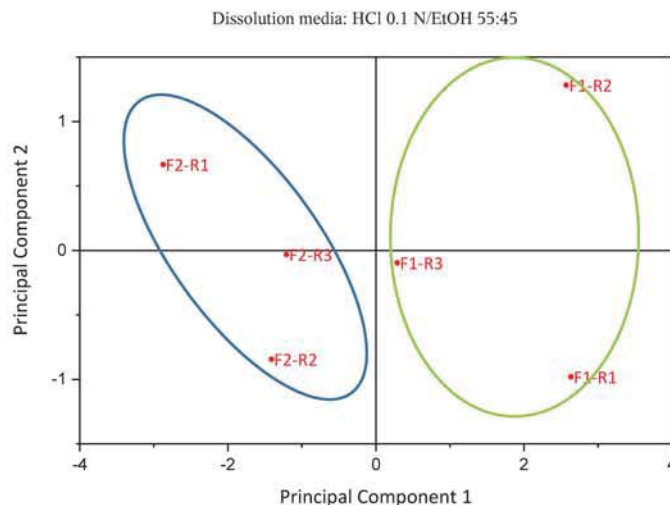
Figure 19 PCA for release profiles in HCl 0.1 N: Ethanol (55:45 % v/v).

PCA grouped the release profiles of both Formulation 1 and Formulation 2 into two clusters regardless the dissolution media employed for the experiments (Figure 17, Figure 18 and Figure 19) suggesting that the addition of HPMC to the films have some effect on the release profiles of progesterone. Further investigation was carried out to understand the effect of EC and HPMC on the release of progesterone and results will be discussed in a later section.

The use of non-aqueous solvents is considered to be unconventional and according to the U.S. Pharmacopeia (USP), high ethanol concentrations may not be desirable for routine characterization studies (FDA, 2014). Although, Formulation 1 and 2 were significantly different, the observed difference was not very pronounced and lack of discriminative capacity when testing formulations with not very pronounced differences was a concern.

Optimization of final experimental conditions was carried out taking into account all information obtained from screening experiments showed above. In order to maintain an

acceptable discriminative ability of the dissolution media, lower ethanol concentration was selected. Table 11 shows the experimental conditions for the optimized release test.

Table 11 Optimized conditions for release test.

Parameter	Specification
Dissolution media	HCl 0.1 N: Ethanol (85:15 % v/v)
Volume of dissolution media (mL)	140
Temperature	Room temperature
Sampling volume (mL)	5
Volume correction	Yes
Sampling time (min)	20, 40, 60, 90, 120, 180, 240, 300

Next step in the development stage takes into consideration the manufacturing process and its possible impact on the physical properties and performance of EC/HPMC composite films.

4.7 Selection of manufacturing process for Progesterone-loaded EC and EC/HPMC composite films

The manufacturing process has been correlated with the release characteristics of HPMC-based dosage forms (Y. Huang, Khanvilkar, Moore, & Hilliard-Lott, 2003). The aim of this part of the study was to evaluate the possible effects of manufacturing process of EC/HPMC composite films on the performance of this dosage form.

Table 12 summarizes critical quality attributes of polymer thin films such as weight, drug content and the amount of progesterone released at t = 240 min from a model formulation made by two different manufacturing processes.

Table 12 Critical quality attributes of EC/HPMC composite films made by two different processes.

Manufacturing process	Weight (mg) (Mean ± S.D.)	Thickness (mm) (Mean ± S.D.)	Amount released at t = 240 min (Mean ± S.D.)
Process 1	57.28 ± 4.98	0.399 ± 0.006	0.4770 ± 0.0433
Process 2	58.63 ± 2.0	0.393 ± 0.009	0.5075 ± 0.0435

A t-test was performed for each response variable and according to the results, there is no significant difference (P-value< 0.05) between product obtained from Process 1 and 2 for all responses. Based on these results we concluded that the manufacturing process does not affect the critical quality attributes of EC/HPMC composite films. Further analysis was carried out in order to observe whether the release performance was affected by changing the manufacturing process.

Figure 20 shows the release profiles of the model formulations made by the different manufacturing processes and Figure 21 shows the PCA of the release profiles for the two manufacturing processes tested.
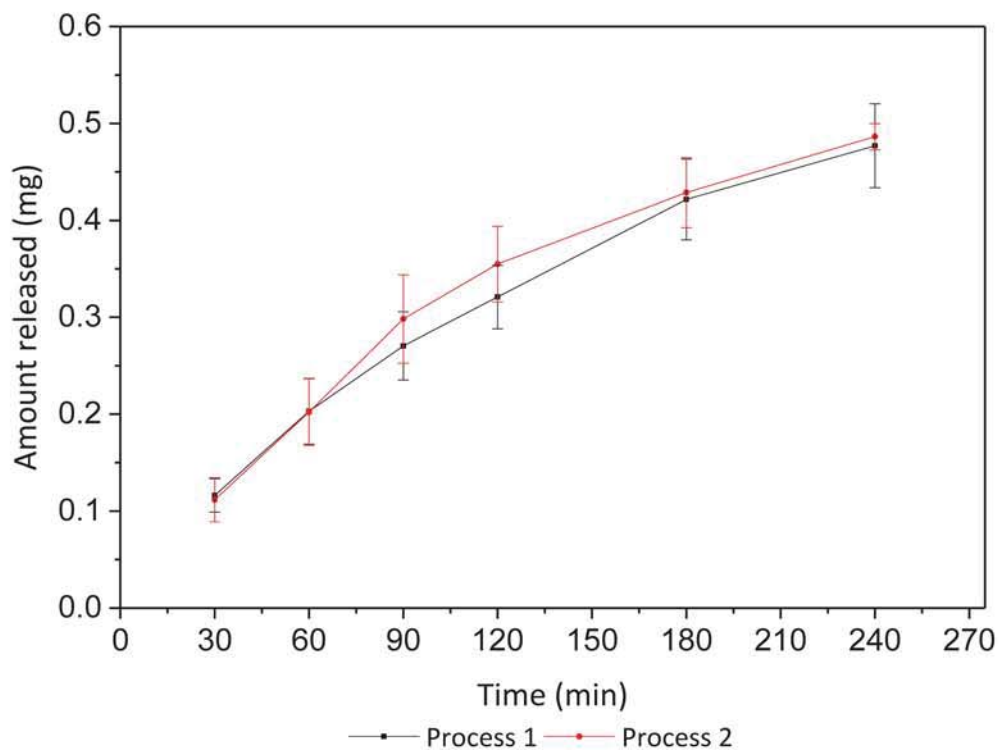
Figure 20 Release profiles of progesterone from EC/HPMC composite films made by two different manufacturing processes.
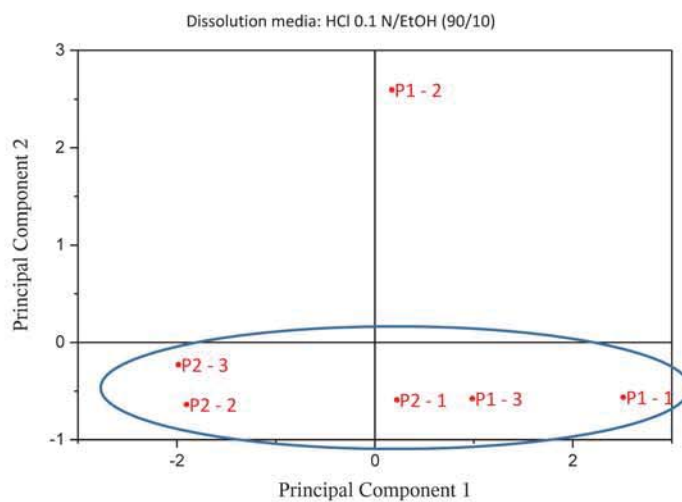

Figure 21 PCA for release profiles of progesterone from EC/HPMC composite films made by two different manufacturing processes.

Figure 20 and Figure 21 show that the drug product produced under two different manufacturing processes have minimal differences in their performance. Optimization of the final manufacturing process was carried out taking into account all information obtained from the experiments showed above. Some other factors such as ease of the process were considered as well. Process 2, requires blending of the solid components, which is an extra unit operation that needs more experiments for optimization. As a consequence, Process 1 was considered to be optimal for the manufacturing of EC/HPMC composite films (see 3.8).

The next step includes the evaluation of EC and HPMC effect on the release of progesterone from EC/HPMC composite films. In summary, from optimization of release test we concluded that HCl 0.1 N: Ethanol (85:15% v/v) was a suitable media for the *in vitro* release test and both process 1 or 2 can be used for manufacturing of EC/HPMC composite films, but process 1 was selected because of its simplicity.

4.8 Evaluation of the EC and HPMC effect on the release of progesterone from EC/HPMC composite films.

The aim of this portion of the study is to understand the effect of EC and HPMC amount on the release rate and release profiles of progesterone. For this purpose, 10 different experiments were performed using different EC/HPMC ratios. Release experiments were carried out using HCl 0.1 N: EtOH (85:15% v/v) as dissolution media. Table 13 shows critical quality attributes of the produced films.

Table 13 Critical quality attributes of EC/HPMC composite films.

	Thickness (mm) (Mean ± S.D.)	Weight (mm) (Mean ± S.D.)	Amount released (mg) at t= 300 min (Mean ± S.D.)
Formulation 1	0.406 ± 0.05	61.20 ± 8.51	0.78 ± 0.01
Formulation 2	0.454 ± 0.016	70.45 ± 3.24	0.76 ± 0.01
Formulation 3	0.392 ± 0.077	59.44 ± 6.90	0.63 ± 0.03
Formulation 4	0.410 ± 0.036	60.55 ± 6.37	0.59 ± 0.04
Formulation 5	0.407 ± 0.027	58.40 ± 5.70	1.02 ± 0.05
Formulation 6	0.410 ± 0.017	59.61 ± 4.61	0.87 ± 0.02
Formulation 7	0.379 ± 0.017	56.80 ± 2.47	0.88 ± 0.01
Formulation 8	0.386 ± 0.014	57.28 ± 2.14	0.75 ± 0.02
Formulation 9	0.363 ± 0.032	50.5 ± 7.07	0.65 ± 0.01
Formulation 10	0.303 ± 0.020	41.96 ± 4.83	0.85 ± 0.02

The analysis of the release profiles showed that under the tested conditions, it is possible to control the release of progesterone from 0.59 and up to 1.02 mg at t= 300 min which means that by changing EC/HPMC ratios we can increase the release of progesterone up to 50%. Figure 22 shows the magnitude and the importance of each variable in the amount of progesterone released at t= 300 min. The chart displays the absolute value of the effects and draws a reference line on the chart. Any effect that extends beyond this reference line is potentially important in the final output (amount of progesterone released at 300 min).
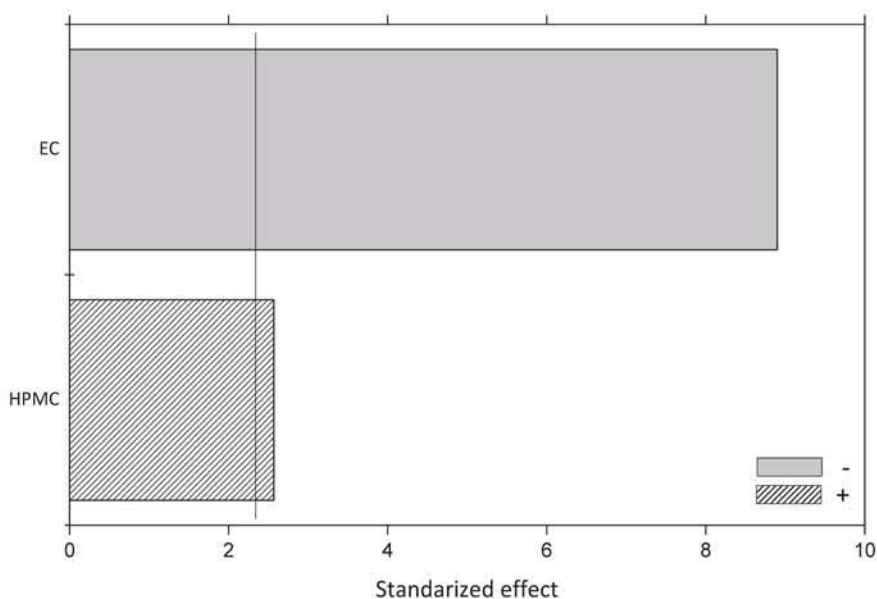
Figure 22 Pareto chart of standardized effects for amount of progesterone released at t= 300 min (α=0.05, d.f. = 7).

Analysis of these results exhibited that the two variables analyzed had a significant effect on the amount of progesterone released at t= 300 min. From the chart we can see that higher amount of EC in the polymer film led to smaller amount of progesterone released from films which is a likely consequence of the increased density of the polymer film that increases diffusion distance and decreases the overall drug release from the polymer film, while addition of HPMC increased the release of progesterone (X. Huang & Brazel, 2001).

The effect on the amount of progesterone released (t= 300 min) can be explained by the following model:

Amount of progesterone released (t=300 min) = 1.36819 - 0.38529* EC + 0.19437*HPMC

Further analysis using ANOVA test indicated a significant effect of independent factors (P-value < *0.05*) on response Amount released at t= 300 min with an adjusted R-square value

of 0.9207. All factors considered in the model were statistically significant (P-value < 0.05). From the model and previous chart, it is possible to conclude that EC is the polymer that controls the release of progesterone, while HPMC can be used as an additive to accelerate this process. This outcome is in good agreement with the results published by other authors who argued that HPMC migrates out of the dosage form increasing the amount of drug released to the dissolution media (Gunder, Lippold, & Lippold, 1995; Raut et al., 2013).

Figure 23 and Figure 24 show complete release profiles for EC and EC/HPMC composite films.
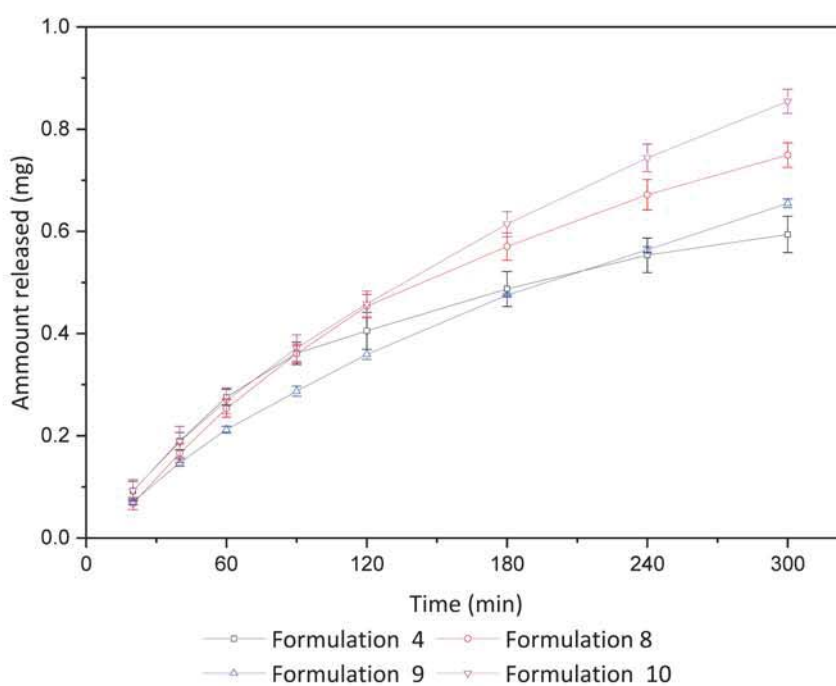
Figure 23 In Vitro release profiles of progesterone from EC films in HCl 0.1 N: Ethanol (85:15% v/v).
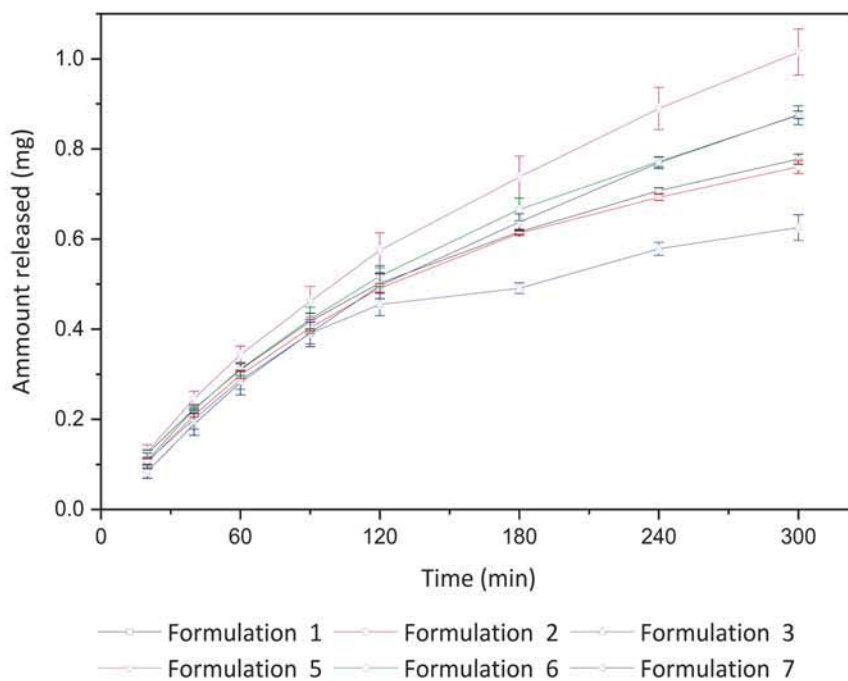
Figure 24 In Vitro release profiles of progesterone from EC/HPMC composite films in HCl 0.1 N: Ethanol (85:15% v/v).

PCA (Figure 25) was able to distinguish between EC and HPMC films confirming the significant difference due to the positive effect of HPMC on the release rate of progesterone from HPMC.
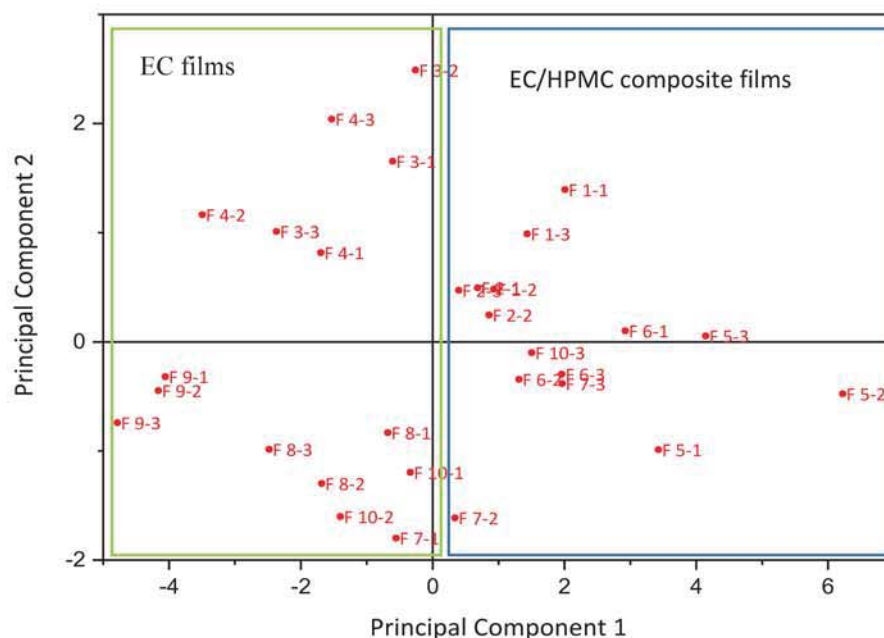
Figure 25 PCA for release profiles of progesterone from EC/HPMC composite films.

In order to analyze the drug release mechanism for films, the release profiles data were fitted to the different kinetic models (Table 14). For EC and EC/HPMC composite films, the drug release profiles showed a suitable fit to Korsmeyer-Peppas model for release kinetics. In all instances, the correlation coefficients (Rsqr_adj) for the data were equal to or greater than 0.95 and showed low AIC values and large MSC values (Zhang et al., 2010).

Table 14 Statistical criteria for selection of the best mathematical model for progesterone loaded EC/HPMC composite films.

Model	Parameter	F 1	F 2	F 3	F 4	F 5
Zero order	Rsqr_adj	0.7511	0.7803	0.5547	0.6182	0.8940
	AIC	21.5694	19.7846	27.9396	23.1476	19.5412
	MSC	1.1640	1.2671	0.5862	0.7180	2.0127
Firstorder	Rsqr_adj	0.7785	0.8032	0.6005	0.6503	0.9141
	AIC	20.6328	18.9010	27.0050	22.4434	17.8636
	MSC	1.2810	1.3776	0.7030	0.8060	2.2224
Higuchi model	Rsqr_adj	0.9690	0.9597	0.9587	0.9658	0.9416
	AIC	5.0082	6.2134	9.1122	3.4042	14.8242
	MSC	3.2341	2.9635	2.9396	3.1859	2.6023
Korsmeyer-Peppas	Rsqr_adj	0.9843	0.9791	0.9558	0.9644	0.9957
	AIC	-0.1248	1.6313	10.4212	4.6129	-5.4824
	MSC	3.8757	3.5363	2.7760	3.0349	5.1406
	n	0.5892	0.6057	0.5186	0.5357	0.6863

Table 14 Statistical criteria for selection of the best (Cont.).

Model	Parameter	F 6	F 7	F 8	F 9	F 10
Zero order	Rsqr_adj	0.8589	0.9141	0.8853	0.9186	0.9273
	AIC	19.2382	15.2152	16.1045	13.0148	18.4964
	MSC	1.7194	2.2806	1.9240	2.2658	2.4255
Firstorder	Rsqr_adj	0.8793	0.9286	0.9005	0.9310	0.9448
	AIC	17.9847	13.6434	14.9559	11.6729	16.2216
	MSC	1.8761	2.4771	2.0676	2.4335	2.7099
Higuchi model	Rsqr_adj	0.9488	0.9217	0.9261	0.9281	0.9218
	AIC	11.1826	14.8499	12.6274	12.0473	19.3063
	MSC	2.7264	2.3263	2.3587	2.3867	2.3243
Korsmeyer-Peppas	Rsqr_adj	0.9896	0.9905	0.9795	0.9949	0.9957
	AIC	-1.1964	-1.1068	2.7306	-8.4048	-3.7534
	MSC	4.2737	4.3209	3.5958	4.9432	5.2067
	n	0.6568	0.7211	0.6907	0.7156	0.7312

The values of the release exponent n which is an indicative of drug release mechanism were in the range of 0.5357-0.7312 for EC films and 0.5186-0.7211 for EC/HPMC composite films. According to Peppas model, for thin films with n values between 0.5 and 1.0, the release mechanism is non-Fickian anomalous transport and the release is governed by diffusion and other mechanisms such as erosion and swelling (Peppas & Sahlin, 1989; J. Siepmann & Peppas, 2001; Juergen Siepmann & Peppas, 2011).

These conclusions are in good agreement with the results published by other authors who concluded that the drug release kinetics for different EC and HPMC dosage forms is governed by diffusion and erosion of polymer matrices (Crowley et al., 2004; Siegel & Rathbone, 2012). In all cases, as a result of its hydrophobic properties, ethyl cellulose reduces the penetration of water in the polymer matrix which causes the reduction in the drug release (Mehta, Missaghi, Tiwari, & Rajabi-Siahboomi, 2014; Patra, Kumar, Pandit, Singh, & Devi, 2007) and adding hydrophilic additives such as HPMC resulted in faster and constant drug release rates (Chambin et al., 2004; Lopes, Manuel Sousa Lobo, Costa, & Pinto, 2006).

Final optimization should be carried out based on the required release rate for a specific formulation that should take into account the intended purpose of the formulation and the needs of the patient. The contribution of this work is the creation of a model that explains and quantifies the effect of EC 7 and HPMC E6 on the release of progesterone from EC and EC/HPMC composite films.

CHAPTER 5. FUTURE WORK

Nanotechnology in pharmaceutical field has attracted enormous interest from researchers due to the multiple advantages that nanoparticles offer. However, as other approaches, nanotechnology have some limitations that need to be overcome in order to fully exploit its potential utility in the development of therapeutic systems. Consequently, there has been an exponential growth of interest on the development of novel drug delivery systems using nanoparticles.

The design and feasibility of a simple process, that consists on incorporating nanoparticles into polymer films is clearly a promising strategy for improving nanoparticles stability, incorporation of drugs with different physical and chemical properties and controlling or enhancing the dissolution rate of poorly water-soluble drugs. Preliminary experiments performed in our laboratory show the feasibility of incorporation of nanoparticles and even microspheres in polymer films (Figure 26).
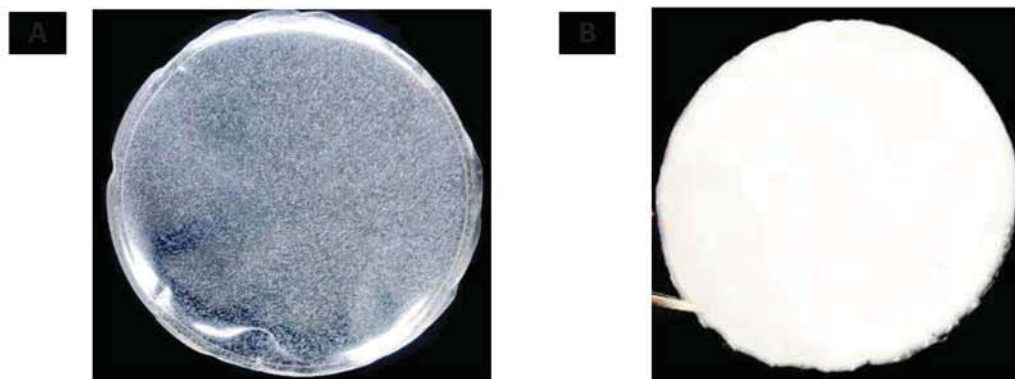
Figure 26 Gelatin films with embedded micro and nanoparticles.

A) *Gelatin film with embedded PLGA microspheres B) Gelatin film with embedded polycaprolactone nanocapsules (*Image courtesy of *Catalina Azcarate, Universidad Nacional de Colombia).*

Further research will focus on incorporating engineered nanoparticles for the design and manufacture of pharmaceuticals taking into account current strategies in the pharmaceutical field, its advantages, and disadvantages to fully achieve the future requirements of pharmaceuticals in areas such as personalized medicine within the current regulatory framework: Quality by Design (QbD).

CHAPTER 6. CONCLUSIONS

- DBZ-loaded nanoparticles and progesterone-loaded EC/HPMC composite films were developed for controlled release.

- DBZ-loaded nanoparticles were composed of PLGA (50:50) which is an FDA approved polymer suitable for pharmaceutical applications because it is biodegradable and biocompatible. Nanoparticles were produced using the nanoprecipitation method. The polymer concentration in the organic phase, temperature, and volume of organic phase are critical parameters and govern the final particle size of the drug product.

- Optimized formulation showed particle size of 212 ± 9.0 nm, polydispersity index of 0.13 ± 0.075, encapsulation efficiency > 94% and zeta potential of -20.2 ± 3 mV.

- Nanoparticles were stable after dilution at different pH values (from 3 to 7.5) for at least 7 days at room temperature.

- The mathematical model that best described the release of DBZ from PLGA nanoparticles was Korsmeyer-Peppas and the predominant release mechanism was diffusion.

- EC and EC/HPMC composite films were produced using the solvent casting method. Dissolution media was optimized for achieving maximum discriminative ability.

Variations in manufacturing process showed to have no effect on the progesterone release profile.

- EC have a strong impact on the release kinetics of progesterone and addition of HPMC as a hydrophilic additive enhances the release rate. Changing EC/HPMC ratios increased the release of progesterone up to 50% when compared to EC films.

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