António Manuel Dias dos Santos Brito
Bachelor in Chemical and Biochemical Engineering

Benchmarking of alternative production techniques for PLGA microencapsulation

Dissertation submitted in fulfillment
of the requirements for the degree of

Master of Science in
Chemical and Biochemical Engineering

Advisers: João Henriques, Team leader, Particle design and formulation, R&D
Hovione Farmaciencia S.A.
Ana Aguiar Ricardo, Full Professor, NOVA University of Lisbon

Examination Committee

Chairperson:	Prof. Dr. Mário Fernando José Eusébio
Rapporteurs:	Prof. Dr. Ana Clara Lopes Marques
Members:	Engineer João Luis Sousa Cardoso Gonçalves Henriques

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UNIVERSIDADE NOVA DE LISBOA

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Abstract

Interest in controlled and extended release parenteral formulations is currently growing in the industry due to the advantages they present in the field of patient compliance. PLA and PLGA microspheres are the most used and well-known type of formulation in this area. They do not present toxicological concerns and have been approved by the FDA for use in commercial applications.

Hot melt extrusion or HME is an emergent technology in the pharmaceutical field. Being known to the plastic industry for decades, this technology has applications in taste masking, solubility enhancement and controlled release formulations.

In this work, two case studies are presented. The first one deals with a controlled release parenteral formulation of PLA and minocycline hydrochloride. This case study focuses on the study of operational parameters of HME, downstream processing in the form of hammer milling and API distribution within the end product. The end product of the extruder is a dense and non-porous material with the API mainly located in the core as checked by SEM-EDS. Hammer milling trials were successfully produced microparticles below 140 micrometers after sieving. These formulations had 20% to 40% of drug released in the first hour and correlation between these values and the operational parameters is inconclusive and requires future work.

The second case study is based upon an extended release formulation of PLGA and diclofenac sodium which was successfully developed. This case study addressed formulation parameters and benchmarking of different IVR testing methodologies. The final microparticle formulations were tested through two different IVR methods. The USP apparatus IV was used to compare two different PLGA grades. A sample-and-separate method was used to correlate particle size of API, drug load and extruder temperature to burst release. The particle size of API was the most significant factor affecting burst release which ranged between 20% and 60%.

Keywords: Controlled release; Hot melt extrusion; Hammer milling; Burst release; Poly Lactic-co-Glycolic acid; Poly Lactic acid; *In-Vitro* release;

Resumo

O interesse em formulações parenterais de libertação prolongada e controlada tem vindo a crescer na indústria, principalmente devido às vantagens que apresentam para o conforto do paciente. Microesferas de PLA/PLGA são as mais conhecidas destas formulações. São aprovadas pela FDA e não possuem qualquer problema de toxicidade.

Hot melt extrusion ou HME é uma tecnologia emergente na industria farmacêutica. Conhecida há décadas na indústria do plástico, esta tecnologia tem aplicações em *taste masking*, *solubility enhancement* e formulações de libertação prolongada.

Neste trabalho são apresentados dois casos de estudo. O primeiro lida com uma formulação parenteral de libertação prolongada contendo PLA e cloridrato de minociclina. O objectivo é o estudo de parâmetros operacionais do HME, processamento adicional através de hammer milling, e a distribuição do fármaco pela matriz. O produto final é denso e não poroso com o fármaco posicionado no interior da matriz, como determinado por SEM-EDS. As experiências de *hammer milling* foram bem sucedidas com microparticulas abaixo dos 140 micrometros após peneiração. As formulações libertaram 20% a 40% do fármaco na primeira hora e a relação entre estes valores e os parâmetros operacionais foi inconclusiva.

O segundo caso de estudo contém PLGA e diclofenac sódico. Este estudo focou-se em parâmetros da formulação e na comparação de diferentes testes de IVR. Como anteriormente dito o produto final do HME foi processado por *hammer milling*. As microparticulas finais foram testadas através de dois métodos diferentes de IVR. O USP *apparatus* IV foi usado para comparar duas formulações com diferentes grades de PLGA. Um método de *sample-and-separate* foi usado para relacionar o tamanho de particula do fármaco, carga de fármaco e temperatura de extrusão com a libertação inicial de fármaco. O tamanho de particula de fármaco foi o factor mais significativo na libertação inicial que rondava os 20% a 60%.

Palavras-chave: Libertação controlada; *Hot melt extrusion*; *Hammer milling*; Libertação inicial; Poli láctido-co-glicólido ; Poli láctido ; *In-Vitro release*;

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Glossary

API Active Pharmaceutical Ingredient.

BSA Bovine Serum Albumin.

CV Coefficient of Variation.

DoE Design of Experiments.

DSC Differential scanning calorimetry.

FDA Federal Drug Administration.

HME Hot Melt Extrusion.

HSA Human Serum Albumin.

IVR *In-vitro Release*.

LOQ Limit of Quantification.

MLR Multi Linear Regression.

MW Molecular Weight.

OFaT One Factor at a Time.

PLA Poly Lactic acid.

PLGA Poly Lactic-co-Glycolic acid.

PSD Particle Size Distribution.

SSE Single Screw Extruder.

T_g Glass Transition Temperature.

TFA Trifluoracetic acid.

TGA Thermogravimetric analysis.

TSE Twin Screw Extruder.

CHAPTER 1

Introduction

Contents

1.1 Motivation

The discovery and subsequent usage of biopharmaceuticals and hydrophobic drugs in the pharmaceutical pipeline is on the rise [1]. These pharmaceuticals possess low oral bioavailability or low solubility. This eliminates a convenient and frequent administration route and heightens the need for parenteral administration. However when dealing with chronic diseases or the necessity of prolonged treatment parenteral administration either intravenous, subcutaneous or intramuscular entails a decrease in patient compliance due to the necessity of frequent and sometimes painful injections.

Controlled and sustained release formulations can be a good solution to this problem. They can defeat the necessity of frequent administration which gives the patient a greater degree of comfort. Not only that but by allowing a greater control on the level of drug in the patient's body, side effects and toxicity worries can be minimized [2].

These formulations are based upon the parenteral administration of a drug encapsulated in a polymeric matrix of some sort. This matrix has the function of releasing the drug into the patients body in a controlled manner that maintains the drug concentration in the patient's body

within therapeutic range. Polymeric carriers can, to some extent, increase stability and apparent (kinetic) solubility [1, 3].

PLA or Poly(lactic acid) and PLGA or Poly(lactic-co-glycolic acid) are part of a biodegradable polymer family currently approved by the FDA for use in controlled and extended release formulations [4].

The great advantage of this polymer lies in its biocompatibility and absence of toxiticity issues. Both of them naturally degrade in the presence of water into their monomers which are innocuous to the body. Additionally, PLGA can be customized to deliver the drug in a customizable time frame [2]. PLA/PLGA matrixes can assume various geometries, but by far the most common are microparticles. These are typically applied by intramuscular or intradermal injections [5].

Generally, PLA and PLGA microparticles are industrially produced by emulsion or phase separation methods which rely on the use of large amounts of organic solvents. Besides the environmental burden, the use of organic solvents warrants downstream drying and large equipment footprint even for small batches. Alternative production methodologies are explored to address these problems.

1.2 Literature review

1.2.1 Polymeric matrices and controlled release

The encapsulation of an Active Pharmaceutical Ingredient (API) in a biodegradable polymeric matrix is a topic that has been studied in the pharmaceutical industry for almost 80 years [3]. A great number of newly developed drugs possess low oral bioavailability, which eliminates the most used and convenient administration route [3, 6]. As such, parenteral application is sometimes required and frequent administration decreases patient comfort and compliance.

Solving this problem can be done using extended and controlled release formulations of API in biodegradable polymeric matrices. This is a topic that brings several advantages to the table especially when dealing with chronic diseases [7] or ailments that require multiple treatments due to the low biological half-life of the API [3]. They can soften the patient's discomfort and also increase compliance due to the decreased treatment frequency and side effects [6, 7].

Countless polymeric carriers have been studied which can also bring to some extent increases in stability [8, 9] and increases in solubility [5, 10]. The list ranges from natural occurring polymers like Bovine Serum Albumin (BSA), Human Serum Albumin (HSA) or chitosan [11, 12], to synthetic polymers such as polyesters, or polyanhydrides [11]. These polymer matrices can have varied morphology from wafers for brain implantation like Gliadel® [8] to new approaches like the development of millirod implants [8]. For controlled release applications the Poly Lactic acid (PLA)/Poly Lactic-co-Glycolic acid (PLGA) family is by far the most prevalent [13]. And, although they possess great physical capabilities to assume a variety of geometries, the most used formulation is comprised of microparticles. [3].

1.2.2 PLA/PLGA microsphere formulations

As of 2008, a total of 12 different controlled release formulations containing microspheres of PLA/PLGA have entered the market [3]. Examples of PLA/PLGA microspheres for controlled release include peptides like Lupron Depot® or small molecules like the one present in Risperdal® Consta [3, 7, 13]. These two products, in 2008, moved 1.9 billion and 1.49 billion US dollars in sales respectively [7]. These numbers prove the potential of these microsphere formulations for sustained release of several types of drugs. A more comprehensible list of mi-

crosphere products still on the market is in table 1.1 in page 3. Note that some products use only one polymer type (p.ex. Risperdal uses PLGA). However, in most of these formulations the usage of PLA or PLGA is dictated mainly by the release target desired as will be discussed in forward chapters.

Table 1.1: List of marketed PLGA microsphere formulations. Data compiled from [3, 7, 13, 14]

	API	Product	Distributor company	Therapeutical indication
Peptides	Ocreotide acetate	Sandostatin LAR Depot	Novartis	Acromegaly
	Lanreotide acetate	Somatuline Depot	Ipsen-Beafour	Acromegaly
	Leuprorelide acetate	Lupron Depot	TAP Pharmaceuticals	Prostate cancer
	Triptorelin pamoate	Trelstar Depot	Allergan; Ipsen [a]	Prostate cancer
	Triptorelin embonate	Pamorelin	Ipsen [a]	Prostate cancer
	Triptorelin acetate	Decapeptyl	Ferring; Ipsen [a]	Prostate cancer
	Buserelin	Suprecur MP	Mochida Pharmaceuticals [b]	Endometriosis
Small molecules	Risperidone	Risperdal Consta	Janssen / Alkermes	Schizophrenia
	Bromocriptine	Parlodel LAR	Novartis	Parkinsonism
	Minocycline	Arestin	Orapharma	Periodontitis
	Naltrexone	Vivitrol	Alkermes	Alcohol dependence

[a] Debiopharm licensees
[b] Aventis licensee

PLA and PLGA are synthetic biodegradable polyesters that are approved by the Federal Drug Administration (FDA). The pairing of a good solubility in a wide range of organic solvents [11] and good mechanical properties allow it to be processed into nearly any shape and size including, but not limited to, rods, discs, wafers, scaffolds, particles, films and even foams [8, 11]. In addition, when present in an aqueous media, they naturally degrade into its monomers, lactic acid and glycolic acid, which can be metabolized and/or excreted safely [11, 13].

Consequently, it is highly biocompatible and poses no toxicological issues [11, 13]. The degradation kinetics correlate directly with the release of the drug and PLGA as a matrix has an advantage in this field when compared to other polymers. PLGA's behaviour is highly flexible and can the polymers can be easily manipulated to tune the degradation and consequently to deliver the required dose in the required time frame [7, 11, 13].

1.2.3 Mechanism of degradation

The degradation of PLGA miscrospheres is thought to be dominated by the non-enzymatic hydrolytic reactions of the ester linkages, which spontaneously occurs in aqueous environment [4, 11]. However, consensus is still not achieved in the scientific community. There is a discrepancy between in vivo and in vitro testing that some authors argue corresponds to a relevant enzymatic contribution. However, the in vivo testing is not standard between all authors and arouses doubts on this reasoning. Much more plausible is a difference between the water uptake being the real culprit of the discrepancy [11, 15].

It's important to distinguish two different kinds of degradation or erosion, the bulk erosion and the surface erosion. Bulk erosion is characterized by an uniform degradation throughout the entire depth of the particle. Surface erosion, however, is characterized by a degradation which is given primarily at the exterior surface of the particle. If the particle has less than 300 microns in diameter the degradation is uniform throughout the particle which is also known as bulk erosion. For parenteral applications the diameter must less than 120 micron preferably

always in the range of 20-100 microns [3,4,12]. Thus, all of the parenteral microspheres degrade by bulk erosion.

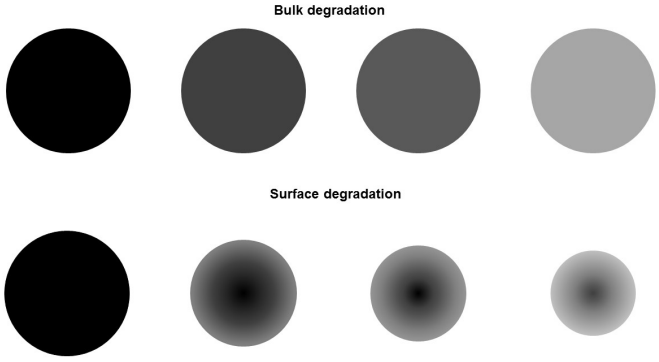


Figure 1.1: Schematic representation of the difference between bulk and surface degradation

The most common mechanism to describe PLGA degradation is based upon bulk degradation and is comprised of three different phases. It starts with a phase where degradation occurs without weight loss, since the hydrolysis breaks polymer strands into smaller but insoluble oligomer strands. This does, though, decrease the molecular weight of the polymer. In the following phase the polymer molecular weight starts to decrease significantly as more and more soluble monomers and oligomers are formed and as such, leave the matrix. The final phase is characterized by the complete polymer solubilization [4, 11].

The drug release depends not only on degradation and erosion but also drug diffusion out of the polymeric matrix. This makes it a much more difficult mechanism to understand.

1.2.4 Burst release

With some exceptions it is of great importance that the drug release is given in relatively constant velocity within the required time frame so as to avoid sharp spikes of drug concentration within the patients body. Typically controlled release formulations suffer from what it's called burst release. It is characterized by a great amount of drug released instantly after administration. While this can be beneficial or even wanted, it's usually a big problem to address since the sharp spikes can lead to potential unwanted effects. [16]

Most of the time the main goal is achieving zero-order release. This is characterized by a constant release of drug into the patient's system. [16] This is to achieve a concentration of drug in the patient's system that is above the minimum effective concentration for the highest amount of time [17] Avoiding burst release is a major topic in controlled release. The key here is understanding the mechanism of drug release and the underlying causes of the appearance of burst. Even then, some ways to avoid burst release have been studied. These are usually additional processing steps that add costs to the process such as surface extraction and/or modification [16].

1.2.5 Modulating the drug release

This added versatility that characterizes PLA/PLGA makes it possible to produce a polymer with specific physicochemical and deterioration properties by changing polymer properties, pro-

duction variables or geometry of the matrix. Different degradation kinetics lead to different drug releases.

The most most well known way of changing the degradation kinetics of this family of polymers is by changing the monomer content. Lactic acid's methyl group, which glycolic acid lacks, adds hydrophobicity to the molecule. Thus, a polymer with higher content of lactic acid in its composition shows slower decomposition due to being more difficult for water to penetrate the matrix. The opposite is also true; a polymer with a higher amount of glycolic acid not only shows more hydrophilicity but also faster degradation [7, 11, 15].

Another way to modulate polymer degradation is by changing molecular weight. Molecular weight is a direct link to chain length. Consequentially, the higher the molecular weight, the higher the time to complete degradation due to the bigger polymer chains to degrade [11, 15].

The drug to be released can interact with the polymer and as such it is always important to consider the drug as a factor in the release [11]. For example, PLGA can degrade more easily in acidic environments or near amine groups and as such the degradation can be accelerated if the API has any of these two criteria [3, 11, 18, 19]. Sometimes the changes can be beneficial such as a drug that effectively works as a plasticizer, lowering the Glass Transition Temperature (T_g), facilitating handling [8]. The exact opposite has also been reported – alkaline drugs that interact with the carboxylic groups of the chains, increasing T_g and slowing degradation [15]. No major interactions between polymer and drug can also happen as in the case of some acidic or neutral drugs that precipitate intro crystals within the matrix [15]. It's clear that many different kinds of interactions can occur and each case must be evaluated alone.

Volume increases more rapidly with size than surface area. The higher surface area is important to consider when dealing with water penetration [11]. In general, smaller particles degrade more rapidly than bigger particles due to having more surface area per volume than bigger particles which are degrade slower and have a sigmoidal release profile [20].

As mentioned before, both acidic and alkaline environments can change the kinetics of the hydrolysis of the ester linkages [11, 15, 18, 19]. Sometimes micro-environments can be originated from the encapsulated drugs and/or from the monomers originated from degradation especially when the matrix is dense and non porous which leads to accumulation of these compounds [7, 11, 15]. The monomers and end groups of polymer chains can then auto catalyze the degradation due to the inherent acidity of carboxylic end groups in water [4, 7, 11].

1.2.6 PLGA microsphere production

A multitude of production methods of PLGA microspheres exist today. Some are already in large scale production while some are considered alternative methods and are still being studied. In the sub-chapters ahead, the more known and used methods of production of PLGA microspheres for controlled release applications are listed, along with pertinent analysis of their steps, ease of usage and application. Additionally, table 1.2 in page 5 compiles the production methods of some products on the market.

Table 1.2: Production processes of marketed PLGA microsphere formulations. Data compiled from [3, 7, 13, 14]

Phase separation	O/W and solvent extraction	W/O/W and solvent evaporation	Hot melt extrusion and cryogenic grinding (Alkermes ProLease)	Spray drying
Sandostatin LAR Depot Somatuline Depot Decapetyl Arestin	Risperdal Consta Vivitrol	Lupron Depot	Trelstar Depot	Parlodel LAR Suprecur MP

1.2.6.1 Emulsion methods

Emulsion methods in general are based upon the formation of one or more micro emulsions followed by removal of the inner phase solvent to a gas phase (evaporation) or extraction to another liquid continuous phase. Removing the inner phase solvent, where the polymer and drug are dissolved, precipitates the polymer and produces microspheres. [3, 4]

The rate of solvent removal is a critical parameter to follow, monitor and control because it greatly influences the final product. Other important factors to have in consideration are the temperature and viscosity of the phases, nature of the drug and the desired final concentration within the matrix. [3]

These production methods suffer from obvious disadvantages concerning their usage of organic solvents which have to follow strict rules concerning their presence in the final product. They also have a high demand for control of the production variables in order to achieve reproducibility. Typical examples are stringent temperature controls and constant monitoring of viscosity of the different phases. Emulsion methods methods are further classified according to the number and nature of the phases they present. [4]

Single emulsion

- **Oil in water** Also called O/W, it is characterized by the formation of a solution of PLGA and API in an organic solvent, usually methylene chloride. This solvent is then added to an aqueous mixture containing a surfactant that acts as an emulsifier. After emulsifying by high shear mixing or sonication the inner phase (solvent) is removed either by evaporation [21] or by continuous liquid-liquid extraction to the aqueous phase [22] .

  This method is usually reserved to low water soluble API's since they are usually soluble in the organic solvents but don't readily diffuse to the aqueous phase. Solvent and emulsifier selection and concentration is paramount. [3, 4]

  This single emulsion method is used for the industrial production of Risperdal Consta and Vivitrol.

- **Oil in oil** O/O is used when the API despite being hydrophobic has a meaningful water solubility that can present problems of low encapsulation efficiencies when using O/W. The production is completely analogous to the O/W but in this case the external phase is also an organic phase. Similarly, microparticles are formed removing the interior solvent by evaporation or liquid-liquid extraction. However, if using liquid-liquid extraction, an additional downstream process to effectively remove the continuous phase is needed (solvent washing usually). [3, 4]

- **Solid in oil in water or solid in oil in oil** These methods are used when the drug does not readily dissolve in either aqueous or organic solutions [3]. Thus, the API remains solid in an organic solution with polymer which is then emulsified in an aqueous (S/O/W) or organic (S/O/O) phase containing an emulsifier. The remaining process is reminiscent of the previous; the interior phase is removed by evaporation or liquid-liquid extraction [23]. The requirement of micronized drug is a hassle especially when considering preclinical API's which may warrant lab scale preprocessing and additional trouble when the drug is hygroscopic or friable or when the particles sediment or float [3]. Considerations on the drug load and on API particle size must be taken to avoid burst release or a non-sustainable encapsulation efficiency [3]. Siepmann et al. encapsulated apomorphine in PLGA using a S/O/W method, with an added focus on avoiding spontaneous oxidation of the labile API [24].

Double emulsion

- **Water in oil in water** When dealing with highly water soluble and hydrophilic drugs, the other methods may be unsustainable when evaluating encapsulation efficiency. When the API is a protein, peptide or generally a sensitive compound, avoiding denaturation and decomposition is also a challenge. This methods sports high encapsulation efficiencies for those compounds while retaining their activity. The first emulsion is of an aqueous solution of API into a organic solvent containing PLGA. This emulsion is then emulsified a second time into an aqueous solution containing a surfactant or emulsifier. The organic solvent is then removed by methods similar to the ones used in single emulsion - evaporation or liquid/liquid extraction. [3, 4, 25]

  Currently this process of double emulsion is used in the production of Lupron Depot.

1.2.6.2 Phase separation

Also called coacervation. This method starts by forming a suspension or solution of an API in an organic solution containing PLGA. The formation of the microparticles is done by adding an antisolvent, called coacervating agent, which despite dissolving the original organic solvent it does not dissolve PLGA. This forces PLGA precipitation by removing its solvent to another phase, encapsulating the drug in the process [4].

This is a production method which is used extensively in the industry and is mainly used with water soluble drugs like proteins or peptides as illustrated by the products on the market [3]. The microparticle size is controlled by varying the mixing speed [4]. After removal of the solvent the microspheres are still too soft. The addition of the soft microspheres to a hardening bath is mandatory [3].

The process despite being widely used has its shortcomings. The final product can easily agglomerate and the usage of organic solvents is incredibly problematic. Lowering the concentration of solvent may not be straightforward but it is a necessary requirement. [4]

Typical materials include methylene chloride, silicon oil and hexane as organic PLGA solvent, coacervating agent and hardening agent correspondingly [4]. Nihant et al. [26] used this setup to study the effect of process parameters in microencapsulation.

Currently this process is used in the production of Sandostatin, Somatuline, Decapeptyl and Arestin.

1.2.6.3 Spray drying

Spray drying consists of the atomization of a solution or suspension of polymer, drug and the appropriate solvent. The solvent is removed by contacting the atomized fluid with a hot gas stream that is usually co current.

Spray drying is a fast, reliable method that presents mild operating conditions. Mass production is feasible and the scale-up is relatively easy. [4]

PLGA/PLA are polymers that are readily available on many organic solvents and spray drying of PLGA is currently done as an industrial production method for the production of Parlodel LAR and Suprecur MP.

1.2.7 Hot melt extrusion as an alternative production method

By far the most prevalent production methods of PLGA microspheres require large amounts of organic solvents. Besides the environmental burden, the use of organic solvents warrants

downstream drying and large equipment footprint even for small batches. To note the exception of Trelstar Depot which uses a modified extrusion process.

Extrusion is a well known process in the plastic industry since the nineteenth century and nowadays it is widespread in the processing of plastic polymers [27, 28]. Interest in extrusion by the pharmaceutical industry sparked during the 1970-1980 and is still currently growing. [27, 28]

1.2.7.1 Process description

Hot Melt Extrusion (HME) is a process in which some kind of polymeric raw material is fed to one or two rotating screws at high temperatures which apply a high shear mixing homogenizing the material input. The operating temperatures are above T_g sometimes even above melting point [28]. The material is simultaneously conveyed along the screw. At the end of the screw the material is forced through a die at high pressure with the purpose of producing a uniform shape product. The shape itself depends on the die opening. [27, 29]

The extruder itself is made out of 4 distinct parts, the motor drive, the screw barrel, one or two screws and a extrusion die. The barrel is heated to a temperature higher than the T_g of the thermoplastic polymer used. More sophisticated extrusion equipment can heat barrel sections independently of one another creating a gradient if necessary. The drive turns the screws which transport the raw material through increasingly small channel depths and pitches. This creates pressure which is necessary to force the material through the die. The rotation has to be stronger than the torque generated by the high viscosity of the polymer in order transport the materials. [27–29]

Extrusion equipment can be classified according to the number of screws, their rotation to one another, their length and the diameter of the screws.

According to the number of screws extruders can be classified as as a Single Screw Extruder (SSE) or as Twin Screw Extruder (TSE). SSE are simpler but do not obtain a good enough homogenization of the materials by pharmaceutical standards and as such are not as used in this context. As a result TSE are more common and more versatile. They allow customization of the screws by dividing them into sections with different purposes. Kneading blocks of different angles and orientations can be coupled to turn extrusion into a powerful homogenization tool even at the molecular level. [27, 29]

TSE can work in two configurations depending on the the rotation of the screws to one another. They can be co-rotating or counter-rotating. Usually co-rotating is preferred over counter-rotating because it imparts higher mixing capabilities to the extruder. [27, 29]

As a powerful homogenizing tool, HME has several uses in the pharmaceutical industry. Ranging from formation of amorphous solid dispersions formulations for solubility enhancement or taste masking applications to the formation of controlled release formulations, HME is growing in the industry. [10, 27, 29]

Table 1.3: Hot melt extrusion pros and cons Data compiled from [28]

Pros	Cons
No solvents required	High energy process
Denser and non-porous end-product	Limited processing of thermal labile materials
Continuous, customizable, scalable and economical	Limited to materials with good flow characteristics
Reduced oxygen exposure	Downstream processing is usually necessary

1.2.7.2 Process parameters

Of all hot melt extrusion process parameters a total of four can be considered critical [29]. They are temperature, screw configuration, screw speed and feed rate.

Extrusion temperature is a critical process parameter since it defines polymer viscosity and thus the torque required to turn the screws is a function of temperature. Extrusion temperature is always above the T_g of the polymer, with a higher temperature equating lower viscosity. Lower viscosity improves mixing capabilities and and a higher quality end product [29]. On the other high temperatures can degrade the feed material be it API or polymer. A compromise between torque and degradation has to always happen, which puts extrusion temperature between T_g and degradation temperature [29, 30].

Screw configuration refers to the zones of the screws which present different geometries and functions. As previously mentioned kneading blocks of different sizes and angles can be added to help with the homogenization [27, 29]. This alters residence time and shear rate. Screw configuration must be changed to suit the application. For example, when a high degree of homogenization and shear is required (amorphous solid dispersions) one kneading block within the screw is a must [29].

Screw speed is important because it influences many other parameters indirectly. Residence time, shear rate, and viscosity in shear-thinning polymers can all be influenced by screw speed [29].

Feed rate is not only a critical process parameter on its own since it must be correlated with screw speed. Both define what is called the filling rate of the extruder. It's defined as the % of the extruder volume which is occupied with material at any giving time. Higher filling ratios equate to lower residence times but higher torques. Filling ratios must be determined in a case by case basis. A rule of thumb for twin screw extruders is to maintain filling ratio between 20-50% [29].

1.2.7.3 Formulation considerations

In pharmaceutical hot melt extrusion the feeding material and consequentially the final formulation is usually composed of to two or three materials. They are the polymeric carrier, the API and plasticizers.

The polymeric carrier that can be used in HME is incredibly varied. The only strict requirements are good thermoplastic behavior and thermal stability [30]. The polymers used can range from alyphatic polyesters (PLA, PLGA), poly(ortho esters), polyuretanes, polyanhydrides, poli(vinilpirrolidones), acrylic polymers and cellulose derivatives (HPMC). Many other options are also being studied [31].

The API's used in HME is mostly limited to it's thermal stability. However a thermal labile drug can still be processed. Care must be taken in choosing the right temperature and a low residence time. However care must be taken in how the API interacts with the polymer of choice. Cases have been reported where the API-polymer interacts affect T_g and handling properties of the end material [27].

Lastly, there are plasticizers. Plasticizers are usually low molecular weight compounds that when mixed with the polymer increase the free space between polymer molecules. This effectively reduces the glass transition temperature of the polymer [27]. Lowering glass transition is important because it also lowers polymer viscosity and allows extrusion and lower temperatures [27, 30]. Typical pharmaceutical plasticizers include citrate esters, fatty acid esters, sebacate esters, phtalate esters, glycol derivatives and many others [27].

1.2.7.4 Downstream processing

Molding the final product into it's final form is also of great importance. Usually the rod like strand that comes out of the extruder has limited applications and a downstream processing step is usually required. Sometimes, the downstream is in-line with the extruder such as calendering or pelletizing that turn the extrudate into sheets or pellets respectively [6, 28]. Other times the downstream is a process in and of itself.

Hammer milling is a milling process that works through the repeated impact of hammers or knives. The material to be milled is fed to a chamber where a rotor rotated at high speeds. The rotor has either fixed or swinging hammers that pulverize, fracture and crush the feed material. At the bottom half of the chamber a mesh or screen is present that allows particles with the desired size to leave the chamber [32]. This works as a way to self-classify the end product and avoids the unnecessary formation of fines.

1.2.7.5 Sterilization

Sterilization of HME end products for parenteral administration is a difficult topic to discuss. There are only two ways of achieving a sterile end product. They are aseptic production and sterilization after production [31].

Aseptic production is incredibly hard to achieve and to control when compared to sterilization after production. On the other hand, downstream sterilization can be achieved by a multitude of ways but most of them are not suitable for polymeric formulations because they are based on processes with high heat and humidity. Option include sterilizing radiation such as gamma radiation or gassing the final product with ethylene oxide for example [31]. Both of these have issues. The first one may degrade the polymeric matrix or crosslink in an undesirable way. The second lives carcinogenic issues of the matrix.

Although aseptic production appears the most straightforward way of sterilizing HME end products, the best technique is not set in stone and depends on the formulation [31].

1.2.8 *In-vitro* release methods

Controlled release parenteral formulations have been used successful in the industry over the past years due to their versatility. However IVR testing on these formulations is lacking in reproducibility and take a lot of time to obtain full data which may impact shelf-life [33, 34]. An absence of compendial IVR methods [33, 34] also makes difficult to obtain comparable date between different authors [33].

Several methods have been proposed in the literature such as sample-and-separate, membrane dialysis and continuous flow. All methods present their disadvantages but the continuous flow one using an USP apparatus IV seems to be the most promising one [33]. It can detect formulation differences and even accommodate an accelerated IVR [33].

1.3 Overview

The main objective of this work is to benchmark hot melt extrusion as an alternative technology to the production of parenteral formulations comprised of PLA/PLGA microspheres.

The main operational parameters of HME technology and the subsequent downstream processing required (milling) will be discussed and will be analyzed by way of several Design of Experiments (DoE).The work can be divided in two distinct parts, both of which will address the quality of the release by studying the occurrence of burst release and the possible reasons of its existence.

The first part of the work will focus on familiarization with the technologies and gaining knowledge of their weaknesses. The work will focus on understanding the difficulties in formulating the final product and the main factors which affect the quality of the end product. The main objectives are evaluating API distribution in the matrix, impact of process parameters on product degradation and impact of milling parameters on particle size and burst release. Experimentally this will entail two simple DoE's to the HME and the milling process as well as analytical characterization of end product and starting materials. This section of the work will utilize PLA and minocycline as a model polymer-drug system. PLA was chosen due to its lower cost which allowed a more liberal use. Minocycline was chosen due to being easily degradable which allowed to check the influence of process parameters on thermal degradation. Also, Arestin, a minocycline/PLGA formulation exists on the market which made the combination of minocycline/PLA as a model polymer/drug system more realistic.

The second part will have it's focus on deepening the knowledge of the process and on the dissolution technologies available. A more thorough DoE will be made on the extruder which will account for formulation parameters. The main objective will be to evaluate the impact of particle size of API, drug load of the matrix and molecular weight of polymer in the drug release profile, especially on burst release. The evaluation and comparison between IVR methodologies will also be tested. This section of the work will utilize PLGA and diclofenac as a model polymer-drug system. PLGA is the most well known polymer for controlled release. On the other hand, diclofenac is a relatively cheap API, with good thermal stability and water solubility which is necessary to make testing and benchmarking easier.

CHAPTER 2

Minocycline Hydrochloride and PLA - First case study

Contents

2.1 Introduction

This case study is based upon the development of a parenteral formulation of PLA and minocycline using hot melt extrusion. In order to produce material that has potential as a parenteral formulation it is required to produce a free flowing powder with microparticles which range from 30 to 120 micrometers [3, 4, 12]. In this work hot melt extrusion end products will be milled by hammer milling, in order to produce the final microparticles. After that a *In-vitro Release* (IVR) test will be used to evaluate the release profile and the burst release.

On of the main goals of this case study will be to gauge the influence of process parameters on the API stability. Minocycline is a tetracycline antibiotic that is known for it's instability, since it easily epimerizes into 4-epiminocycline. The polymer choice of PLA is primarily based upon the price of the polymer which is cheaper than PLGA and allows a more liberal use.

Another goal of this case study is to study the morphology of the matrix and the distribution of API within the matrix. It is known that processes that involve solvents, such as spray drying

or emulsion methods, can produce hollow or more porous particles due to solvent migration [5]. This porosity can lead to higher burst release due to increased surface area. It is evaluated if the hot melt extrusion end-product is denser and where API is located within the matrix.

The final major goal of this case study is to evaluate milling parameters of hammer milling in the final Particle Size Distribution (PSD) of the particles. Feed rate and rotation speed of the mill will be the main process parameters studied.

In order to accomplish these goals, two DoE's are planned, one to the extruder process parameters and the other one to the hammer milling process parameters.

The first DoE will gauge extruder process parameters. Out of all process parameters of the laboratory extruder used only temperature, screw speed, feed rate and residence time are controllable. Usually residence time is dependent on the feed rate and screw speed, but the extruder used in this part of the work possesses a pneumatic valve that allows recirculation of the melt material and permits artificial manipulation of the residence time. In order to better evaluate degradation causes the more relevant factors are temperature and residence time. Consequentially screw speed the feed rate will remain constant for all extruder trials. The temperature will be estimated based upon thermal analysis of the feedstock. Residence time is defined based on a worst case scenario. Breintenbach [6] mentions 2min as a standard residence time for a twin-screw extruder. These experiments will gauge what happens when residence time is bigger than 2 min. In this DoE the main responses will be the % of drug degraded and the % of drug released as burst.

A second DoE will ascertain the optimal milling parameters. Hammer milling possesses three different controllable factors which are critical to the end product. They are the rotational speed of the milling hammers, the feed rate, and the mesh or sieve below the hammers. The objective here is to produce microparticles ranging from 30 to 120 micrometers. The smallest sieve for the model of hammer milling available was 400 micrometers and was fixed for all trials. The remaining factors will be put to the test in order to produce the optimal milling conditions. The response will be the Dv50 defined as the particle diameter in which 50% of the volume of particles exist.

Summarizing, the DoE on the extruder will be a simple full factorial with 2 factors. However this DoE will contain five center points leading to nine experiences. The downstream processing will take the form of hammer milling. To choose the optimal milling parameters the five center points of the previous DoE will be the starting material of the DoE on the milling parameters. As such this DoE will be a two factor full factorial with one center point. The optimal milling settings will be maintained for the remaining 4 extruder batches.

As a feasibility study, one of the extruder batches will be done on the bigger Pharma 11 extruder. This batch will be as similar do the Haake Minilab II as possible. This batch will also be the focus of a battery of SEM-EDS analysis in order to gauge the morphology and API distribution.

2.2 Materials and methods

For this work PLA was bought from Evonik (Resomer 202 H). Micronized minocycline was kindly provided by Hovione Farmaciencia SA. Solvents and other reagents were of analytical grade or higher.

Differential scanning calorimetry (DSC) was performed using a TA DSC 250 equipment. PLA and PLA/API physical mixture was analyzed using pinhole aluminum pans loaded with approximately 5 mg of sample material. The sample was heated 10^{o}C/min until 90^{o}C and then quenched to 10^{o}C. After that, the pans were heated 3^{o}C while modulating the temperature 0.48^{o}C for 60s until the temperature reached 250^{o}C.

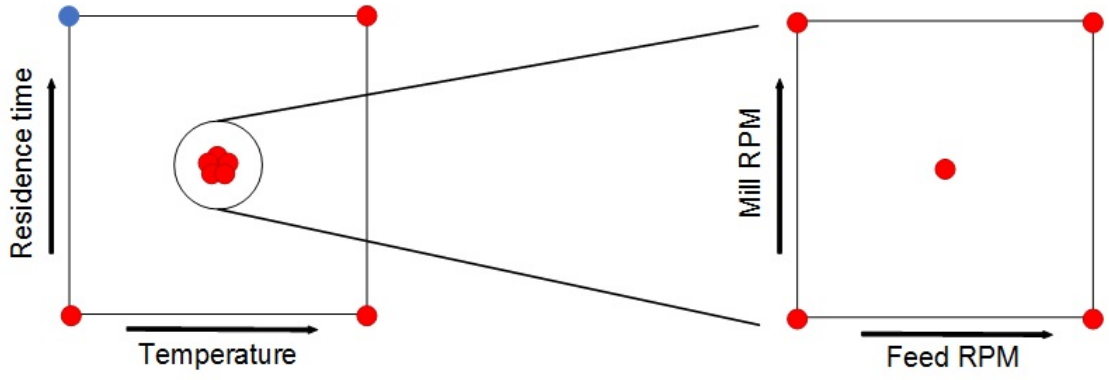


Figure 2.1: Schematic representation of the design of experiments of the first case study. The blue point corresponds to the Pharma 11 feasibility study.

Thermogravimetric analysis (TGA) was performed using a TA TGA 550. A 10mg sample of PLA/API was heated 10ºC/min until 250ºC was reached.

The several PLA/API blends that are to be extruded were accurately weighed in a Quintix Sartorius balance. A total of nine blends of 15 grams each were made with a total of 15 grams and 20 % of API. This solid blend was homogenized using a T2F Turbula at 40 RPM for 15min.

HME was done using an Haake Minilab II extruder from ThermoScientific. It's screws are conical intermeshing screws with a top diameter of 1cm and a length of approximately 11cm. This extruder sports a pneumatic operated valve that allows for the recirculation of the material and an artificial manipulation of the residence time. The extruder was operated at different, temperatures and residence times as shown in 2.1. The barrel of the extruder was filled with feedstock material and left to recirculate for the amount of time established. After that the pneumatic valve was opened and the barrel was emptied. This was repeated until the material had ended. In all batches screw speed was maintained at 50 rpm.

Table 2.1: Design of experiments used for hot melt extrusion trials. The order shown corresponds to the run order. Under parentesis the level of the factor is shown.

Batch number	Temperature (ºC)	Residence time (min)
06NY01.003	80 (-)	10 (+)
06NY01.004	100 (0)	6 (0)
06NY01.005	120(+)	10 (+)
06NY01.006	100 (0)	6 (0)
06NY01.008	100 (0)	6 (0)
06NY01.009	100 (0)	6 (0)
06NY01.010	120 (+)	2 (-)
06NY01.011	100 (0)	6 (0)
06NY01.012	80 (-)	2 (-)

A single test extruder test was made using a Pharma 11 extruder from ThermoScientific. This extruder sports an 11mm diameter screw being significantly bigger than the previous. This batch was kept as close as possible in all aspects to the previously described ones. The feed port of the extruder was moved towards the die to only use a portion of the screw's length. The

Table 2.2: Minocycline burst release during the first hour

Batch number	% of drug released at t=0		Average (%)	Standard Deviation (%)
06NY01.003	27.3	26.3	26.8	0.5
06NY01.005	20.9	22.8	21.9	1.0
06NY01.009	41.1	42.9	42.0	0.9
06NY01.010	33.1	40.2	36.6	3.6
06NY01.011	41.3	44.0	42.6	1.3
06NY01.012	18.5	18.6	18.5	0.1

screw speed was 50rpm. The feed was automated using a ThermoScientific volumetric screw feeder. The feeder screw speed was calibrated to keep the 10min residence time and was kept at 15rpm.

A Frewitt modular milling machine was used to hammer mill the extrudates. The Frewitt machine was equipped with a HammerWitt-Lab head and a 0.4mm circular mesh. The material was automatically fed into the machine using the provided automatic electric feeder. The machine was operated at different speeds and feed rates according the experimental plan. Between batch mills the hammers were checked for accumulation of material but if not present the milling chamber casing was not cleaned.

Table 2.3: Design of experiments used for hammer milling experiments. Under parentesis the level of the factor is shown

Batch number	Milling RPM	Feed RPM
06NY01.004	2500 (-)	12 (+)
06NY01.006	7500 (+)	12 (+)
06NY01.008	5000 (0)	9 (0)
06NY01.009	2500 (-)	6 (-)
06NY01.011	7500 (+)	6 (-)

The SEM-EDS analysis was made using a Phenom Pro-X equipment. A minimal amount of sample was spread on a carbon adhesive sheet. The sheet was then vacuumed to remove the material that did not stick to the sheet.

Particle size distribution was measured using a laser diffraction and dry dispersion method. A Sympatec Helos/BR instrument equipped with the Aspiros module as the dosing unit and the Rodos/L as the dry dispersion module. The speed of the dosing unit, the pressure of the dispersing unit and the lens of the HELOS/BR module were varied until an accurate and reproducible result for the specific powder was obtained. After fixing the measuring condition all measurements were done in duplicate and only the average is shown. For each measurement the conditions will be shown in the caption.

The UPLC measurements were performed using an Acquity Waters UPLC with a BEH C18 50mm x 1.7um column. The mobile phases used were 0.1%Trifluoracetic acid (TFA) in water and 0.1%TFA in acetonitrile in a 80/20 proportion. The total flow was 0.4 ml/min and the run time was 1 minute with an injection volume of 1 microliters. The method linearity for minocycline was assessed by making a concentrated solution of 0.75mg/ml pure minocycline in milliQ water. Five more solutions of 0.5 mg/ml, 0.25 mg/ml, 0.1 mg/ml, 0.05 mg/ml, 0.025 mg/ml were done by diluting the original solution. These solutions were injected three times each with the middle

one, 0.1 mg/ml, injected 5 times.

The drug load assays was made using the previous UPLC method already described. A known amount of formulation (20-40mg) was dissolved in approximately 10ml acetonitrile. To this solution was then added 10ml of water. This precipitated the polymer. A small aliquot was filtered through a syringe with a 0.4 micrometer pore filter into an HPLC vial. This was then analyzed using the UPLC method previously described.

The *in-vitro* release of the final formulations was accomplished using an eppendorf procedure. Per formulation, a total of 14 eppendorfs were filled with approximately 10-20mg of formulation. The eppendorf was filled with 1mL of phosphate buffer solution 7.4pH and put into an orbital shaker rotating at 100rpm at 37ºC. Every 24h, two eppendorfs of each formulation were removed and centrifuged at 13400rpm for 10min. An aliquot of 250 microliters of the supernatant was diluted in 750 microliters of a solution of 0.1% TFA in acetonitrile. This was then analyzed by UPLC using the method previously described.

Every DoE was analyzed using the software MODDE by Umetrics.

2.3 Results and discussion

2.3.1 Thermal Analysis

The first results to be analyzed will be DSC and TGA analyses done on the physical mixture of PLA and minocycline. They are presented in fig. 2.2, fig. 2.3 and fig. 2.4.

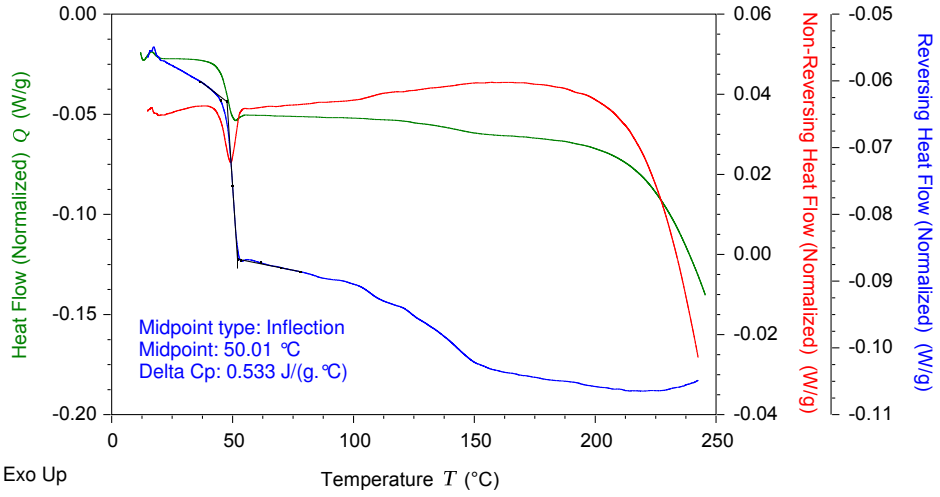


Figure 2.2: DSC graph of pure PLA.

Hot melt extrusion impacts a high amount of thermal energy onto it's starting material. Choosing a temperature that does not impart thermal instability to the feed is of incredible importance. This is especially true when referring to the API. On the other hand the temperature should be high enough so that the polymer viscosity does not throttle extruder performance through high torque requirements [29]. Concluding, temperature has to lie on the interval between API degradation and T_g. These DSC's and TGA's allows us to choose the temperature intervals.

The DSC's shows us that the T_g of PLA when in a physical mixture with minocycline is 48ºC when alone is 50ºC. These values are not significantly different and shows us no significant

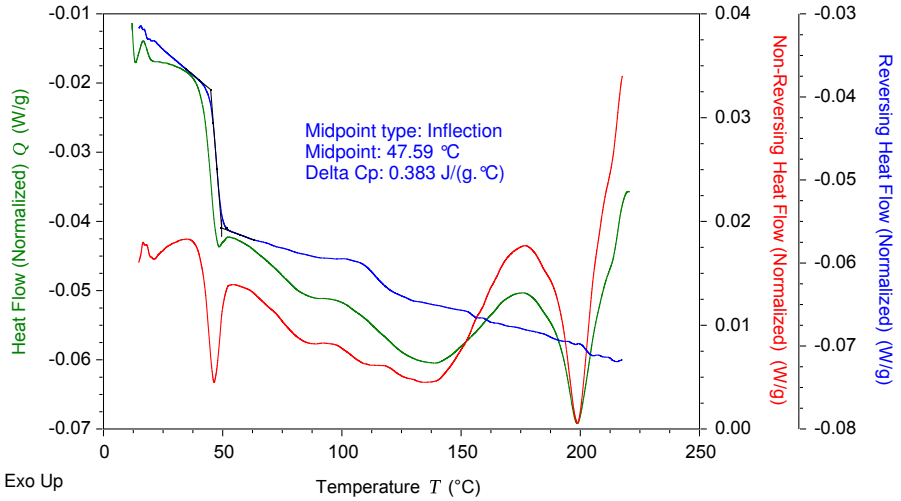


Figure 2.3: DSC graph of PLA/minocycline 20% physical mixture.

interactions between drug and polymer that may affect glass transition. As a rule of thumb minimum extrusion temperature is to be chosen as 20°C to 40°C above glass transition [30]. As such, 80°C will be the low value taken for the extruder DoE.

The TGA shown in fig. 2.4 shows us mass loss due to temperature degradation. This will define the maximum temperature that can ever be delivered to the feed material. Significant mass loss starts to occur around 180°C. This is still a high temperature and care should be taken, since degradation without mass loss can occur. A conservative 120°C will be chosen as the higher value taken for the DoE.

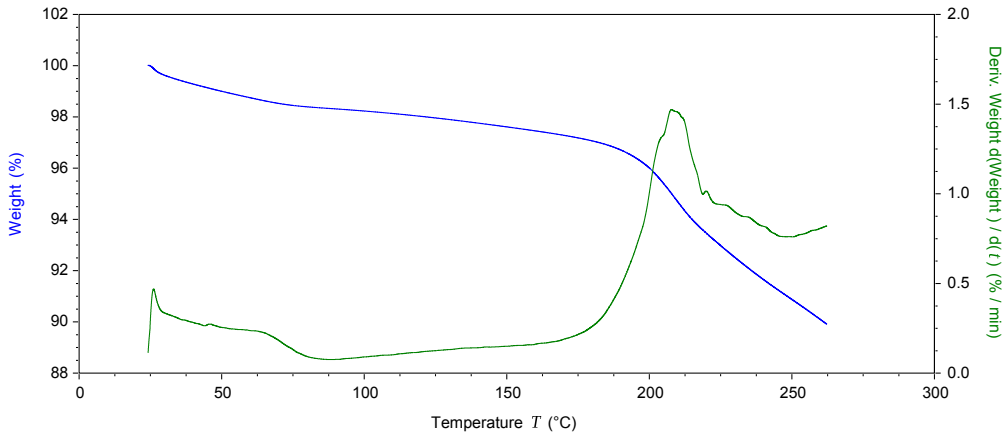


Figure 2.4: TGA graph of PLA/minocycline 20% physical mixture.

2.3.2 UPLC method linearity

A previously developed UPLC method for tetracyclines was tested for linearity for minocycline. The linearity of the method was proven with a regression coefficient (R^2) of 0.999. The

Coefficient of Variation (CV) of the middle point of the curve was also evaluated and presented a good value of 0.55%, inferior to the 2% maximum criteria. All the data is presented in table 2.4 and plotted on fig. 2.5.

Table 2.4: Average peak areas of the minocycline linearity chromatographs

Concentration (mg/ml)	Average peak area
0.025	111991
0.05	194523
0.1	393595
0.25	1024778
0.5	1983362
0.75	2928783

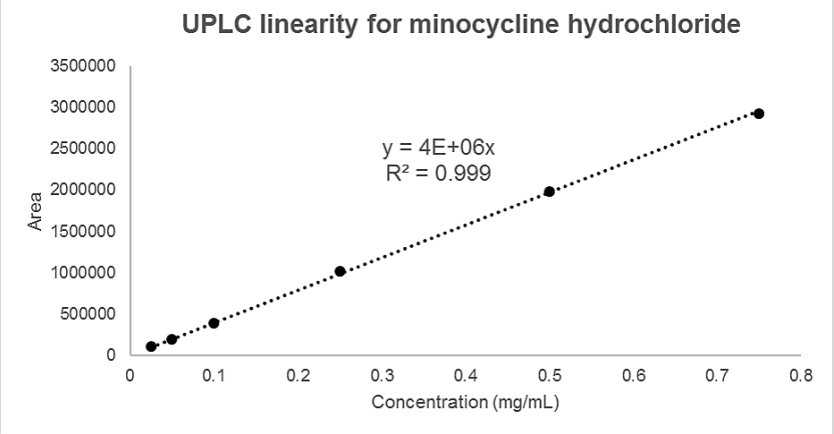


Figure 2.5: UPLC method linearity check for minocycline hydrochloride

2.3.3 Hot melt extrusion trials

The batch size was 15 grams for all experiments. The starting material was an homogeneous light yellow powder. The material was easily extruded but some difficulties appeared in the feeding of the material related to poor powder flowability. These problems affected the yield negatively. The yields ranged from 55% and 76% with no apparent statistical pattern involved. The yields are presented in table 2.5. Higher yields are hard to achieve due to the recirculating chamber holding a large amount of material leftover. The final extrudate material was a brittle yellow rod with a quadrilateral cross section akin to the die of the extruder.

2.3.4 Hammer milling

As previously mentioned the five center points of the extruder will be the five experiments needed to conduct a small DoE on milling parameters. The material was readily milled with no apparent milling problems even at low milling speeds. The 10, 50 and 90 volumetric percentile diameters of the five experiments are presented on table 2.6.

Table 2.5: Extruder yields for the PLA/minocycline batches

Batch number	Yield (%)
06NY01.003	70.5
06NY01.004	73.3
06NY01.005	70.0
06NY01.006	72.1
06NY01.008	68.3
06NY01.009	76.0
06NY01.010	64.7
06NY01.011	76.5
06NY01.012	54.7

Table 2.6: Results from the hammer milling trials. All values except span are in micrometers.

Batch number	Dv10	Dv50	Dv90	Span
06NY01.004	36	158	299	1.7
06NY01.006	61	181	281	1.2
06NY01.008	35	158	281	1.6
06NY01.009	33	160	309	1.7
06NY01.011	35	136	247	1.6

The shape of the PSD curve was similar between all tests and a sample PSD that corresponds to the batch 011 is shown in fig. 2.6. A cutoff around 400 micrometers is noticeable in the shape of all curves. This is expected due to the 400 micrometers sieve used in the hammer mill.

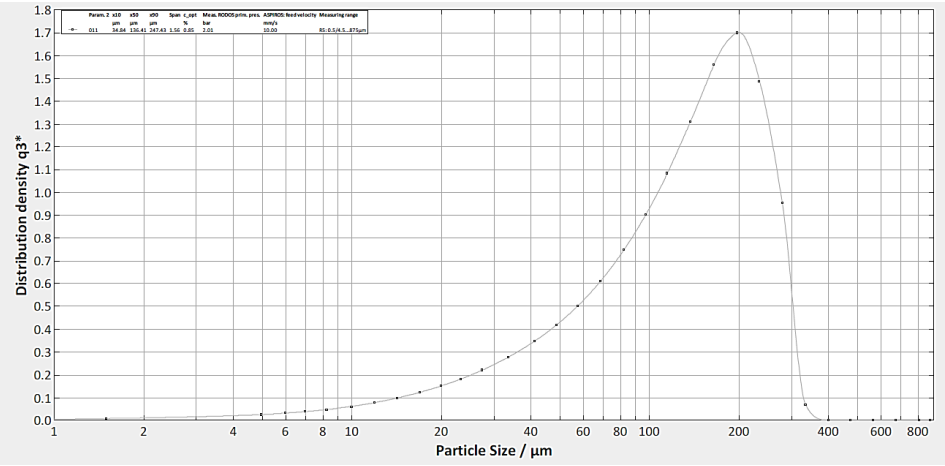


Figure 2.6: PSD of batch 011 of PLA/Minocycline; Measured at 2 bar, 10 mm/s.

SEM images of the milled extrudates confirm the PSD measurements. The particle morphology is jagged and irregular which is consistent with the shattering of particles by hammer milling. In 2.7 a SEM image is shown of batch 011. All the batches had similar SEM images.

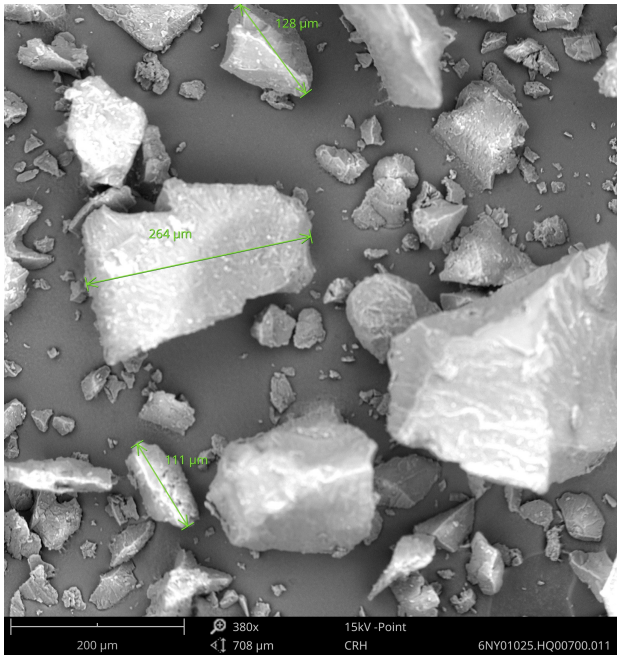


Figure 2.7: SEM image of milled batch 011 PLA/minocycline extrudate.

In order to relate these results to the factors in cause a Multi Linear Regression (MLR) model was fitted to the Dv50 data. In Figure 2.8 the two main statistics for model evaluation are shown.

The regression coefficient (R^2) allows us to say if the regression fitted the raw data successfully. A value of 1.0 is good but this statistic can't be evaluated alone. The goodness of prediction (Q^2) is a estimative of how good the model is at predicting new outcomes. Since it uses a cross validation technique it ends up as a much better representation of how useful the model is. In this model it's value of 0.964 is a good outcome. A general criteria calls for $Q^2 >$0.5 and $R^2 - Q^2$ of approximately 0.2, which these data comply.

The model also allows us to check how do the factors affect the Dv50. This will confirm if they are significant, and if the interaction between the factors is indeed relevant as thought before. The linear coefficients of each factor and their interaction is shown in fig. 2.9.

The error bars correspond to the 95% confidence interval. As seen by the values of the coefficients the mill rotation is not a significant factor because zero is within the confidence interval (p>0.05). However, the feed RPM and the interaction between the two factors are significant.

Interpreting the results the lowest median particle size can be reached using a low feed rate and a high mill speed. We can reduce the milling process to a very simplistic process with two simple elements or components. The energy component per unit of time, where the mechanical energy conveyed by the hammers to the feed materials shatter the particles; and the mass flow component, that in this case is given by the feeder speed. Per particle, if more energy is given the more they shatter and the smaller is the end product.

The remaining extruder batches were milled with the combination of factors that maximized energy per gram, i.e. high mill speed and low feed rate.

The powder itself is free flowing and does not form agglomerates which are prerequisites for parenteral formulations. However the particle size is still too large since it should be in the

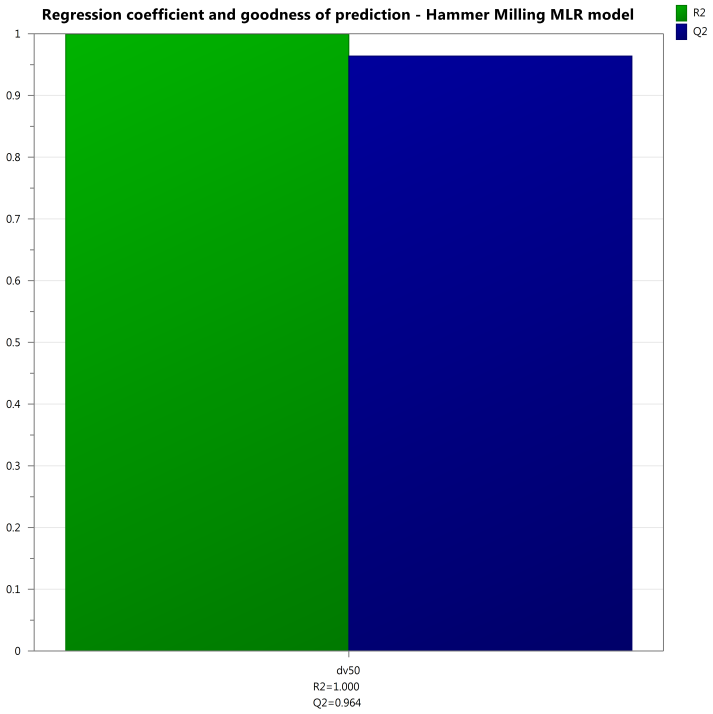


Figure 2.8: Regression coefficient and goodness of prediction of the MLR model applied to hammer milling.

30 to 120 micrometer range at most. This is probably due to the large mesh since it should have been smaller. In order to continue the trials, the raw powder was sieved through a 150 micrometer sieve. This should shift the particle size distribution enough so that it lies closer to the particle size required. Sieving was first tested with the batch 011 since it's the one that had the smallest particles. The PSD of the sieved batch 011 is shown in fig. 2.10. The sieved end product showed a much lower PSD which is more aligned with the requirements for parenteral formulations.

The following tests, such as assays and dissolutions were carried out using the sieved batch 011 as a centerpoint for having the lowest particles size. The remaining batches (003, 005, 010 and 012) after being milled using the best levels and sieved through a 150 micrometer sieve were also used. In order to have two centerpoints the batch 009 was also used in the dissolution tests. The 10, 50 and 90 percentile diameters of all the batches used are compiled in table 2.7.

2.3.5 UPLC assay

In order to gauge the effective drug load and compare it to theoretical drug load an UPLC assay was done on the samples.

As can be seen the assays showed an unexpected stability of minocycline to the high energy HME process. Not only are the values coherent between different assays of the same formulation but are also coherent between themselves.

Having in consideration the inherent stability of minocycline, showing almost no drug degra-

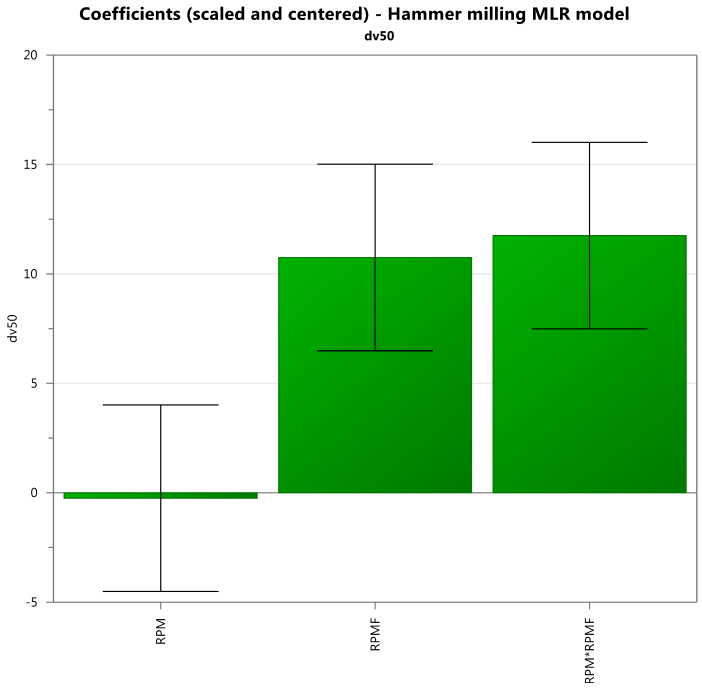


Figure 2.9: Linear coefficients of the hammer milling MLR model

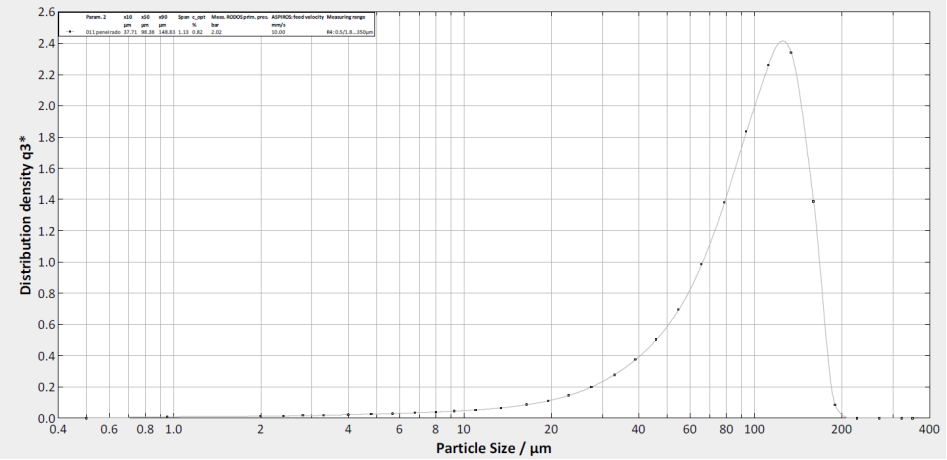


Figure 2.10: PSD of batch 011 of PLA/Minocycline after sieving; Measured at 2 bar, 10 mm/s.

Table 2.7: Particle size percentiles for the final sieved formulations. All values except span are in micrometers

Batch number	Dv10	Dv50	Dv90	Span
06NY01.003	30.8	98.7	184	1.47
06NY01.005	51.2	115	190	1.21
06NY01.009	32.2	101	181	1.55
06NY01.010	38.1	104	179	1.35
06NY01.011	46.8	114	194	1.29
06NY01.012	37.7	98.4	148	1.13

Table 2.8: Drug load assay results for the PLA/minocycline formulations

Batch number	Drug Load Assay 1 (%)	Drug Load Assay 2 (%)	Average (%)	Standard Deviation (%)
06NY01.003	16.7	19.3	18.0	1.3
06NY01.004	19.6	19.3	19.4	0.1
06NY01.005	18.4	19.7	19.0	0.6
06NY01.006	18.7	18.4	18.5	0.1
06NY01.008	18.5	18.9	18.7	0.2
06NY01.009	19.1	18.9	19.0	0.1
06NY01.010	19.1	20.2	19.7	0.6
06NY01.011	18.8	18.9	18.9	0.0
06NY01.012	19.2	19.3	19.2	0.1

dation between these operating conditions is a good sign for the HME process. However it did not show the limitations of the process which was one of the goals of these trials. This denotes that the intervals of the process parameters need to be broader.

2.3.6 *In Vitro Dissolution*

Due to the degradation of the minocycline in the dissolution media it was impossible to proceed with the tests since no reliable quantification could be achieved after the first day. As such only the t=1 hour and t= 24hours time points were able to be measured. Subsequent analysis focused only on the burst release which is considered as the t=1 hour time point (Table 2.9)

Table 2.9: Minocycline burst release during the first hour

Batch number	% of drug released at t=0		Average (%)	Standard Deviation (%)
06NY01.003	27.3	26.3	26.8	0.5
06NY01.005	20.9	22.8	21.9	1.0
06NY01.009	41.1	42.9	42.0	0.9
06NY01.010	33.1	40.2	36.6	3.6
06NY01.011	41.3	44.0	42.6	1.3
06NY01.012	18.5	18.6	18.5	0.1

A burst release ranging from 20 to 40% is reported. Between the duplicates of each formulation the values are coherent between them. This can suggest the appearance of a pattern and can figuring out the reason for its appearance can elucidate the mechanisms of drug release.

It is not clear whether this result is negative or not, since it depends on the time of release desired and therapeutic concentration. However, ascertaining what could be the reason for this burst can elucidate the mechanisms of drug release. In order to do that the burst release data was tried to be correlated with process parameter data using MODDE.

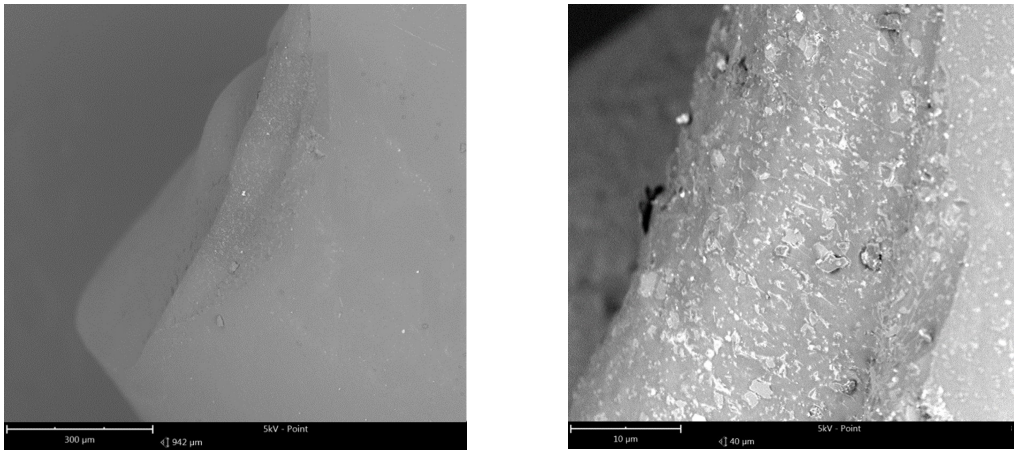
No MLR model can be accurately fit to the data. Analyzing the data seems to suggest that quadratic terms to the model must be added in order to accurately explain the results. However this cannot be done which such low amount of experiments. With this factorial the quadratic term of each factor are confounded with each other.

In order to explain this data it is required to complement the DoE with more experiments by considering an additional factor or even a third level to the existing factors. That way the possible quadratic terms that may exist can be calculated.

An additional factor that was not considered was the particle size of the API. This was not measured and differenced in this variable could explain the results obtained. The next case study will consider it as a factor.

2.3.7 API distribution within the matrix

Batch 003 in particular was done in the Pharma 11 HME. The end product is not incredibly different from the other batches presenting itself as a yellow rod but this time with a circular cross section due to the different die shape.



(a) Surface and core of the extrudate **(b)** Close-up of the extrudate core

Figure 2.11: SEM images of batch 003 PLA/minocycline done on the Pharma 11 HME

In order to study API distribution, a small sample of this material was analyzed by SEM-EDS. The SEM image in fig. 2.11a shows the surface of the extruder as well as a small cross section. The surface is smooth and non-porous and zooming in on the core of the extrudate (fig. 2.11b) shows us the majority of the drug crystals are located within the core. To accurately check if the drug is concentrated within the core EDS measurements were performed.

EDS is a analytical technique used for elemental analysis. An electron beam excites the atoms of the sample in a single point. The X-rays that are emitted allows us to accurately measure the chemical composition of that point. Minocycline hydrochloride contains in it's chemical structure numerous nitrogen atoms. On the other hand PLA is solely composed of carbon, hy-

drogen and oxygen atoms. Detecting the presence of nitrogen is the same as detecting the presence of API.

A total of 34 EDS measurements were made, 14 in the core of the particle and 20 at the surface. The great majority of the minocycline detected was located at the core (see fig. 2.12).

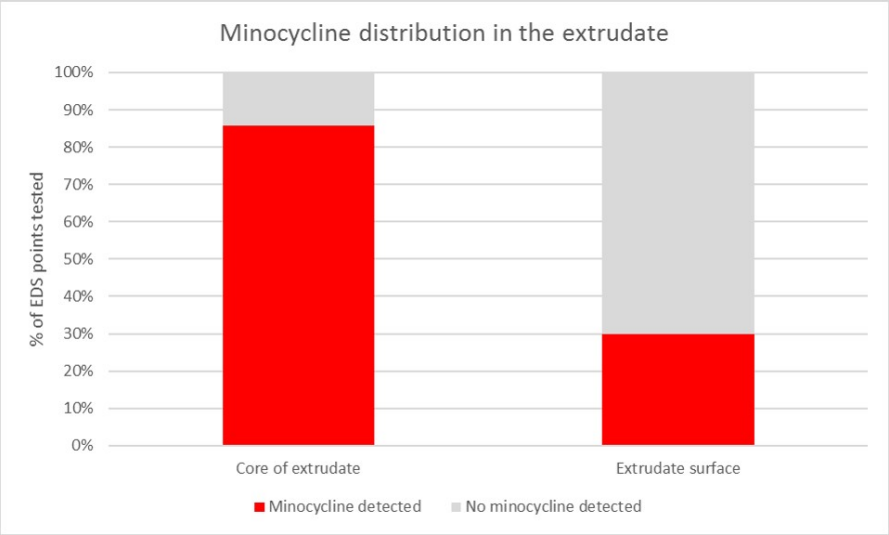


Figure 2.12: API distribution in the extrudate as measured by EDS.

This results allows us to confirm that the extrudate material is dense and non-porous and may allow less surface area exposed leading to less burst. However it also denotes a potential disadvantage with the HME/hammer milling combination. Hammer milling exposes a large amount of drug to the surface which was otherwise located at the core of the material and therefore protected by the polymeric matrix. Surface drug is thought to be a major cause of burst release [16].

A possible explanation for this imbalance in the drug distribution is the migration of polymer during the extrusion process. It does not matter if a HME polymeric matrix is denser and non-porous because if it ends up with a higher concentration of surface drug, the burst will inevitably be higher.

2.4 Conclusion

Ultimately this case study allowed to reach a new understanding of the HME process. Although not all of the goals proposed were achieved, the work elucidated a few weaknesses of the process and opportunities to improve. It also revealed the need for further testing by highlighting important factor.

The extrusion trials in general went unexpectedly well with no API degradation. This example highlights the possibility of using an high energy process like HME on thermal labile drugs if the process parameters are well chosen and the exposure of the drug to the harsh conditions is restrained.

Hammer milling was deconstructed as a downstream processing step although it failed to reach the required particle size without sieving. However this is a standard procedure and it is not a great hassle. On the other hand the API distribution study indicated that the API is mostly

located within the core of the extruder and the example displayed how milling can lead to an overall rise on surface drug in the final microparticles.

Lastly, IVR was not conclusive. A considerable amount of burst release is reported. However it sparked the discussion of the importance of API particle size and it's impact on dissolution. This is will be studied in the next case study.

CHAPTER 3

Diclofenac Sodium and PLGA - Second case study

Contents

3.1 Introduction

This case study is based upon the development of a parenteral formulation of PLGA and diclofenac sodium using hot melt extrusion and hammer milling.

The previous case study surfaced the necessity of studying with greater detail formulation parameters in order to accurately predict and understand burst release.

In particular, this case study will have one of it's goals in understanding the influence of the drug PSD and drug load. Temperature will also be a factor to study in tandem with these parameters to check if it's influence is really as underwhelming as it was in the minocycline/PLA case. This case study entails a two level full factorial with three factors and 2 centerpoints equating 10 experiments (see fig. 3.1). To note that the API particle size is a qualitative factor.

A secondary goal of this case study is focused on the benchmarking of different IVR technologies. This secondary goal came from the necessity for a reproducible IVR test for parenteral controlled release formulations.

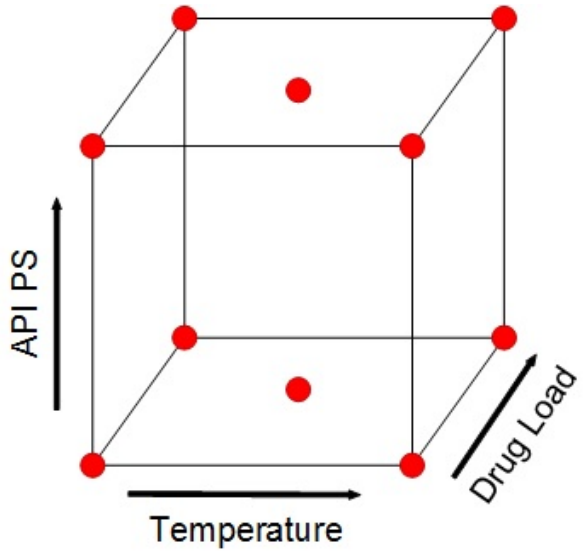


Figure 3.1: Schematic representation of the design of experiments of the second case study.

To achieve this last goal two different IVR tests will be performed. One will be performed using the sample-and-separate method described in [33]. All 10 previously mentioned formulations will be tested during 7 days.

The second IVR test to be performed will be done with the USP Apparatus IV flow through cell. One of centerpoints of the previously done formulations will be tested in triplicate alongside an equal formulation that used a lower Molecular Weight (MW) PLGA, in a One Factor at a Time (OFaT) type of experiment. This test will last 15 days.

As such, it is necessary that the API chosen is thermally non labile as a way to easily remove thermal degradation as a potential factor. It is necessary as well that the drug chosen is relatively water soluble so as to facilitate IVR studies. Diclofenac sodium easily sports all of these characteristics and will be the API of choice in this case study. The PLGA chosen will be a 50/50 ratio of lactic/glycolic monomers. PLGA itself is the most well known controlled release polymer and this PLGA grade sports the lowest degradation time which is useful as a testing material.

3.2 Materials and methods

For this work PLGA 50:50 was bought from Evonik (Resomer 503 H and 504 H). Diclofenac sodium was purchased from Sigma Aldrich. Solvents and other reagents were of analytical grade or higher.

Differential scanning calorimetry analysis was made using a TA DSC 250 equipment. PLGA was analyzed using pinhole aluminum pans loaded with approximately 5 mg of sample material. The sample was heated 10ºC/min until 90ºC and then quenched to 10ºC. After that, the pans were heated 3ºC/min while modulating the temperature 0.48ºC for 60s until the temperature reached 250ºC.

Thermogravimetric analysis was accomplished using a TA TGA 550. A 10mg sample of API was heated 10ºC/min until 350ºC was reached.

Diclofenac sodium was micronized using a laboratory fluid jet mill J-20 with DS20 from Tecnologia Meccanica. The pressure at the chamber and pressure in the venturi feeder were iterated by feeding small amounts of API and checking the particle size of the end product. When the particle size desired was achieved the conditions were set. The mill was operated with 4 bar of pressure in the chamber and 6 bar of pressure in the venturi feeder. A total of four 10 grams batch were done. After checking for similar PSD between the batches the three were homogenized using a T2F Turbula at 40 RPM and 15min.

The several PLGA/API blends that are to be extruded were accurately weighed in a Quintix Sartorius balance. A total of ten blends using Resomer 504 H were made with a total of 10 grams of either 20, 30 or 40% drug load. Five had micronized diclofenac and the remaining five had standard diclofenac. An additional solid blend this time with Resomer 503 H was also performed. All solid blends were homogenized using a T2F Turbula at 40 RPM for 15min. See table 3.1 for more information about the 11 blends and how they fit into the DoE.

Table 3.1: Design of experiments for the second case study; batch 011 corresponds to a replicate of batch 008 with a lower MW PLGA.

Batch number	Temperature (ºC)	Drug load (%)	API PS
001	80(-)	20(-)	High
002	120(+)	20(-)	High
003	100(0)	30(0)	High
004	80(-)	40(+)	High
005	120(+)	40(+)	High
006	80(-)	20(-)	Low
007	120(+)	20(-)	Low
008	100(0)	30(0)	Low
009	80(-)	40(+)	Low
010	120(+)	40(+)	Low
011	100(0)	30(0)	Low

Hot melt extrusion used an Haake Minilab II extruder from ThermoScientific. The extruder was operated at different temperatures and all other controllable factors were maintained. The screw speed was set at 50 rpm and the residence time at 10 minutes.

A Frewitt modular milling machine was used to hammer mill the extrudates. The Frewitt machine was equipped with a HammerWitt-Lab head and a 0.4mm circular mesh. The material was automatically fed into the machine using the provided automatic electric feeder. The machine was operated at 13 500 RPM and the feeder at 2 RPM.

The SEM analysis was made using a Phenom Pro-X equipment. A minimal amount of sample was spread on a carbon adhesive sheet. The sheet was then vacuumed to remove the material that did not stick to the sheet.

Particle size distribution was measured using a laser diffraction and dry dispersion method. A Sympatec Helos/BR instrument equipped with the Aspiros module as the dosing unit and the Rodos/L as the dry dispersion module. The speed of the dosing unit, the pressure of the dispersing unit and the lens of the HELOS/BR were varied until a accurate and reproducible result for the specific powder was obtained. After fixing the measuring condition all measurements were done in duplicate and only the average is shown.

The UPLC measurements were accomplished using an Acquity Waters UPLC with a BEH C18 column. The mobile phases used were 20 mM phosphoric acid in water and acetonitrile

in a 50/50 proportion. The total flow was 0.4ml/min and the run time was 10 minute. The method linearity and limit of quantification for diclofenac sodium was assessed by making a stock solution of 0.1975 mg/ml and diluting it 5 times. The concentrations tested were 0.00004 mg/ml, 0.000075 mg/ml, 0.04 mg/ml, 0.12 mg/ml, 0.15 mg/ml, 0.1975 mg/ml. All of these solutions were injected once with the 0.04 mg/ml being injected seven times. The more

The *in-vitro* release of the final formulations was accomplished in two distinct ways. The first one used a Sotax USP 4 flow-through cell apparatus. In this equipment formulation 008 and 011 were tested in triplicate. In each cell approximately 30mg of sample were dispersed in glass beads. 250 ml of phosphate buffer solution 7.4pH at 8ml/min was pumped through each cell in closed-loop. At each time point a Sotax autosampler removed approximately 1ml of media intro HPLC vials.

Another IVR test using 250ml Falcon flasks was used. A total of 10 formulation were testes this way in duplicate. Each flask was filled with 250ml of phosphate buffer solution 7.4p H and approximately 30mg of formulation. At each time point a small aliquot of 1ml was taken and centrifuged. 750 microliters of the supernatant was removed. What remained in the eppendorf was resuspended with an equal amount of media and returned to the original flask. All samples were analyzed by UPLC.

3.3 Results and discussion

3.3.1 Thermal analysis

As previously done the first results to be analyzed are the DSC and the TGA presented in Figure 3.2 and Figure 3.3 respectively.

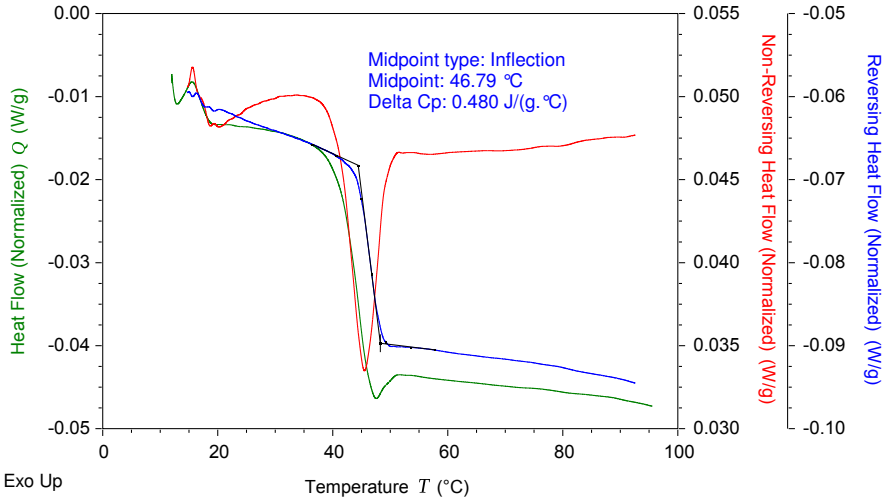


Figure 3.2: DSC graph of pure PLGA.

PLGA's T_g is approximately 47°C while diclofenac starts to readily degrade at temperatures above 150°C. The rationale that was used in the previous case study will also be used here. That is, the extrusion temperature will be contained inside this interval.

In the last case study the behavior of all the trials was acceptable with good yields and almost no degradation. So the same levels of temperature, that is 80°C, 100°C and 120°C are taken for this case study.

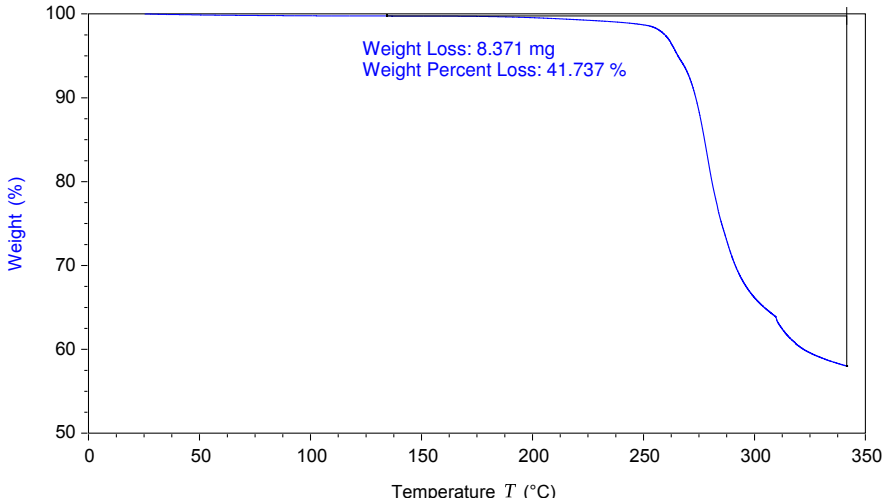


Figure 3.3: TGA of diclofenac sodium

3.3.2 Jet milling

In order to gauge the particle size and the morphology of the starting diclofenac sodium material a SEM analysis was performed and it is shown in Figure 3.4.

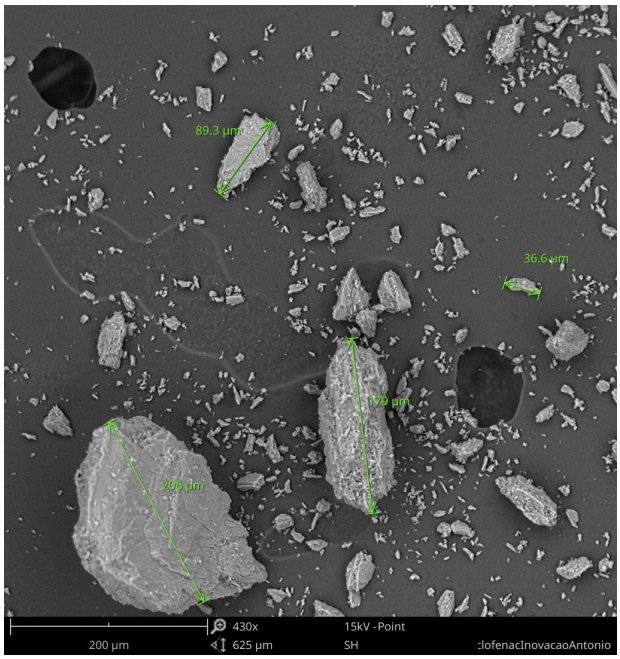


Figure 3.4: SEM image of diclofenac sodium as purchased

The diclofenac sodium came in a micronized form. Although some large particles are present the majority of the particles are smaller than 30 micrometers. To accurately check, the PSD was

measured and is presented in Figure 3.5.

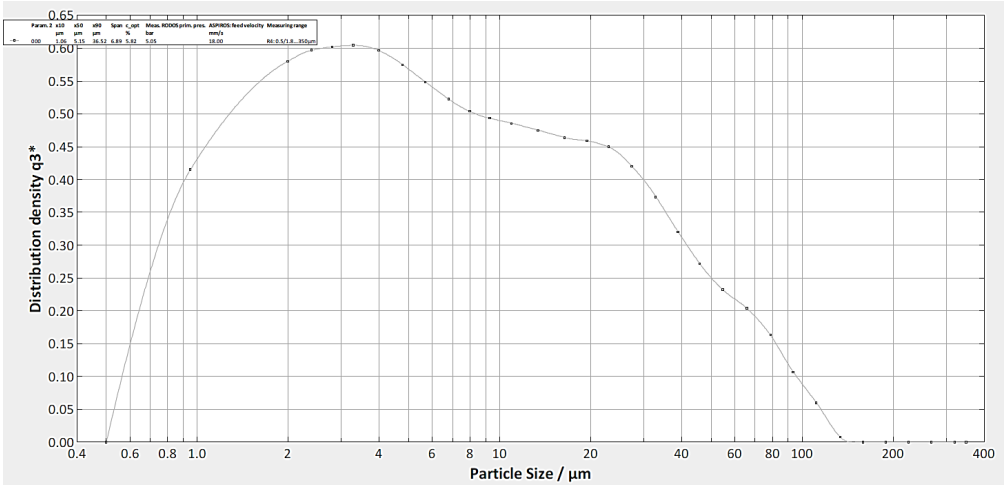


Figure 3.5: Particle size distribution of starting diclofenac. Measured at 5 bar, 18mm/s.

This measurement confirmed that the API came in micronized form. Diclofenac sodium as is will be considered the high level of the API particle size factor. In order to produce the low level API particle size, diclofenac sodium was milled by jet milling.

The jet milling trials were done without any major issues. The final material was visibly less dense and much more electrostatic which was a nice indication of a smaller particle size. The total yield after mixing all the batches was 34.5 grams which equates to 86%. The final particle size distribution is presented in Figure 3.6.

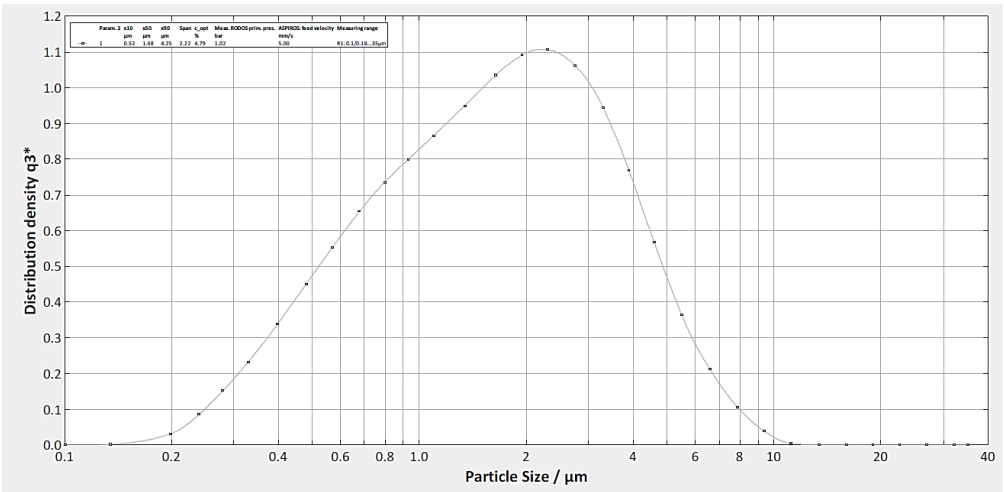


Figure 3.6: Particle size distribution of micronized diclofenac. Measured at 1 bar, 5mm/s.

Disclosed in Table 3.2 is a comparison of the particle size percentiles between the starting material and the micronized one herein called high and low level of API particle size.

Table 3.2: Comparison between the high and low API particle size. All values except span are in micrometers.

API particle size level	Dv10	Dv50	Dv90	Span
Low	0.52	1.68	4.25	2.22
High	1.06	5.15	36.52	6.89

3.3.3 Hot melt extrusion trials

The extrusion trials proceeded with minor issues arising from high amounts of torque. This is especially evident in low temperatures and high drug loads where the polymer viscosity is higher. To counter act this problem the screw speed was sometimes lowered to 10-15 rpm.

The yields were also problematic. The high polymer viscosity sometimes leaded to low yields to the an increased difficulty in the clearing of the barrel by the screws. Batch 003 in particular was problematic in terms of yield due to leaks in the barrel caused a bad seal of the barrel matrix.

The yields of the 10 extrusion trials are presented in table 3.3.

Table 3.3: Extruder yields for the PLGA/diclofenac batches

Batch number	Yield (%)
001	58%
002	58%
003	23%
004	45%
005	57%
006	59%
007	64%
008	67%
009	42%
010	58%
011	67%

3.3.4 UPLC method development

The UPLC method described was tested for linearity and produced the areas presented in Table 3.4 and plotted in Figure 3.7.

The regression coefficient (R^2) presented a good value of 0.999. The CV of the middle point had a value of 1.14% which passes the criteria.

In addition, the smallest concentration was also used to determine Limit of Quantification (LOQ). To serve as a LOQ the chromatogram peak must have a signal to noise ratio (S/N in short) above 10. The 0.00004 mg/ml had an S/N of 12 which passed the criteria.

3.3.5 Hammer milling

The hammer milling trials were done at a higher velocity and lower feed than what previously done before. This was an attempt at making smaller particles than what was done previously.

Table 3.4: Average peak areas of the diclofenac sodium chromatographs

Concentration (mg/ml)	Average peak area
0.00004	1109
0.000075	3817
0.04	2750424
0.12	7909252
0.15	9556293
0.1975	12741868

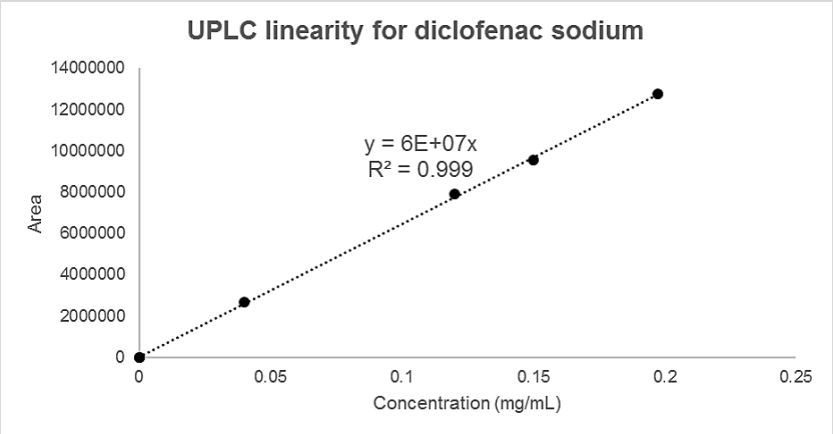


Figure 3.7: UPLC method linearity check for diclofenac sodium

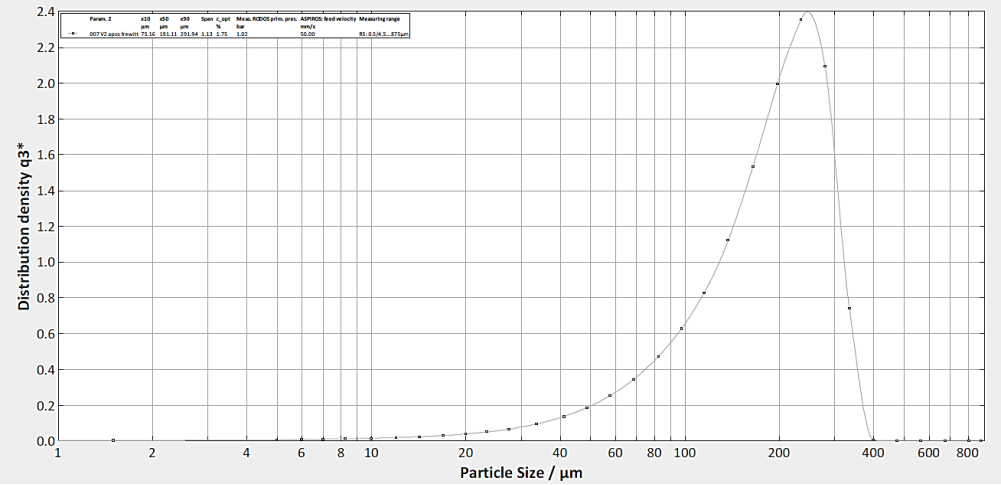


Figure 3.8: Particle size distribution of batch PLGA/diclofenac batch 007 after hammer milling. Measured at 1 bar, 50 mm/s.

To check for the particle size distribution of the hammer milling end product, a random batch was tested and the result can be seen in Figure 3.8.

Despite the change in the operating conditions the final particle size was unfortunately of the same range of what was achieved before. The 10, 50 and 90 percentiles are shown in Table 3.5. It's hard to gauge effectively the reason for why the particle size was not smaller. The feed material is not the same and as such a baseline to compare them to does not exist.

As before, the hammer milling batches were sieved through a 150 micrometer sieve and the result was also analyzed using the same sample batch as before and is shown in Figure 3.9.

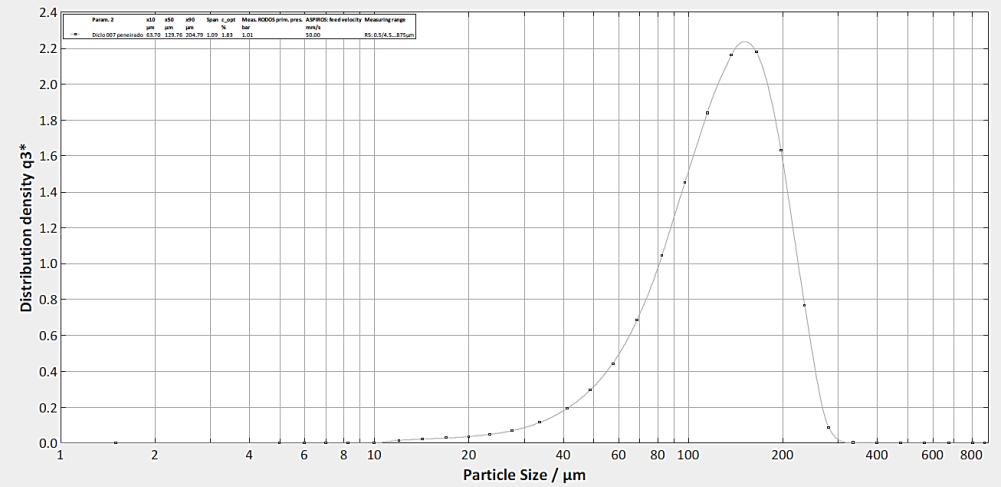


Figure 3.9: Particle size distribution of batch PLGA/diclofenac batch 007 after sieving. Measured at 1 bar, 50 mm/s.

The 10, 50, and 90 diameter percentiles of the sieved final product sample is shown in Table 3.5. This value is still high for a usual parenteral formulation and is high in comparison to the sieved batches of the previous case study. To assess possible reasons a SEM analysis to the same sample batch was made to check particle morphology and is shown in Figure 3.10

Table 3.5: Comparison between sieved and unsieved PLGA/diclofenac formulations. All values are in micrometers.

Batch 007	Dv10	Dv50	Dv90	Span
Before sieving	75	191	292	1.13
After sieving	64	130	205	1.09

The particle morphology is consistent with what was expected, being jagged and irregular. However, in contrast with the previous case study, this SEM shows us bigger particles. It's clear in Figure 2.7 present in Page21 that many smaller particles are present which is not the case here.

The reason for the bigger particle size is not clear. As mentioned the toughness of the material may not be the same and may not shatter as easily leading to a bigger and narrower particle size distribution than what was achieved before.

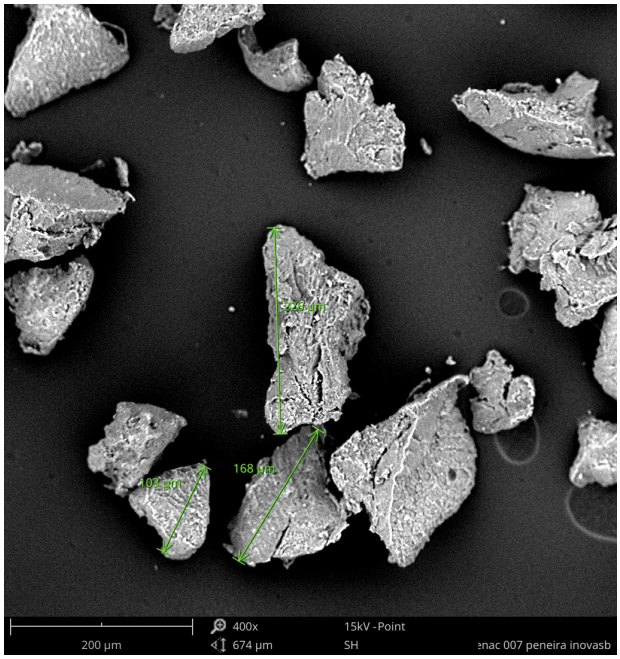


Figure 3.10: SEM image of batch 007 of PLGA/diclofenac after sieving.

3.3.6 Apparatus IV IVR

The USP apparatus IV used contained 7 vessels and only 6 were used herein called V1, V2, V3, V4, V5 and V6. The first three correspond to the lower MW formulation 011 that is ran in triplicate Consequentially the last three correspond to the higher MW polymer formulation 008.

It's expected a similar burst due to similar amounts of surface drug, but a higher release velocity for the lower MW polymer after that.

The results yielded the plot disclosed in Figure 3.11. All of the trials showed the same dissolution pattern, however the plateau they achieve is slightly different. It's clear that the V1 result may be an outlier and some imprecision or error was made along the way. This is especially evident by the impossibly high plateau achieved of 120%. Possible reasons for this outlier can range from imprecision in weighing, to errors in the uncalibrated machine auto-sampler.

In Figure 3.12 we can see the average release with error bars for each formulation. In comparison Figure 3.13 shows us the same average without the V1 outlier in the measurements.

Considering V1 in the measurements the average release follows the same pattern but with different plateaus. The lower MW formulation, which as Resomer 503 H as the PLGA is the fastest as expected. However, without V1 in the calculations, both of the formulations have an average release that is insignificantly different.

The IVR results are inconclusive since a significant difference in the release profile is not evident. Work on different PLGA grades and their release profiles is common in the literature and it's well known that increases in MW lead to slower release. The reason why there is no difference in the release profiles in this case is unknown. A possible cause can be a lack of sensitivity to such a small difference in MW.

Important to also note is the absence of an incomplete release. Some literature on the matter [] report the appearance of a secondary release phase that is caused by polymer erosion.

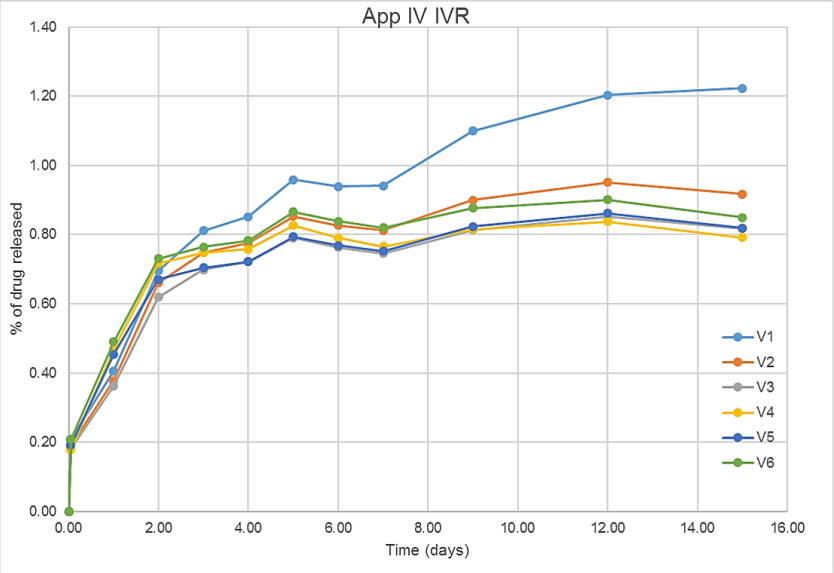


Figure 3.11: IVR releases for the USP apparatus IV trials

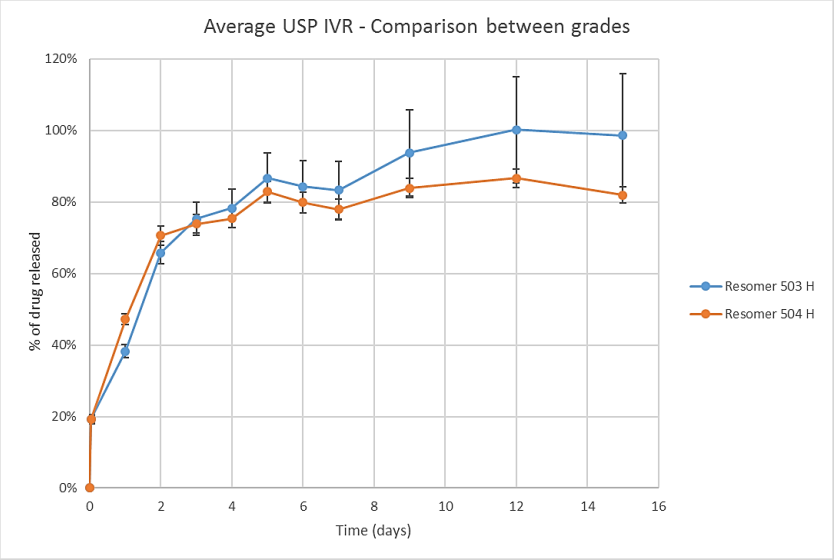


Figure 3.12: Average IVR results for the USP apparatus IV trials with the V1 measurement

This could explain this phenomena. It also highlights the need for a more extended IVR to gauge if this is a factor to have in consideration.

Some discussion can be made on the USP apparatus IV relating to it's mode of operation. The flow on the cell appears laminar which can help maintain particle morphology. The automation reduces man made errors. Lacking compendial knowledge, literature on the subject of parenteral formulations mentions this method as the most reliable and similar to *in-vivo* release.

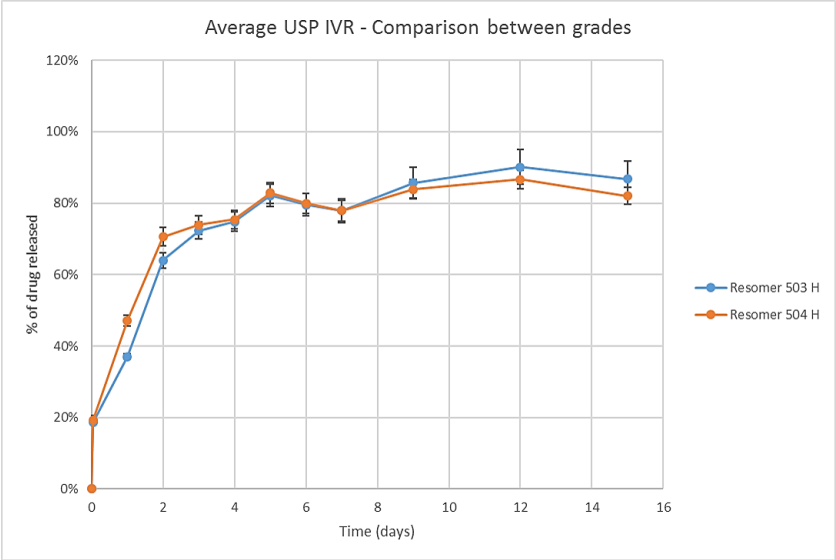


Figure 3.13: Average IVR results for the USP apparatus IV trials without the V1 measurement

3.3.7 Sample-and-collect IVR

The 10 formulations were tested in duplicate and the final averaged results are plotted in Figure 3.14.

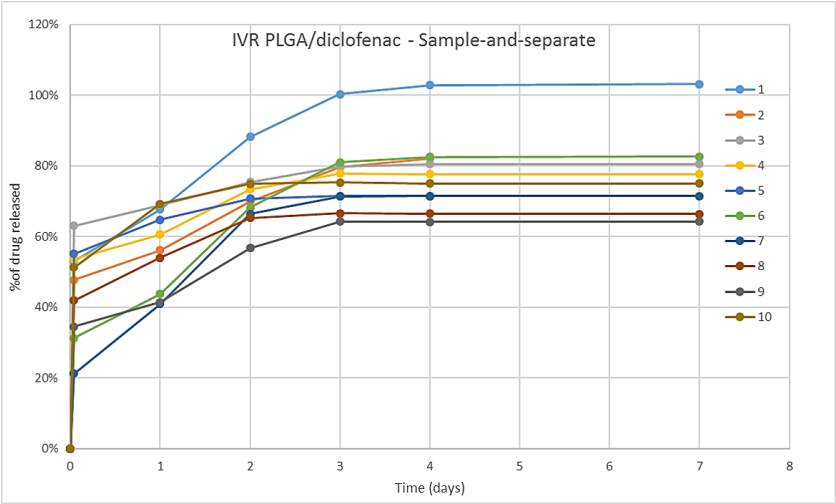


Figure 3.14: Average IVR results for the sample-and-collect trials

Looking at the release along the 7 days of study we can divide the release into three separate phases. The burst release, the zero order release that happens along the first three days, and lastly the plateau achieved after the first three days. Each of these phases will be analyzed separately.

Looking at the burst release, which is defined as the release at the first time point (t=1 hour),

we can see it ranges from 20% to 70%. To understand if this variation has any correlation with the formulation parameters a MLR model was fitted. In fig. 3.15 we have a plot of several statistical variables that serve as a criteria for the fit of the model to the data.

The regression coefficient (R^2) and the goodness of prediction coefficient (Q^2) take values of 0.793 and 0.648. These values give us a metric of how good the model fit is. These values comply with the general criteria mentioned earlier and thus the model is well fitted.

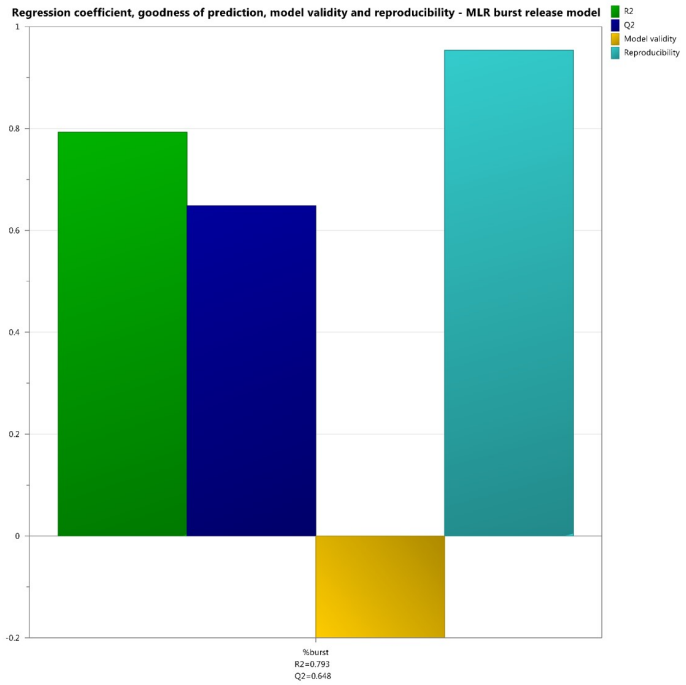


Figure 3.15: Regression coefficient, goodness of prediction, model validity and reproducibility of the MLR model applied to the burst release.

The model validity statistic has a value of -0.2 which does not comply with the general criteria of at least 0.25. This statistic represents that the model has a lack of fit, i.e. that the model error is higher than the replicate error. This concretely means that the DoE chosen was not the most appropriate to study the data. Complementing the design is required to obtain a better fit and this could begin by making more experiences by adding a third level each factor. This is required to determine if any square terms are statistically significant.

Reproducibility general criteria calls for a value higher than 0.5 which is achieved in this case. This is a measure of how good the replicates are, that is to say, how similar they are to one another.

In general the model is well fitted to the data. Nonetheless the model could be substantially improved if the square terms that may be present are determined. To this end complementing the design with a third level is a must.

In Figure 3.16 the values for the model's linear coefficients are plotted. The interaction terms that are not significant were removed from the model in order to improve the fit. In the end the most significant terms are the API particle size and the drug load. There is an interaction term between temperature and drug load that can't be ignored and will be evaluated later. Extrusion

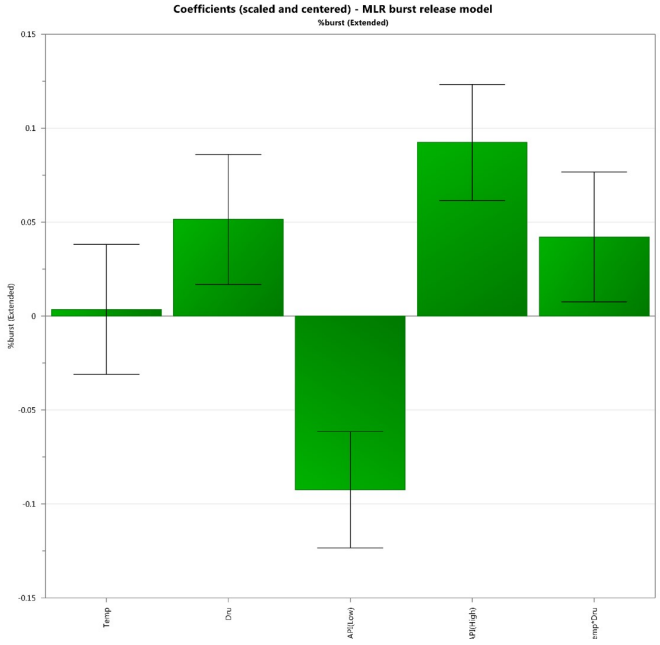


Figure 3.16: Linear coefficients of the burst release MLR model

temperature alone is not statistically significant. In Figure 3.17 a contour graph relating all variables to the burst release is presented.

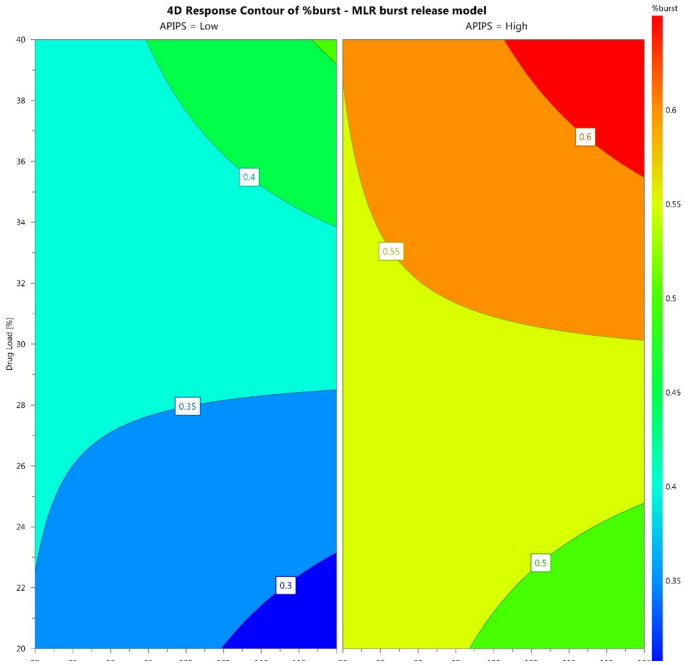


Figure 3.17: Contour graph relating all of the factors with burst release

Increasing the particle size of the API leads to a higher burst. This may seem counter intuitive. The higher the size the lower the surface area to volume ratio and the slower the dissolution of a single API particle. However this does not have in consideration the polymeric matrix. When the surface area to volume ratio is bigger we maximize the contact surface area between polymer and API. The API, is then more easily protected by the polymer when its particle size is smaller. This could lead to a lower amount of drug exposed to the dissolution media.

The drug load is another significant factor to the model. An increase in drug load leads to an increase in burst which is an expected result. A bigger drug load leads to an increased chance of having surface drug.

Lastly we have the least significant model term but one that is still worth analyzing. The interaction between temperature and drug load is better shown as plotted in Figure 3.18.

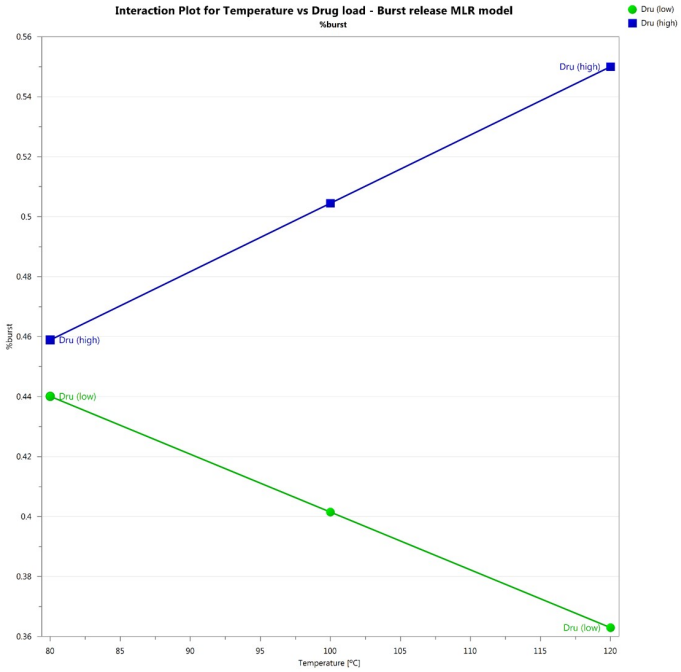


Figure 3.18: Interaction plot between drug load and extrusion temperature

We can observe that the drug load is a much more significant factor when the extrusion temperature is high. When the extrusion temperature is lower, the drug load has little impact on the burst. How extrusion temperature can interact with burst or with drug load is not at the moment clear. However, it is a studying how extrusion temperature can affect the release by modifying the matrix's physical properties is an opportunity that will remain as future work.

Focusing our attention on the zero order release phase of the IVR we can identify that it lasts until approximately day three. For each of the 10 formulations a straight line was fitted to the average of the four measurements that happened until day three. The slope of the line is herein considered as the zero order release velocity for each of the formulations. Each velocity and the regression coefficient for each is in Table 3.6.

The next step is fitting the velocities to a MLR model to see if any statistical relationship exists between the velocities and formulation parameters. Figure 3.19 shows the regression

Table 3.6: Zero order release velocities and linear regression coefficients

Batch number	Zero order velocity (%/day)	R^2
001	16.4	0.996
002	11.1	0.996
003	5.7	0.997
004	8.6	0.984
005	5.5	0.936
006	17.6	0.991
007	17.8	0.972
008	8.6	0.956
009	10.6	0.989
010	7.8	0.880

coefficient and the goodness of prediction of the referred model.

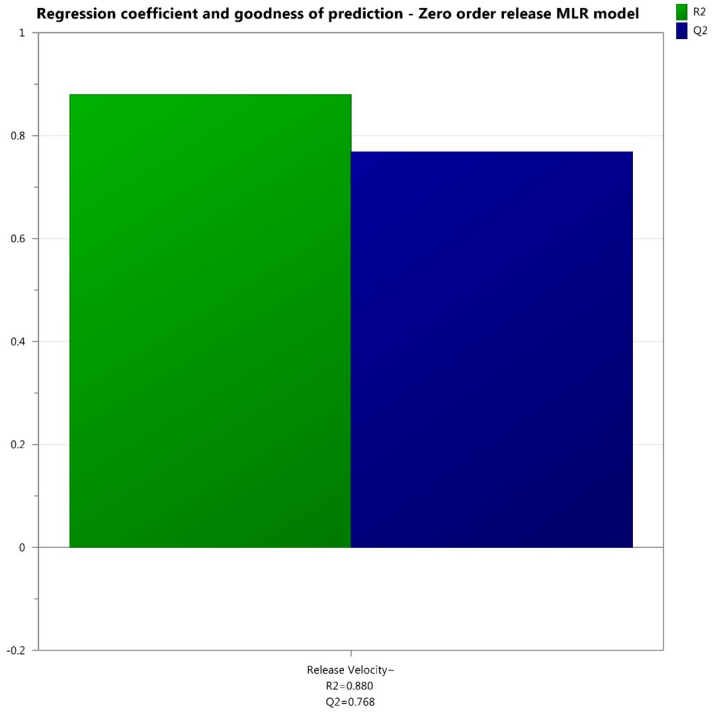


Figure 3.19: Regression coefficient, goodness of prediction of the MLR model applied to the zero order velocity.

With a R^2 and a Q^2 or 0.880 and 0.768 respectively the model has a good fit that complies with all general criteria.

Analyzing the coefficients in Figure 3.20 we can clearly see that although drug load and extrusion temperature are significant factors the interaction between the two is negligible. Drug load and temperature affect inversely the release velocity. That is to say that an increase in

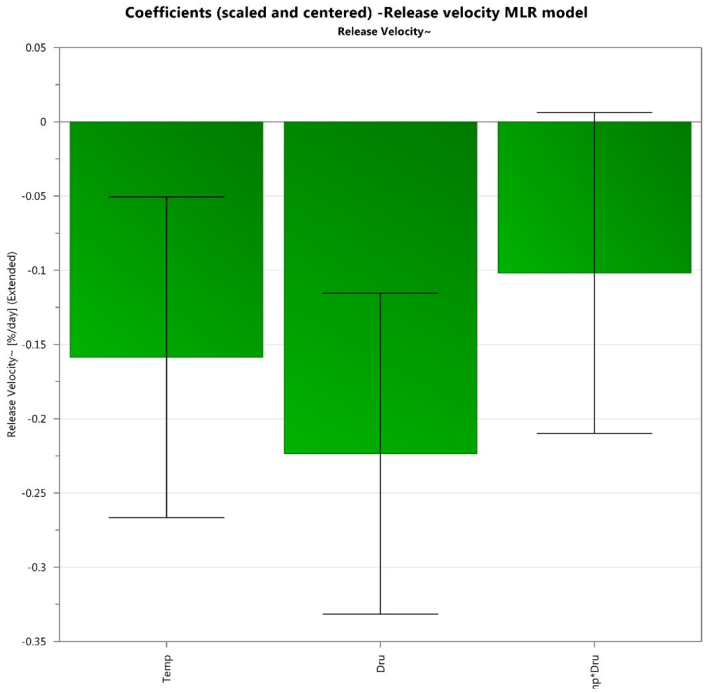


Figure 3.20: Linear coefficients of the zero order release velocity MLR model

either extrusion temperature and drug load, leads to a decrease in release velocity. This can also be seen in Figure 3.21.

The drug load and extrusion impact on the velocity is not straight-forward. There is no apparent simple physical reasoning that can explain the results. One possibility is to assume that the plateau is achieved at the same time and at the same % of release. A decrease in drug load leads to a decrease in burst. So when the zero order release begins, in order to reach the plateau in the required day the velocity must be higher. Relating extrusion temperature in a similar way is not feasible at the moment. Future work may be required in order to explain how the temperature influences matrices' physical properties and thus how they impact the velocity. One possibility can be related to polymer migration being heightened by the extrusion temperature due to increased molecular movement.

This model is similar to the previously fitted one that addressed burst. With the exception of particle size of the API this polymer is an inverse of the previous.

The third phase, that is characterized by a plateau is also worth to analyze. Returning to the Figure 3.14 present in 40 we can observe that all formulations slow down and maintain their release after day three. The final value is different for all formulations but revolves around the same interval of 80% to 60%. Formulation 001 is different being the only one to achieve 100% release.

Understanding the reason for incomplete release is important. As before, it may be needed to perform longer IVR. However this could also be cause by an incorrect IVR methodology. Observing the flasks after the 7 days had passed shows that the microparticles are no longer in suspension. Instead they agglomerated with each other and became stuck to the edges of the flask and its lid. This problem is recurrent of a sample-and-separate methodology [33] and may have adulterated the results. A longer IVR, this time in the presence of a surfactant to pre-

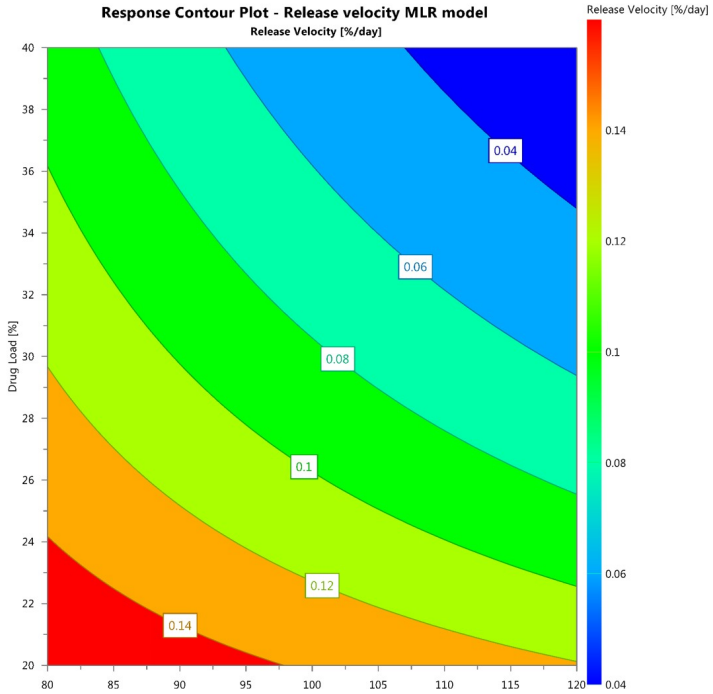


Figure 3.21: Contour graph relating temperature and drug load to release velocity

vent microparticle aggregation, is recommended. This can figure out the reason for incomplete release and shed light on microparticle release mechanisms.

3.4 Conclusion

Summarizing, although this case study did not answer all the proposed questions, it shone light on the importance of formulation parameters on the controlled release of a PLGA formulation. The importance of a standardized IVR technique is also shown.

IVR tests of two different formulations that differed only on the PLGA molecular weight was done using the USP apparatus IV. Although it is expected a slower release from the bigger MW polymer, this method showed no significant difference between both of the formulations. More testing is required in order to gauge the sensitivity of the method to several formulation parameters. No formulation achieved full drug release.

Using a sample-and-separate methodology, the release of a total of 10 formulations was tested. The factors varied were the particle size of the API, the extrusion temperature and the API load. This method showed that these factors impact the burst release quantity and the zero order release velocity. The particle size of the API affected heavily the burst release while for the zero order velocity the more significant factors are the drug load and the extrusion temperature. For the great majority of the formulations the release was not complete.

Benchmarking both technologies highlighted several issues that need to be addressed in future work. The reason for incomplete release needs to be examined. It appeared in almost all formulations and in both IVR methods. Verifying if the release is incomplete due to a IVR method that is lacking or if it is just a matter of a second release phase further down in time is important to understand the release mechanisms.

CHAPTER 4

Conclusion

The results showed above highlighted how hot melt extrusion can be a good alternative technique for PLGA microencapsulation. Developing a parenteral PLGA formulation through is shown to be feasible by studying the process and formulation parameters and their impact on the IVR profile.

The first case study emphasized the importance of choosing the right process parameters. Extrusion temperature was chosen through thermal analysis allowing the successful processing of a thermal labile drug. The hammer milling process parameters were chosen in order to yield the right particle size. On the other hand it also showed the importance of a proper IVR technique for the full characterization of the end product. However, one of the most important findings is the API distribution within the polymeric matrix. SEM-EDS measurements showed that although the extrudate is dense and non porous the core of the extrudate is API enriched when compared with the surface. This is probably because of a polymer migration during the cooling process. Downstream processing can expose the drug that was otherwise present in the core and lead to burst release due to surface drug.

The second case study put its focus on formulation parameters and on benchmarking different IVR technologies. It showed how the same API formulated in a different way can lead to different burst release results. Drug load is important but the particle size of the API cannot be underestimated. It must considerably smaller than the final microparticles desired in order to obtain a good encapsulation. A good IVR technique is also required in order to accurately test parenteral formulations. An example is the USP apparatus IV. It is reproducible, easy to perform and with similarities to *in-vivo* which may result in more biorelevant data.

Although this work shed light on many issues some weren't explained to the fullest and as such require future work. Understanding the exact mechanism for the API enrichment in the core and the full extent of physical properties changed by extrusion temperature can help understand the HME process parameters. On the other hand understanding reasons for incomplete release sheds light on the degradation mechanisms. Further studies on IVR techniques for parenteral formulation can help develop the most in-vivo similar technique. It is also important to develop accelerated IVR techniques can help to characterize long acting formulation on a smaller time frame.

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