Universidade de Lisboa

Faculdade de Farmácia

Preparation of drug loaded biodegradable films

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Mestrado Integrado em Ciências Farmacêuticas

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Abstract

Dexamethasone is a drug with several therapeutic indications, which may require different dosages and routes of administration. Therefore, the control of its release profile is advantageous to improve the effectiveness of the treatment. This control can be achieved using biodegradable polymers, such as poly(lactic acid-co-glycolic acid) (PLGA), since drug release may depend on the state of dispersion and on its diffusion from this polymer, but also on its erosion profile.

So, in this work, it was attempted to approach the state of a solid dexamethasone solution in PLGA films, manufactured using a solvent method. Among the variables that have been studied to reach the state of solid solution are the drug/polymer ratio, the used co-solvent to dissolve the drug, the drying/evaporation method of solvents during the formation of films, the concentration of drug in the solutions, with the increase of solvents volumes in the mixture that was used to make the films and the film casting method. It was observed the drying process of films, as well as the short-term stability of the obtained solid solution. The solid solution state was studied by means of macro and microscopic observation, looking for the existence of crystals. With this study, it is concluded that the solid-state solubility of dexamethasone in PLGA increases with the decrease of the drug/polymer ratio, with the increase in the solubility of the drug in the co-solvent and with the increase in the rate of solvent evaporation. Also, when samples are heated during drying, solid-state solubility decreases. When developing an assay for dexamethasone in the films, it was concluded that acetonitrile is the one of the solvents that were studied, which provides a more linear calibration curve and the films that were analysed showed an amount of drug close to the theoretical value.

Tests for the release of dexamethasone from these biodegradable films weren't carried out, due to the interruption of the internship, due to the current pandemic.

Keywords: dexamethasone; poly(lactide-co-glycolide) (PLGA); solid solutions; films

Resumo

A dexametasona é uma substância ativa com muitas indicações terapêuticas, que podem requerer diferentes posologias e vias de administração, sendo o controlo do seu perfil de libertação um aspeto vantajoso para melhorar a eficácia do tratamento. Este controlo pode ser obtido através do uso de polímeros biodegradáveis, como o poli(ácido lático-co-ácido glicólico) (PLGA), já que a libertação da substância ativa pode depender do estado de dispersão e da sua difusão a partir deste polímero, mas também do seu perfil de erosão.

Assim, neste trabalho, pretendeu-se obter o estado de solução sólida de dexametasona em filmes de PLGA, produzidos utilizando um método de solvente. Entre as variáveis que foram alteradas para atingir o estado de solução sólida estão o rácio fármaco/polímero, o co-solvente utilizado para dissolver o fármaco, o método de secagem/evaporação dos solventes durante a formação dos filmes, a concentração do fármaco nas soluções, com o aumento do volume de solventes na mistura utilizada para produzir os filmes e o método de produção dos filmes. Foi observado o processo de secagem dos filmes e também a estabilidade a curto prazo da solução sólida obtida. O estado de solução sólida foi estudado por meio de observação macro e microscópica, procurando pela existência de cristais. Com este estudo, conclui-se que a solubilidade no estado sólido da dexametasona em PLGA aumenta com a diminuição do rácio fármaco/polímero, com o aumento da solubilidade do fármaco no co-solvente utilizado e com o aumento da taxa de evaporação dos solventes. Adicionalmente, quando as amostras são aquecidas durante a secagem, a solubilidade no estado sólido diminui. Ao desenvolver-se um método de doseamento da dexametasona nos filmes, concluiu-se que o acetonitrilo é, dos solventes estudados, aquele que permite uma curva de calibração mais linear e os filmes analisados apresentaram uma quantidade de fármaco próxima ao valor teórico.

Os testes de libertação da dexametasona a partir destes filmes biodegradáveis ficaram por realizar, devido à interrupção do estágio, consequente da atual pandemia.

Palavras-chave: dexametasona; poli(ácido lático-co-ácido glicólico) (PLGA); soluções sólidas; filmes

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Acronyms

ADA: adenosine deaminase

CLF: chloroform

DDS: drug delivery systems

DM: dexamethasone

$[DM]_i$: concentration of DM in the initial solution

DSC: differential scanning calorimetry

EA: ethyl acetate

EtOH: ethanol

logP: partition coefficient

MeOH: methanol

PEG: polyethylene glycol

PGA: polyglycolic acid

PLA: polylactic acid

PLGA: poly(lactic-co-glycolic acid)

$[PLGA]_i$: concentration of PLGA in the initial solution

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

Tg: glass transition temperature

THF: tetrahydrofuran

1. Introduction

1.1. Dexamethasone

1.1.1. Characteristics

First, it's given a description of the drug that was used in the practical work, where approaching a solid solution of dexamethasone in poly(lactic-co-glycolic acid) (PLGA) was attempted. This drug is, along with prednisolone and hydrocortisone, from the glucocorticoids group. With the molecular formula of $C_{22}H_{29}FO_5$, this drug has a molecular weight of 392.47 $g.mol^{-1}$. This polymorphic substance is presented as a non-hygroscopic crystalline white to practically white, odourless powder that is practically insoluble in water, sparingly soluble in ethanol, methanol and acetone and slightly soluble in dichloromethane. (1)

Having low aqueous solubility and consequent low bioavailability and oral absorption, this drug molecule may require an alternative administration route, when a higher potency is needed, namely intravenous and intramuscular routes, which present disadvantages in terms of patient compliance. (2)

1.1.2. Mechanism of action, therapeutic indications and pharmaceutical dosage forms

Dexamethasone has a very weak mineralocorticoid effect, yet it presents a glucocorticoid effect that is up to 30 times more potent than the natural corticoid, cortisol. (3) This molecule binds to specific receptors in the cytoplasm, which then, in the nucleus, control transcription, thus protein production. This leads to an anti-inflammatory reaction, since it interferes with apoptotic pathways. (4) In fact, it lowers the number of neutrophils in the inflammation area, while lymphocyte production is reduced. In addition, it decreases permeability of capillary membranes and inhibits prostaglandins and some cytokines. (5)

Regardless, it can not only be addressed as an anti-inflammatory agent, since it is also considered an antiemetic, an antineoplastic, an immunosuppressive and an adrenergic agent. (4) Therefore, dexamethasone has a wide range of indications, e.g. in the treatment of acute inflammatory exacerbations, it is indicated for allergies, cerebral oedema and shock and it can be used as a test for Cushing Syndrome. (5,6) As for off-label use, it's used for nausea and vomiting that occur during chemotherapy, as well as altitude sickness and for the treatment of spinal cord compression due to metastases. (5,7) More recently, it has been recommended for use in treatment of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, particularly in patients with severe respiratory symptoms, reducing deaths in patients requiring ventilation or oxygen. (8,9)

This drug can have topical or systemic uses, the last of which can be tablets or solutions, administered by oral route, or injections as alternative routes. (10) In particular, via intramuscular and intravenous injections, it is indicated in endocrine, rheumatic, dermatologic, allergic, ophthalmic, gastrointestinal, respiratory, hematologic, neoplastic, oedematous and other conditions. (11)

The required daily dosage has a wide variation. As follows, an example of the variability of required dose, in acute medical conditions. Inflammation should be initially treated starting at low doses of 0.75 mg/day and possibly increased to 9 mg/day. Moreover, circulatory shock has a range of 1 to 6 mg/kg of intravenous dexamethasone as a one-time bolus, or 40 mg given intravenously every 2 to 6 hours, as needed. (5)

As local effects, to avoid systemic side effects of steroids and achieve effective concentrations on the target tissue, dexamethasone has been successfully used in otic (e.g. for acute otitis, with ciprofloxacin (12)) and ophthalmic diseases. (8)

There are also non-conventional formulations, like intravitreal implant Ozurdex® (Allergan Inc), consisting of the PLGA polymer, that erodes, while 700 µg of dexamethasone are gradually released, for 6 months. It is indicated for the long-term treatment of macular oedema associated with retinal vein occlusion or with uveitis, diabetic macular oedema after vitrectomy, persistent macular oedema, non-infectious vitritis and age-related macular degeneration. (13,14)

1.1.3. Relevance of polymer-based implants

Polymer-based drug delivery systems (DDS) aim to optimize physicochemical properties, hydrolysis rates and drug release profiles to accommodate diverse biomedical treatments. For example, they can lead to an adequate constant concentration and decreased frequency of administrations, which improves efficacy in a certain long-term treatment, in terms of patient compliance, too. Just like the above-mentioned example of Ozurdex®, DDS also allow a lower required dose. (15)

Therefore, implants of drugs like dexamethasone can be relevant in certain medical conditions, where constant levels of drug, thus constant efficacy is crucial for their treatment. For example, using chemotherapeutic agents, these implants can help prevent cancer recurrence, with local therapy, since they provide for a continuous release of drug in the intended location. (16,17)

1.2. Polymers in drug delivery systems

DDS are formulations or devices that should selectively deliver a drug to the target site, in a way that reduces problems associated to conventional administration in chronic treatments. Their advantages comprise: slower release of water-soluble drugs and better bioavailability of

low soluble drugs; possibility of more than one drug in the formulation; controlled and targeted release of toxic drugs.

The use of polymers in these DDS allows for higher versatility, as this is a characteristic of polymers. (18) These can be inert or biodegradable polymers. (19) The disadvantage of inert polymers is that, although they allow a more predictable release, only by slow diffusion, (20) they need surgical removal after total drug delivery. Although biodegradable polymers can be seen as undesirable for certain applications, as is the case of nylon (polyamide) prostheses (21), they can be useful as controlled release systems, since, whether natural or synthetic, they have the advantage of not requiring removal surgery (in the case of implants), as they are susceptible to hydrolysis, *in vivo*. The products of these polymers' erosion should be eliminated from the body or metabolized and should give rise to non-toxic substances. Another improvement, regarding biodegradable polymers, is the possibility of releasing drug molecules that have high molecular weight or poor solubility in polymer. (15)

Polymeric DDS can be classified, according to structure, into three groups: polymer-drug conjugate systems; reservoir-based systems; monolithic matrix systems. (20,22,23) The first type reports to water-soluble and biodegradable polymers that, through covalent bonds (that can be physiologically labile (18)), can improve bioavailability of poorly soluble drugs or that are susceptible to fast degradation. (22,24) An example of these conjugates the use of polyethylene glycol (PEG)-adenosine deaminase (ADA), to treat ADA deficiency, that causes severe combined immunodeficiency syndrome. (25,26) Despite the enhanced efficacy, these conjugates can show heterogeneity, in terms of drug loading and site of modification. (24)

Reservoir-based systems consist of a polymer-covered drug core, out of which the drug undergoes diffusion. Thus, the concentration gradient of the drug between the reservoir and the medium, is the limiting step of the release rate. If this surrounding polymer membrane is eroded only after all the drug has been released, then it should be a zero order kinetics, i.e., with constant release of drug. (20) On the other hand, changes in the release rate are observed, if there's polymer erosion before the exhaustion of the entire amount of drug. (23) This way, the release rate is modifiable, as needed. A disadvantage is the possible rupture of the membrane and consequent rapid release of the drug. (20) An example of this type of systems is an intravitreal implant made of Poly(vinyl alcohol) (hydrophilic), surrounded by a release-controlling coating of Ethylene-vinyl acetate (hydrophobic), that allows the entry of fluid, making a saturated solution of ganciclovir, that is released in a constant rate, for 5 to 8 months, to treat cytomegalovirus infection. (27)

As for monolithic matrix systems, they have a dispersion or solution, in which the drug is evenly distributed throughout the polymer, which is in the solid state, without resorting to covalent

bonds. This way, the release rate is conditioned, both by the diffusion of drug to the outer media and by the erosion of the polymer. If the drug is immobilized within the polymer, the release rate is controlled by the erosion rate, only. On the contrary, depending, for example, on the drug solubility in the external media, if the diffusion rate is much higher than the erosion rate, the diffusion will be the limiting step. (23)

1.2.1. Existing polymers

In order to achieve optimal delivery of numerous recent molecules, new biodegradable polymers may be required. Aside from inert or biodegradable, polymers can also be synthetic or natural. Cellulose and chitosan are examples of natural biodegradable polymers. Within the synthetic group there are polyesters and polyamides, for example. They can also be classified as either hydrolytically or enzymatically degradable polymers, the last of which are commonly natural. (28)

However, new polymers, without proven safety and efficacy studies, will have high costs and risk, as well as time investment for the needed clinical research and industrial development, all of which will contribute for the stagnation of therapeutic innovation. Other challenges of the several polymers are the possible issues with toxicity and immunogenicity, drug-polymer compatibility, low drug loading and poor preclinical performance. Nevertheless, introducing new polymers also has the potential to improve therapy. (29)

1.2.2. PLGA

Poly(lactic-co-glycolic acid) (PLGA) is an aliphatic copolymer, that contains polyesters lactic acid (α-hydroxy propanoic acid) or lactide and glycolic acid (hydroxy acetic acid) or glycolide. Aside from PLGA, both polylactic acid (PLA) and polyglycolic acid (PGA) are biodegradable and synthetic polymers. Additionally, PLA has two enantiomers and the synthetic blend is DL-lactide. The stereochemistry of PLA will affect the crystallinity of the polymer, which in turn determines the degradation profile. In fact, PLA is semi-crystalline and PLA derived from 50 to 93% of L-lactide is amorphous. (30)

PLGA is used in DDS, of which the release profile is influenced by the drug's structure and solubility. (31) PLGA can be obtained by a ring-opening co-polymerization of lactic and glycolic acid or using cyclic dimers as a starting material. (32)

This and other biodegradable polymers are, because of this property and for their biocompatibility and toxicological safety, adequate for sustained drug delivery applications, from days to several months. (33–37) In fact, PLGA's ester bonds undergo simple hydrolysis, resulting in lactic and glycolic acid, which are removed from the body by normal metabolic pathways, such as tricarboxylic acid cycle (or Krebs cycle). (37–39) As for glycolic acid, it is

either excreted unchanged in the kidney or, just like lactic acid, enters the Krebs cycle and after metabolization it's eventually eliminated as carbon dioxide and water. (40)

1.2.2.1. Advantages and disadvantages

This polymer is suitable for the present work and has considerable attention, for its advantageous properties. PLGA is currently one of the few synthetic polymers that have been approved for clinical use, including in drug delivery systems for parenteral administration, by both the European Medicines Agency and the United States Food and Drug Administration. That makes it ideal to develop more pharmaceutical products of parenteral long-acting release (29,37) and it's due to being biodegradable and biocompatible. There are described formulations with PLGA and methods of production adapted to various types of drugs, e.g. hydrophilic or hydrophobic small molecules or macromolecules. (39)

When using PLGA, continuous release is possible, if the drug remains solubilized in the polymer, in high amounts and if the polymer is of low molecular weight. (41) This way, sustained drug levels can be maintained in the blood or target tissue for months following a single injection, instead of daily injections of the solution dosage form or weekly dosing of products resulting from peptide modification strategies. It can be used to maintain systemic levels of a certain drug, but also for local delivery. (29)

Besides providing sustained drug release, its relevance also lies in its ability to avoid drug degradation and to target specific organs or cells. It can also modify surface properties, allowing a better interaction with biological materials. (42) In fact, its adhesive properties allow binding to gastrointestinal mucosa, which is useful in gastroretentive oral formulations. (39) All these aspects, along with its high mechanical strength, makes of PLGA one of the biodegradable polymers of most interest. (43)

PLGAs are easily processed and configured in terms of their physical, chemical, mechanical, and degradative properties, by means of their composition, molecular weight, crystallinity and environmental parameters (e.g. pH, temperature, organic solvents). (40,44) This processing of the polymer will vary according to its purpose, which can be cell growth and tissue regeneration, drug release and host response. (38)

As disadvantages of PLGA, however, there's low drug loading capacity, tendency for aggregation and broad particle size distributions. (45) Besides low drug loading, there's also the high initial release in PLGA formulations, e.g. microparticles containing small hydrophilic drug molecules, (39,46) which may or not be desirable, depending on the intended release profile. (45) Large molecules, namely proteins, aren't easily incorporated in PLGA. In opposition to non-degradable polymers, PLGA reacts with water, which can be a disadvantage if degradation products interact with the drug. (39)

Designing a commercial PLGA formulation can be challenging. For example, excipients, such as poorly soluble bases (e.g. $MgCO_3$) can affect release kinetics and solvents that may result from the manufacturing process can be toxic. Other challenges include needle size that may be required for injection of PLGA depots, as well as drug stability. (29)

1.2.2.2. Specifications and their impact

Both solid-state solubility of the drug in the polymer and drug load can be increased, by changing the following parameters in the PLGA: an increase in lactide content (and consequently a decrease in glycolide content); a lower molecular weight; the presence of ester end groups instead of free acid end groups. Both higher lactide content and higher ester end-groups content will make a more hydrophobic polymer, resulting in better solid-state solubility for hydrophobic drugs. (34) Higher solubility with lower molecular weight can be explained by the increased entropic contribution, where solubilizing the drug requires lower free energy, (47) due to lower viscosity. (48,49) Accordingly, differential scanning calorimetry (DSC) results show that dexamethasone is about two times more soluble in the low molecular weight (12000 Da) polymer than in the high molecular weight (143000 Da) polymer. (34)

Degradation of this polymer by water uptake can be enhanced by a higher surface area/volume ratio. Furthermore, thickness of PLGA films shouldn't be too high, to prevent heterogeneous bulk degradation, due to an autocatalytic effect. (38) This causes the accumulation of carboxylic groups over time inside the film and thus contributes towards a much faster degradation of films, than the homogeneous degradation, in thinner films. (50) Degradation rate can be relevant, for example, in PLGA films used as barriers in guided tissue regeneration, since a significant amount of tissue needs to be regenerated before the polymer is completely degraded. So, for this case, increased hydrophilicity (higher PGA content) and higher thickness may not be as adequate, because degradation occurs faster. (38)

1.2.2.3. Applicability

PLGAs' importance lies in their applications in tissue engineering and controlled drug delivery, whether as temporary scaffolds for cell transplantation, regenerating various tissues or as carriers with the purpose of delivering drugs. (43) These copolymers have been approved for bioabsorbable sutures (23) and bone fixation devices. (43,51) In fact, PLGA is currently used in paediatric surgery as a resorbable suture material, due to its biocompatibility. (29)

An example of their use as the previously mentioned temporary scaffolds for cell transplantation, is the use of PLGA thin films, in previous studies, serving as a substrate for human retinal pigment epithelium cell culture *in vitro*, that is important for the maintenance of normal functions of photoreceptors. (52) Or the use of PLGA films with Schwann cells and salidroside (neuroprotective glycoside) for peripheral nerve regeneration. (53)

Again, being important agents in guided tissue regeneration, PLGA films can prevent other tissues from interfering with periodontal ligament and alveolar bone's regeneration in a maxillofacial defect, due to their barrier effect. (54) Besides, their osteoconductive properties are also relevant in the reconstruction of the bone. (38,55,56) LactoSorb® (Biomet Manufacturing Corp) is an example of craniomaxillofacial fixation device in the form of implantable screws and plates, using PLGA (82% of polyL-lactic acid and 18% of polyglycolic acid), that is fully degraded by the end of less than 12 months. (56–58)

Furthermore, PLGA has the potential to provide systemic sustained delivery to several substances, like hormonal peptides (e.g. leuprolide and octreotide), glucagon-like peptide-1 as a therapy for type-2 diabetes, growth hormones, (29) blood coagulation factors, monoclonal antibodies, enzymes and cytokines. In most cases, the incorporated drug is present in a dispersed state. (34)

As an example, leuprorelin-depot PLGA microparticles allow for monthly injections, instead of daily injections, which are also available. Leuprorelin is indicated for palliative treatment of advanced localized or metastatic prostate cancer. (37) Zoladex® (AstraZeneca) is a second example of an implant, that contains goserelin in millimetric PLGA cylinders, which can be administered every 12 weeks. The less frequent exposure to the needle and consequent better compliance, along with more stable blood levels of drug, will lead to an improved efficacy and better lifestyle for patients. (29) However, contrary to monthly injections, daily injections don't show reports of handling errors, which may prove as a disadvantage, requiring a healthcare professional to perform each administration, even if they are reduced to a monthly basis administration. (59,60)

1.3. Release profile from PLGA

In DDS, the release profile depends on the polymer, on the drug's physicochemical properties and its mobility in the polymer. (27)

The profile of drug release from PLGAs can have the three following phases:

a) initial fast release, that can go from one day to a week and is influenced by diffusion;

b) lag or induction phase, that undergoes slow erosion of PLGA and possibly some residual drug release, by diffusion;

c) active erosion phase, with continuous mass loss of polymer, which turns into continuous release of drug, so overcoming the lag phase is important for continuous release.

The initial fast release happens when there's adhered drug on the polymer's surface or it's free among it, so there's nothing delaying its release. (29) This phase accelerates when the drug is very hydrophilic or exists in high amounts and when surface area is higher (e.g.

microparticles). (27) This initial phase can be advantageous for single dosage treatments. However, it could lead to low drug levels within PLGA, which, in turn, isn't wanted in long duration treatments, e.g. cancer treatments. (39)

Among other approaches to reduce the initial fast release of drug, (29,61–67) there is the option of increasing interactions between the drug and the polymer (PLGA), thus increasing the dissolved amount of drug in the polymer matrix. Simultaneously, these two components' interactions with solvents are to be decreased. (39) This can be accomplished by several approaches: alteration of the drug, to increase organic solvent partitioning; alteration of the polymer or use of a binder, in order to increase drug binding. Peptide modification with PEG or lipid tails is an example of the first approach, in case of drugs that are peptides, since it will help co-dissolve the peptide with the polymer. (68) The use of PEG can also be interferent of PLGA, showing higher drug encapsulation in nanoparticles and lower initial fast release. (69)

The second phase of the release profile occurs at an approximately constant rate, when the drug molecules within the PLGA diffuse out and over time that rate eventually starts to decrease. On the other hand, as polymer degradation unfolds, drug particles start gaining more mobility, which will increase the diffusion rate, i.e. the release rate, over again.

Lastly, during the third phase, drug release ends with the erosion and disintegration of polymer particles, resulting in a fast and complete drug release. (39) This can be minimized, adding a higher molecular weight PLGA, that lasts longer than a second PLGA with lower molecular weight. (27)

Additionally, the drug release profile can also be bi-phasic, when the second and the third phase are indistinguishable, because both diffusion and polymer degradation simultaneously occur, (39,40) or when there's only a diffusion phase and an erosion phase. (70)

1.3.1. Effect of drug parameters

Both release rate and drug loading are influenced by the drug's solubility, i.e., its hydrophilicity. (39,71) The higher the solubility of the drug in the aqueous release medium, the higher the release rate. On the contrary, the more soluble the drug in the polymer, the lower the release rate, assuming that the polymer is hydrophilic and the drug is released by diffusion. (23) However, drug's solubility in solvents will determine whether it's dissolved, or partially dissolved or crystallized. This will subsequentially influence the release profile, from the PLGA, because when crystallized, the drug takes time dissolving, before diffusing out, contrary to when it's completely dissolved. Decreasing drug particle size is, due to an increased surface area, a way of achieving its dissolution in the solvents and in the polymer, (71,72) leading to a higher release rate. (73)

If drug particles are suspended in the polymer, release profile should be less controlled, since some of the particles may be at the surface. On the contrary, if there is a molecular dispersion, there is a more uniform distribution of the drug, leading to a more predictable and constant release rate. (20)

1.3.2. Effect of PLGA parameters

As previously mentioned, certain parameters of PLGA can be changed to make the drug more soluble in the polymer, which will change release rate of the drug.

When increasing lactide/decreasing glycolide content of the PLGA in nanoparticles, which leads to higher solid-state solubility for the drug, there is a lower percent of released encapsulated drug (Figure 1.1). However, drug release is almost similar, when comparing 50:50 and 75:25 lactide/glycolide ratio, possibly because there isn't a significant change in the nanoparticles' properties. (34)

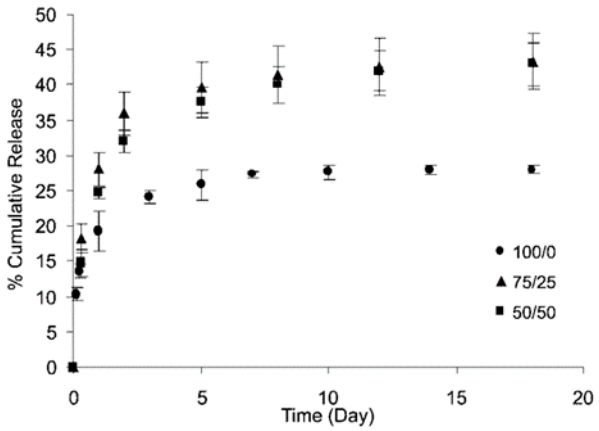


Figure 1.1. Effect of PLA:PGA ratio on the *in vitro* release of dexamethasone from nanoparticles. Adapted from Panyam et al., 2004 (34)

However, release rate also depends on the polymer's degradation rate, not just on the diffusion process, as with non-biodegradable polymers. So, anything that affects this degradation will be important, like PLGA's crystallinity and hydrophilicity, which in turn are influenced by the stereometric composition and the PLA:PGA ratio. (27) For example, the higher content of hydrophilic glycolic units in PLGA shows increased degradation, by absorption and diffusion of water, facilitating hydrolysis of the ester bonds. (32) In fact, degradation of PLGA and, therefore, drug's half-life, make a graph, as a function of PLA:PGA ratio, in the shape of "U", i.e., when the ratio is 50:50, half-life is shorter, thus drug release is faster. On the other hand, as the proportions of PLA and PGA become more and more different, this rate of degradation decreases, whether the proportion of lactic acid or glycolic acid increases. This also means that the necessary administration frequencies will be higher when the ratio is 50:50, because half-life is shorter. (23)

Aside from crystallinity, there are other influencing factors, like glass transition temperature (Tg), determining the glassy/rubbery state; molecular weight, determined by the amount of carboxylic groups and consequent autocatalytic degradation; porosity, affecting water permeability; implant dimensions and composition, since basic components will catalyse hydrolysis of ester bonds. (27)

1.3.3. Effect of temperature

The temperature at which solid polymeric systems are prepared, by melting of components, is relevant for the release profile, since if this temperature is lower than the melting point, there is higher probability of existing undissolved drug particles, leading to a slower drug release. (74)

1.4. Solid dispersions

A solid dispersion is a mixture of one or more drugs, that are generally hydrophobic, in one or more carriers, in a way that improves drug release, due to the polymer properties and an increased surface area of drug. (75) Drug release can also be delayed, using a hydrophobic or insoluble carrier, instead of a hydrophilic carrier. (76) Solid dispersions can be classified according to their components' physical state. In fact, the matrix (carrier) can be crystalline or amorphous and drug(s) can be molecularly dispersed, or in particles, which in turn can be amorphous or crystalline, as well. When the drug is molecularly dispersed, there is only one phase and the carrier can be crystalline (solid solution) or it can be amorphous (glassy solid solution). However, only the second usually occur. (76,77) The solid dispersion is very stable when the drug is dispersed as crystals, i.e. in its favoured state, in the amorphous polymer phase. If both the drug and the polymer are in an amorphous state but not in a molecular dispersion, the solid dispersion is metastable, since the drug recrystallizes during processing or storage, because, usually, its solubility in the polymer is below 10%. (78)

1.4.1. Solid Solutions

Aside from decreasing particle size and agglomeration and improving wettability, solid dispersions can also allow changes in physical state of the drug, leading to a molecular dispersion. (79) Ideally, solid solutions only have one phase, just like liquid solutions, which means that the dispersed component in a solid solution is molecularly dispersed, but in this case in a carrier at the solid state. (80,81) Achieving a solid solution depends on the components' physicochemical properties and interactions, as well as the preparation method. (79) A characteristic that differentiates solid solutions from solid dispersions in general is the absence of crystallinity of the drug. (81)

In the glassy solid solution, best for poorly soluble drugs, the drug is molecularly dispersed and immobilized in the amorphous polymer. The choice of polymer will affect the stability of the system. Particularly, the polymer's Tg should be high, so that stability of the amorphous state, at room temperature, is increased. Also, physical stability can be achieved, through drug-polymer interactions. (82) Some determining characteristics of polymers for the achievement of solid dispersions are:

a) thermal stability and thermoplastic characteristics, for hot melt extrusion method;

b) high solubility in organic solvents, for solvent method. (79)

When discussing the distribution of the molecules of the solute in the solvent, the solid solution can be substitutional, interstitial or amorphous. The first type refers to when the solute molecules can substitute the solvent crystalline molecules, or occupy the interstitial space between them, for being of similar molecular diameter to them (difference <15%). (81,83) The interstitial type has bigger solute molecules, with a molecular diameter of less than 59% of the solvent's molecular diameter, so the solute is only present in the interstitial space between the solvent crystalline molecules. Finally, the amorphous solid solution has the solute molecules irregularly dispersed in an amorphous carrier.

Both the components' solubilities as well as the drug load influence solid solution's relevance, because there could be a mass limit for a certain dosage form and low solubility may need more amount of carrier. (81)

1.4.2. Methods of characterisation of solid dispersions

The most widely applied methods to differentiate solid solutions, solid dispersions in which drug is only partly molecularly dispersed and physical mixtures of drug and carrier are: differential scanning calorimetry (DSC), X-ray diffraction, infrared spectroscopy and measurement of the release rate of the drug. (81) Structure probing capabilities, relatively easy operation and short analysis time are the reasons why these methods are the most used. (82)

DSC method quantitatively determines drug's solid-state solubility in polymers, (34,84) taking in consideration the thermal properties of materials such as melting and recrystallization. (82) From all thermoanalytical methods, i.e., that examine temperature variations to characterize the system, DSC is the most appreciated one. (81) By means of instrumental software the heats of melting associated with the melting endotherm can be calculated and plotted as a function of drug concentration, where the intercept on Y-axis represents the solubility of the drug in the polymer at its melting temperature. (34) The reference and samples depart from the same temperature and when a phase transition such as melting (endothermic transition) or conversion of one polymorph to a more stable polymorph (exothermic transition) happens,

extra heat is applied to the sample, in order to keep its temperature the same as the reference. If the drug is amorphous, a melting peak won't be obtained. (81)

Sample amount can be a limiting factor for DSC, since a signal can be difficult to detect or be undetectable, when the sample is too small. (82) Furthermore, scanning rate is also a critical parameter for DSC, because it can influence drug recrystallization kinetics. (85) At last, an important set back is that, before reaching drug's melting temperature, there may be melting of excipients and dissolving of the drug, leading to an absence of melting peak and a consequent false result.

X-ray diffraction determines the amorphous or crystalline nature of a sample. Here, crystallinity is detected according to interference bands from the diffraction of an X-ray beam as it passes through the sample intercepting an X-ray beam. Accordingly, the eventual crystallinity of the sample can be detected, thus differentiating solid solutions, where the drug is amorphous. However, it's not sensitive enough to detect crystallinity under 5±10%. (81)

Infrared spectroscopy is a known technique for compound identification, by identifying crystalline structural changes, (81) as the wavelength of absorbed infrared radiation differs. This fast and reproductible technique is used in the study of intermolecular interactions between solid dispersion components, like hydrogen bonding. (82)

1.4.3. Advantages

Drug loading and its release profile can be influenced by the solid-state solubility of the drug in polymer. (34)

Solid solutions can improve oral bioavailability of a poorly water-soluble drug, like dexamethasone, if carrier has relatively good aqueous solubility and also because they will significantly increase the dissolution rate, due to the maximum reduction in particle size (dispersion on a molecular level), but also because less energy is required, since there is no crystal structure of the drug to be broken up. Increasing bioavailability can thus enable to lower the administered dose. In addition, they can be relevant, because of the increasing number of poorly soluble drugs and because of the improvements in the manufacturing methods of solid dispersions.

On the other hand, the choice of the carrier can also allow delaying the release of the drug, which is beneficial for modified release dosage forms. (81)

1.4.4. Challenges and disadvantages

Scaling-up of the manufacturing method, physical stability and required amount of carrier to facilitate the required increase in the release rate are significant challenges of solid dispersions, in general. (81)

Regarding solid solutions, since the amorphous state has high energy, having a tendency to change to the lowest energy state, (76) it is susceptible to be partially recrystallized during storage of the systems, leading to reduced drug fluxes. Drug recrystallization can be prevented by reducing drug load, but sometimes, to an extent that isn't intended, since it requires a bigger delivery system. However, this can be overcome by prodrug formation (86) or use of crystallization inhibitors, for example. (87) Dexamethasone has a partition coefficient (logP) value of 1.83, that is lower than the logP of PLGA, which, depending on the polymer's composition, can vary from about 2.6 to 4. Consequently, approaching a solid solution containing high amounts of this drug in PLGA becomes more challenging. (88)

1.4.5. Manufacturing techniques

The production of solid dispersions can be executed using methods that require organic solvents or methods that rely on the fusion of components to obtain a mixture. (89)

1.4.5.1. Melting or fusion method

Here, there's heating of the components to a temperature higher than their melting temperatures or Tg, after which they are mixed, temperature is reduced (79) and mixture is milled. This way, drug is entrapped in the polymer. A possible solid solution is obtained if the cooling is fast enough to reduce the degree of supersaturation of the drug. (81)

This method is less expensive and less toxic, because it doesn't require the use of organic solvents. Nevertheless, it may subject drugs to degradation, as a result of high temperature. So, substances should be thermostable and they should also be miscible, when melted. (81)

1.4.5.2. Hot melt extrusion method

This technique is a version of the melting method that became more popular, because it's more applicable in an industrial level. (81) It has the advantage of being a scalable one-step continuous process. (89) This method subjects the components to an elevated temperature for less time, which is better for more thermolabile drugs. (81) It allows for a more uniform dispersion, due to the intense agitation and consequent disaggregation of the particles. (90) If, on one hand, it's more economic and safe, as well as environmentally less harmful, it's also challenging for drugs with high melting points, thermolabile drugs and viscous polymers. (78)

In addition to the previously mentioned conventional method, this involves the extrusion of powders that have resulted from the melting of the components, leading to the production of granules, pellets, cylinders, or films. (81)

1.4.5.3. Hot-spin-melting method

It allows the melting to occur a lot faster, by means of a high-speed mixer. Air or an inert gas are used in the cooling process. Therefore, this method presents as well as an alternative to the classical hot melt method, for thermolabile substances. (81)

1.4.5.4. KinetiSol® technology method

This is a new method based on the fusion method, to prepare amorphous solid dispersions. It consists of a high mechanical force, caused by the rotation of blades that impose friction and shear to the components. In just seconds, an amorphous mass is formed, without resorting to an external application of heat energy. This results in more stable products and, in fact, makes the technique useful for thermolabile drugs. It's also advantageous to viscous polymers, drugs with high melting points or with low solubility in organic solvents. Another advantage is its scalability to a semi-continuous mode. KinetiSol® requires careful monitoring of processing conditions. (78,89)

1.4.5.5. Solvent method

This method has emerged after the melting method, where both the drug and the carrier are dissolved in a common organic solvent, which evaporates under vacuum, leading to a solid state. This method is useful, not only for thermolabile substances and polymers with a high melting point, but also because it grants the use of highly lipophilic drug with a water-soluble carrier. As a prerequisite of this method, both components should be soluble in the solvent. Aside from evaporation under vacuum, freeze-drying and spray-drying can also remove the solvent. The use of organic solvents, with their toxicity issues and changes in product performance, brings a disadvantage to this method. (81) Besides, to mix the drug in the matrix, particle size can be decreased and low concentrations of drug are used, leading to a low drug load. It may also require the use of cyclodextrins or surfactants to increase solubility. (83)

Spray drying has the following stages: atomization of liquid; interaction between liquid and drying gas (e.g. compressed air or nitrogen), which will spray drug particles, resulting in fine droplets; evaporation of liquid from the mixture, when droplets are inside a chamber with heated air; separation of dried particles from drying gas, through centrifugation. (89) There are several factors that can be changed according to the intended final product. For example, the solvent system will affect drug solubility, toxicity and ease of evaporation (e.g. solvents with low boiling point); inlet and outlet temperatures, when too different will lead to higher humidity in the obtained particles. Aside from this possibility to configurate the process, this method has another advantage, which is the obtaining of uniform small spherical particles, which is also due to the properties of the selected polymer. (91)

Freeze drying or lyophilization is another method of removing the used solvents during the manufacture of solid dispersions. After the compounds have been mixed, they undergo freezing and then sublimation, resulting in evaporation of the solvent and a dry solid dispersion. This method has the advantages of using lower temperatures and of decreasing the chance of phase separation. (83,92)

An alternative technique is spray-freeze drying, which combines the two above, where the formed droplets are frozen and subjected to sublimation. This will decrease even more the possibility of phase separation. (83)

1.4.5.6. Melting solvent method

This method will benefit from advantages of both the fusion method and the solvent method. The drug is dissolved in a solvent and then incorporated into the melt polymer. After which the solvent is evaporated. It's only relevant when low drug loads are required and thermolabile drugs or with high melting points. (83)

1.4.6. Crystallization process

Crystallization of solid dispersions occurs in two stages: nucleation and crystal growth, which occurs at higher temperatures than the first. (79) Nucleation is the formation of a new phase, where drug molecules assume a crystal-like atomic arrangement, forming so called nuclei. (93,94) For nucleation to initiate, not only is required a certain degree of supersaturation, but also an activation energy must be overcome. This activation energy is, in turn, influenced by interfacial tension, that is caused by the interaction of small curved particles and the solvent. In fact, nucleation rate increases with higher degree of supersaturation (because a lower number of molecules is necessary to induce effective nucleation) or with higher solubility and, consequently lower interfacial energy, thus lower activation energy, due to higher affinity between medium and crystals, i.e. higher probability of intermolecular collisions. (79,94)

Crystal growth follows nucleation, consisting of the diffusion of molecules from the solution to the nuclei surface and their integration. This diffusion of molecules can be influenced by the viscosity of the solution. (79) Both nucleation and crystal growth are affected or inhibited by polymer-drug interactions. (95)

1.5. Films

Films are a recent pharmaceutical dosage form that consists of a thin and malleable layer or sheet, which is made of one or more polymers and possibly a plasticizer. Films can be used as DDS or topically applied (e.g. wound dressings) and they are produced usually resorting to the previously mentioned solvent method. (96) These can be a versatile alternative to conventional dosage forms, for being easier to swallow, self-administrable, and quickly

dissolvable. This dosage form can be applied as both a systemic and local action delivery system, via several routes such as oral, buccal, sublingual, ocular, and transdermal routes. (97) In this work, they were used as a simple form to work with and manufacture, where it would be studied the release profile of the drug, as a step that precedes more complex forms, like extrudates.

1.5.1. Production methods

As previously mentioned, the typical method to produce films is the solvent method. At an experimental level, the solvent-casting method is used. Essential elements of film production are, in addition to the drug and solvents, polymers, such as PLGA. In this case, two different solutions, one with drug and an organic solvent and the second solution with PLGA and another organic solvent, for instance chloroform, (38) are mixed together and spread onto a glass (e.g. microscope slide) or aluminium surface (98) and dried under different conditions. (34) The films can also be subjected to compression, in case they are multi-layered. (98)

Another possible method refers to the injection of 50 µL of a solution with drug, PLGA and tetrahydrofuran (THF) as a solvent, directly on the water surface. If both PLGA and the drug aren't water-soluble, being THF water-soluble, then the film is obtained on the water surface, upon THF diffusion into the water. (37)

1.5.2. Advantages and disadvantages

Some advantages of films are: high surface area, softness, absorbency, and ease of fabrication into many product forms (37) (e.g. multi-layered films (66,98); fibres as wound dressings (99); injectable depot formulations for long-term controlled drug release (29)). When containing high concentrations of drug, these products generally promote high drug fluxes and lead to high daily dosages from relatively small, and thus attractive, systems. (87)

Despite the many advantages of this dosage form, the design and manufacturing of efficient thin films requires a comprehensive knowledge of the pharmacological and pharmaceutical properties of drugs and polymers. (97)

2. Aim of this investigation

This work was carried out as part of a laboratory internship of Introduction to Research, which took place as an activity of the Erasmus + program, at the Department of Biology-Chemistry-Pharmacy, in the Pharmaceutical Technology division, of the Institute of Pharmacy, of the Freie Universität Berlin. The laboratory work started on January 31st of 2020 and ended earlier than planned (April 29th), on March 19th of 2020, due to the covid19 pandemic situation. It was carried out within the research group of Professor Doctor Roland Bodmeier and supervised more closely by Friederike Bach.

The main purpose was to obtain deeper understanding of the manufacturing process of dexamethasone loaded PLGA films. The drug was intended to be dissolved in this biodegradable polymer, to which several variables were taken in consideration. It should be noted that this discussion is the result of interpretation of macro and microscopic observations of the films.

Other investigation goal would also be to address eventual results of release tests. However, due to the unexpected shortening of the practical internship, these were not accomplished, with only the film manufacturing tests and the assay of dexamethasone in the final formulation having been realized, wherein the second was a step that would precede the release tests. The experimental procedure (Chapter 3.), results and discussion (Chapter 4.), conclusions (Chapter 5.) and recommendations for future work (Chapter 6.) are described hereafter.

3. Experimental procedure

3.1. Materials

Dexamethasone (DM) was supplied by Caelo (Hilden, Germany); PLGA Resomer RG 502 H by Evonik (Essen, Germany); chloroform (CLF) and ethanol (EtOH) by Carl Roth GmbH + Co. KG (Karlsruhe, Germany); methanol (MeOH), acetonitrile, acetone and ethyl acetate (EA) by VWR Chemicals (Darmstadt, Germany).

To manufacture the films, glass vials, a balance (Sartorius MC210P), a vacuum oven (Heraeus VT 5042 EKP), a film making knife (Proceq ZUA 2000 Universal Film Applicator, Zehntner GmbH Testing Instruments), micropipettes (Eppendorf AG) and microscope slides (VWR International) were used. Film characterisation resorted to a Zeiss polarized light microscope (Axioskop), with use of the ZEN software program. For the assay development, a UV-spectrophotometer (Shimadzu UV HP 8453, Shimadzu Japan), a magnetic stirrer (IKA-Werke GmbH & CO. KG) and a quartz cuvette were used.

3.2. Methods

3.2.1. Film preparation

Films were prepared using the solvent method. DM and PLGA were weighed and two solutions were made: DM in methanol, with a concentration of 25 mg/mL and PLGA in chloroform, with a concentration of 30 $mg.mL^{-1}$. Then, to make a DM:PLGA solution with a ratio of 10:90 (w/w), for example, 0.2 mL of the first solution (with 5 mg of drug) were added to 1.5 mL of the second solution (with 45 mg of PLGA), in a new glass vial. In this case, methanol was the co-solvent, since it contributed to the dissolution of DM in CLF, because of its low solubility in the latter (1 $mg.mL^{-1}$). (100) Different DM:PLGA ratios were used, changing the concentration of dexamethasone in the initial solution, with a variation of the mass of DM (Table 3.1), according to Equations 1 and 2. After mixing of the solutions, the film casting took place. Two methods were used: a micropipette was used to spread the solution in the glass microscope slide and a second method involved the use of a film casting knife, to spread a certain volume of the final mixture that had been placed on the microscope slide, also with a micropipette. This film casting knife allowed to adjust the height, i.e. thickness of the created films and could spread the solution uniformly throughout the surface. There was also film casting using PLGA solution, only. Based on a previous article, samples were left to dry overnight, under the hood. (34) But this drying method was also compared to vacuum drying, for 1 hour, using a vacuum oven, at room temperature and at the temperatures of 50ºC and 35ºC. The drying process was also observed in films from 6% to 9% DM (w/w), throughout the first hour after film casting with a micropipette and 7 and 24 hours later. Stability was also studied, by observing the increase in

size and number of crystals, 3 days after vacuum drying of films, using formulations of Table 3.1. Also, different co-solvents were used for dexamethasone's initial solution, where methanol was compared to ethanol, in 5% and 10% DM (w/w) films (according to Table 3.1), that were dried under the hood, overnight. Drug/polymer ratio of 10% DM (w/w) formulations were also prepared, while changing solvents volumes and maintaining constant the weights of DM and PLGA in the final mixture (Table 3.2).

$$mass\ of\ DM\ in\ final\ mixture = x = \frac{45 * DM\%}{PLGA\%}\ (mg) \quad (1)$$

$$concentration\ of\ DM\ in\ intitial\ solution = \frac{x}{0.2} = \frac{45 * DM\%}{0.2 * PLGA\%}\ (mg.mL^{-1}) \quad (2)$$

Table 3.1. Formulations of films according to the variation of the concentration of DM in the initial solution ($[DM]_i$) with methanol and consequent variation of its mass in the final mixture.

DM initial solution			PLGA initial solution			Ratio / %(w/w)	
$[DM]_i$ / $mg.mL^{-1}$	Volume /mL	DM weight /mg	$[PLGA]_i$ / $mg.mL^{-1}$	Volume /mL	PLGA weight /mg	DM	PLGA
25.00	0.2	5.000	30	1.5	45	10	90
22.25	0.2	4.451	30	1.5	45	9	91
19.57	0.2	3.913	30	1.5	45	8	92
16.94	0.2	3.387	30	1.5	45	7	93
14.36	0.2	2.872	30	1.5	45	6	94
11.84	0.2	2.368	30	1.5	45	5	95

Table 3.2. Formulations of films with different solvents volumes, with constant drug/polymer ratio.

DM initial solution			PLGA initial solution			Ratio / %(w/w)	
$[DM]_i$ / $mg.mL^{-1}$	Methanol volume /mL	DM weight /mg	$[PLGA]_i$/ $mg.mL^{-1}$	Chloroform Volume /mL	PLGA weight /mg	DM	PLGA
25	0.2	5	30	1.5	45	10	90
25	0.2	5	6	7.5	45	10	90
5	1	5	30	1.5	45	10	90
5	1	5	6	7.5	45	10	90

3.2.2. Film characterisation

To observe crystals, in order to determine if there is a solid solution, microscopical pictures were obtained. Additionally, films were observed macroscopically regarding transparency, colour and streaks. Like previously mentioned, films were usually observed after being dried, but also during drying, to describe the drying process. For the stability analysis, i.e. the

evaluation of the evolution of the amount of crystals, films were observed after 1 or 3 days. When drug/polymer ratio was gradually changed, the results were classified from 0 to 2, according to size and amount of crystals in the totality of each film, where 0 meant that inexistent or rare crystals were observed, 1 meant that few crystals were irregularly present in the periphery of the films and 2 meant that several crystals were constantly found in the periphery of the films.

3.2.3. Assay determination

An initial stock solution was made using 30 mg DM and 270 mg PLGA in 100 mL of acetonitrile. This solvent was used instead of chloroform, because the last is very volatile, which could make the solution more concentrated. The solution was left with a magnetic stirrer overnight, after which it was analysed over the UV-spectrophotometer. A factor 10 dilution was made, because the peak was above the detection limit. With a concentration of 0.03 mg/mL, the peak was at 234 nm. An undetermined amount of PLGA was added to the previous solution, where the peak was very different from the previous solution's peak. Therefore, a new wavelength was determined, where absorbance wasn't very different from the previous solution, which was 270 nm, with a background correction of 400 nm. The quartz cuvette was washed every time the following solution was more diluted.

To make a calibration curve, several dilution factors were used to make the standard solutions (Table 3.3). To calculate the volume of stock solution (V_i) for the dilution, Equation 3 was used, where f was the dilution factor and C_f was the concentration of dexamethasone in the diluted solution, the volume of the diluted solution (V_f) was always 5 mL and the concentration of dexamethasone in stock solution (C_i) was 0.3 mg/mL. The calibration curve that was achieved using acetonitrile was linear (Table 3.3; Figure 3.1).

$$V_i = \frac{C_f \times V_f}{C_i} = \frac{C_f \times 5}{0.3} = \frac{5}{f}\ (mL) \quad (3)$$

Acetonitrile was replaced with acetone and ethyl acetate, using the same volume of solvent in order to attempt even more linear curves. Two spectra were made with each of these solvents. Acetone wasn't used, because the absorbance values were too high. Ethyl acetate also showed high values, but slightly lower than acetone and when PLGA was added to the solution with EA, the difference of absorbance at λ=270 nm was lower than when acetonitrile was used. So, a second calibration curve was made using ethyl acetate as a solvent. However, the calibration curve where acetonitrile was used was more linear, thus more suitable to do the assay.

Table 3.3. Concentrations of standard solutions, that were used to make a calibration curve, using acetonitrile and respective absorbance at 270 nm.

Dilution factor	Volume stock solution (mL)	Volume standard solution (mL)	Final concentration/ mg.mL^{-1}	Absorbance at 270 nm
20	0.250	5	0.0150	0.11572
16,67	0.300	5	0.0180	0.13139
14,29	0.350	5	0.0210	0.15483
12,5	0.400	5	0.0240	0.19564
11,11	0.450	5	0.0270	0.21844
10	0.500	5	0.0300	0.23409
8	0.625	5	0.0375	0.28409
5	1.000	5	0.0600	0.43226
4	1.250	5	0.0750	0.53517
2	2.500	5	0.1500	1.0603

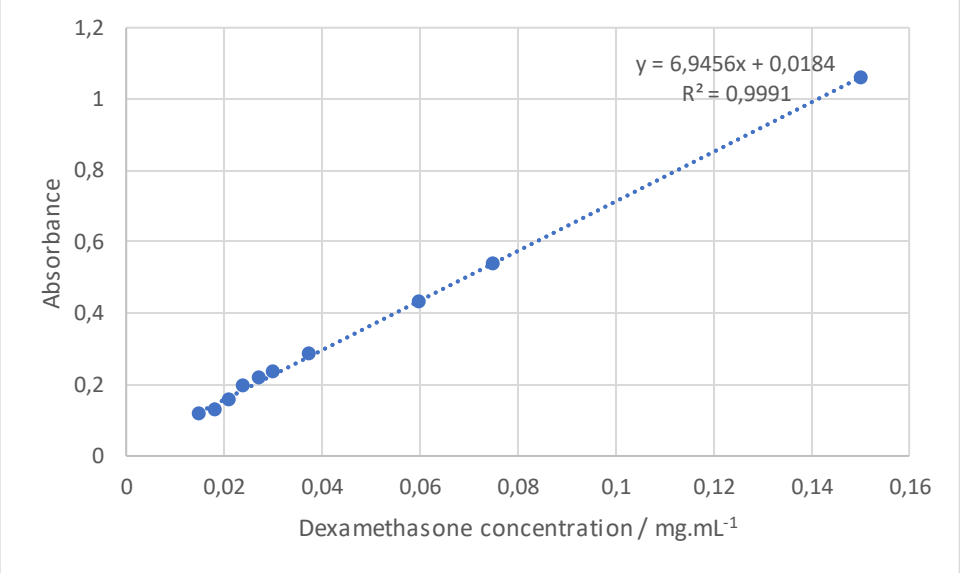


Figure 3.1. Calibration curve, using acetonitrile, according to Table 3.3.

After film micro and macroscopic characterisation, it was selected the formulation and method that provided films with less observed crystals, which was the intended goal to approach a solid solution. Three films were produced as samples and subsequently each sample was dissolved in 2.5 mL of acetonitrile, after which they were analysed over the UV-spectrophotometer at 270 nm. Real concentration of DM in acetonitrile ($DM_{concentration}$/mg.mL^{-1}) was determined, using Equation 4 from the above-mentioned calibration curve (Figure 3.1), upon the resultant absorbances. The mass of DM per film was then calculated, using Equation 5.

$$Absorbance = 6.9456 \times DM_{concentration} + 0.0184 \quad (4)$$

$$DM_{mass\ per\ film} = DM_{concentration} \times 2.5\ (mg) \quad (5)$$

4. Results and discussion

4.1. Preparation and observation of films

4.1.1. Effect of drug loading on film solid state

When there was an alteration of the concentration of dexamethasone in the initial solution, there was also a change in the amount of crystals in the films. As Table 4.1. shows, when gradually decreasing the concentration of this solution, there was an equivalent decrease in crystal amount. The higher drug loading mixture (10% DM (w/w)) originated films with several crystal agglomerates in the line or streak that was observed without a microscope, surrounding the films. The following mixture with 9% DM (w/w) shows lower density and amount of crystals, than the previous one. The films that were created with the mixture with 8% (w/w) of drug loading, where 3.91 mg of drug was in the final mixture, had smaller crystals than the previous films. A fourth formulation had 3.38 mg of drug in the final mixture and followed the tendency, presenting slightly less crystals than the one before, regarding that one of the samples had almost no crystals. All these four formulations showed similar macroscopic results, presenting white and opaque spots or areas in the one circular streak of the films, with a completely transparent and colourless area in the middle of the films. The fifth formulation had 2.87 mg of dexamethasone and a lot less crystals and showed no opacity and few streaks, as well as the last films with 5% (w/w) of drug, i.e. 2.37 mg in the mixture, which only had crystals in one spot, being almost undetectable, at the microscope. Figure 4.1. exemplifies films that are completely transparent, with slightly thicker edges than the centre (Figure 4.1.a) and films that have white, opaque streaks (Figure 4.1.b).

Table 4.1. Qualitative analysis of crystal amount, introducing the variables of drug concentration in the initial solution and drying method

Drug loading/ % (w/w)	Drug final mix/mg	PLGA/ mg	Drug solvent/ mL	PLGA solvent/ mL	Hood, overnight/ crystals	Vacuum, 1h/ crystals	Vacuum, 1h, after 3 days/ crystals
0	0	45	0	1.5	0	0	0
10	5	45	0.2	1.5	2	1	2
9	4.45	45	0.2	1.5	2	1	2
8	3.91	45	0.2	1.5	2	1	1
7	3.38	45	0.2	1.5	1	0	1
6	2.87	45	0.2	1.5	0	0	0
5	2.37	45	0.2	1.5	0	0	0

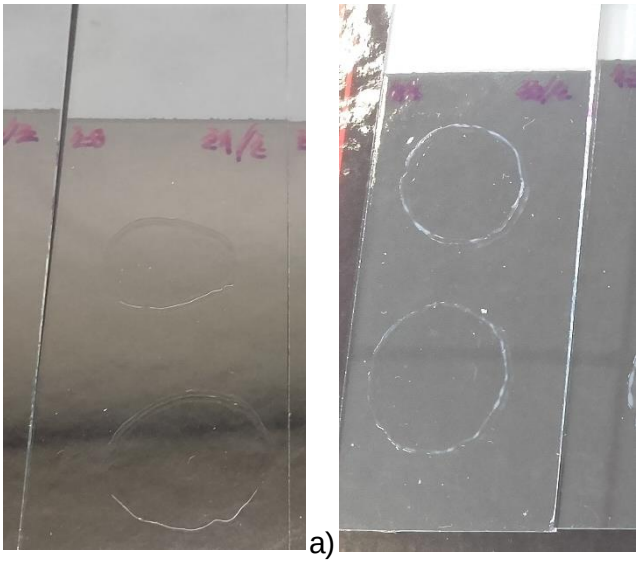

a) b)

Figure 4.1. Films casted with a micropipette, a) completely transparent and b) with a white streak in the periphery.

In conclusion, as initial drug solution gets more diluted, while initial PLGA solution remains constant, crystal amount decreases, as well as the opacity of the films. In this case the amount of drug in the film also decreases and the amount of PLGA is constant. This can be related to the simple fact that the final mass of DM in the films is lower, leading to the existence of less crystals. In this case, it can also be the lower saturation degree of the drug in methanol and in the final mixture (also with chloroform) that would also influence the higher solubility, since all these other components remain constant. Furthermore, the lower drug-polymer ratio in the films, i.e. the lower amount of drug in the same amount of polymer, leads to an increased stabilization of the amorphous drug, by increasing drug-polymer interactions, (101) namely hydrogen bonds, (102) resulting in lower free energy of the amorphous drug, thus less crystal growth. (101) These results are compatible with previous literature, where less than 10% drug is soluble in PLGA. (34)

4.1.2. Effect of co-solvent type on film solid state

When films were being dried under the hood, methanol was compared to ethanol as the co-solvent and it took more volume of ethanol to dissolve the drug, than methanol. When methanol was replaced by ethanol, both films with 5% (w/w) (Figures 4.2 and 4.3) and 10% (w/w) (Figures 4.4 and 4.5) drug load had a greater number and larger crystals, as well as more opacity. Furthermore, a concentration of 10 mg DM/mL ethanol, with 5 mg DM in 0.5 mL ethanol in the final mixture, was used instead of a 25 mg/mL concentration (5 mg DM in 0.2 mL of solvent), as was done when methanol was used.

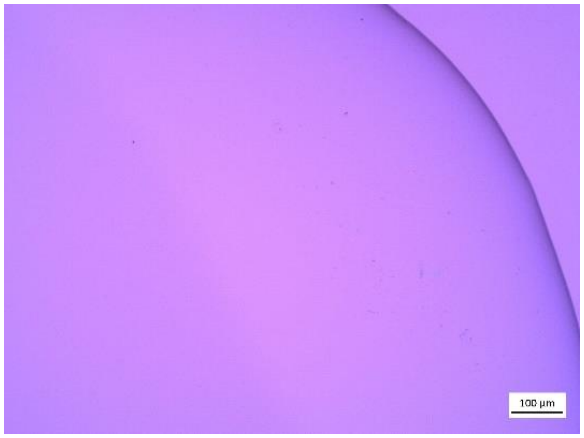
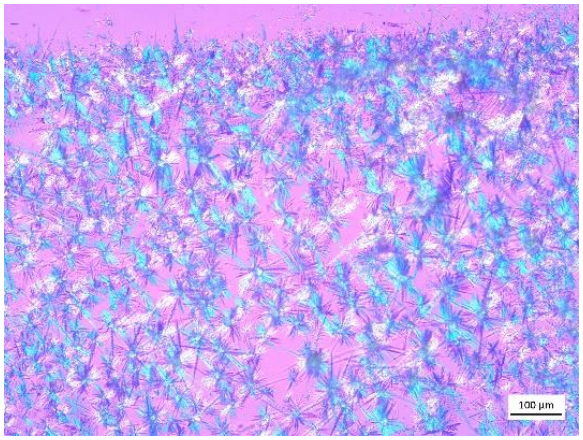
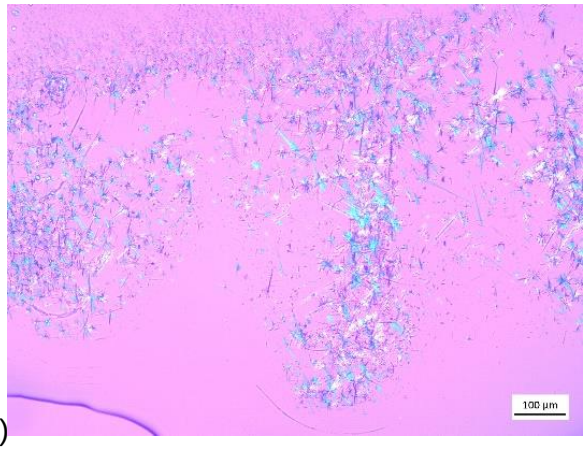


Figure 4.2. Microscopical observation of the peripheral region of 5% DM (w/w) loaded PLGA films using methanol as a co-solvent, dried under the hood overnight.



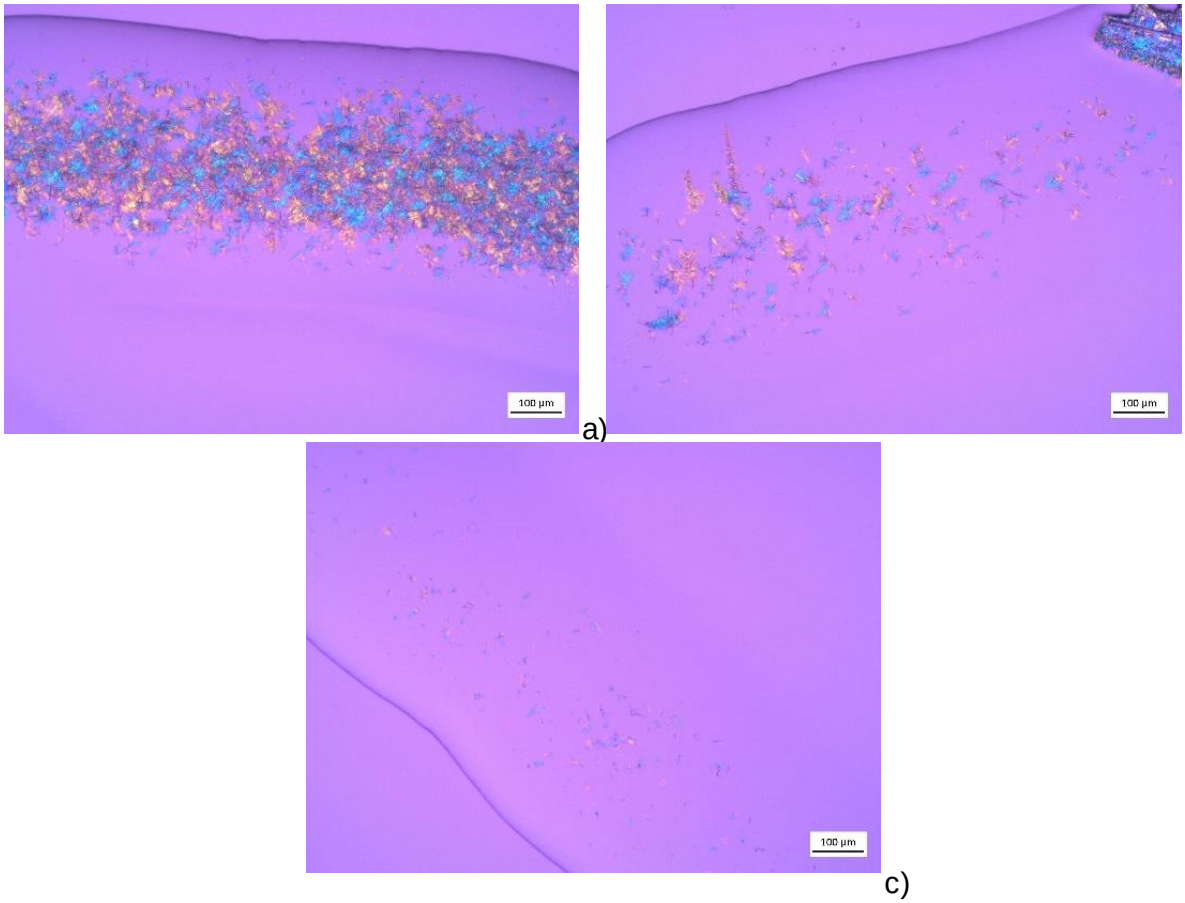
a)



b)

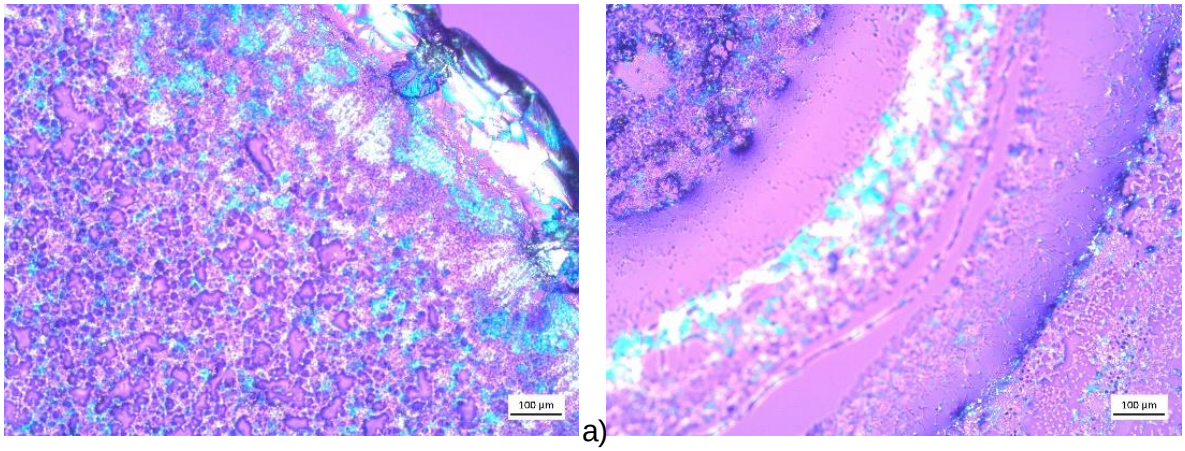
c)

Figure 4.3. Microscopical observation of the peripheric a) denser regions, b) regions with medium density of crystals and c) the central part with no visible crystals of 5% DM (w/w) loaded PLGA films using ethanol as a co-solvent, dried under the hood overnight.



a) b) c)

Figure 4.4. Microscopical observation of the peripheric a) denser regions, b) regions with medium density of crystals and c) more frequently, with less crystals, of 10% DM (w/w) loaded PLGA films using methanol as a co-solvent, dried under the hood overnight.



a) b)

Figure 4.5. Microscopical observation of the a) peripheric regions and b) streak regions and 10% DM (w/w) loaded PLGA films using ethanol as a co-solvent, dried under the hood overnight.

Replacing methanol with ethanol, as the co-solvent for dexamethasone, was an attempt to decrease crystal amount in the films. If the solubility of the drug in the solvent mixture was

lower, then, with an increased interfacial energy, the partitioning of the drug from solvent to PLGA matrix would be shifted into the direction of PLGA and therefore the drug loading would be higher. (96) Indeed, ethanol didn't dissolve the drug as well as methanol did. However, it led to bigger and more crystals, because the co-solvent's function was to keep the drug from crystallizing before solidification of PLGA, while chloroform evaporated, after casting. So, methanol continued to be the chosen solvent for dexamethasone.

4.1.3. Drying characterisation

When crystal growth was observed during approximately 1 hour since right after the films were casted, a clear evolution in size and number of crystals was observed during this first hour after film casting and also 7 and 24 hours after (Figures 4.6 – 4.9). Crystals were observed in all samples, with an increase in amount over time and with increasing dexamethasone concentration from 6% to 9% (w/w). After 1 hour, all samples were transparent and appeared to have dried over about 2 minutes, being that the centre seemed to dry before the periphery, which had an elevation/streak, whereas the centre was a plane, less thick surface. After 7 hours, white opaque spots were observed without the microscope. 24 hours later, the white spots had evolved to lines/streaks, being that the 6% (w/w) samples only had one white streak. The white spots and streaks that were observed without the microscope (Figure 4.1.b) correspond to the areas with higher concentration of crystals, observed under the microscope. In conclusion, opacity, thus crystals increase over time and it's usually observed in an outer streak and the centre of films is usually without any crystals. It is also noteworthy, that crystals also change colour and shape and that crystals were only seen in rare locations of the films.

The higher evaporation rate in the centre will determine a lower crystallization rate (Figures 4.6.d and e). In the edges the lower evaporation rate leads to a less homogeneously distributed viscosity, that forms folds over the film's surface, tendentially in the shape of streaks. These are the resultant edges or margins of deep channels or grooves. Here, there's higher interfacial tension than in smooth surfaces, which induces nucleation. In the centre of the films, where evaporation occurs faster, these channels are less deep and, subsequently their edges, streaks, are less sharp. (101)

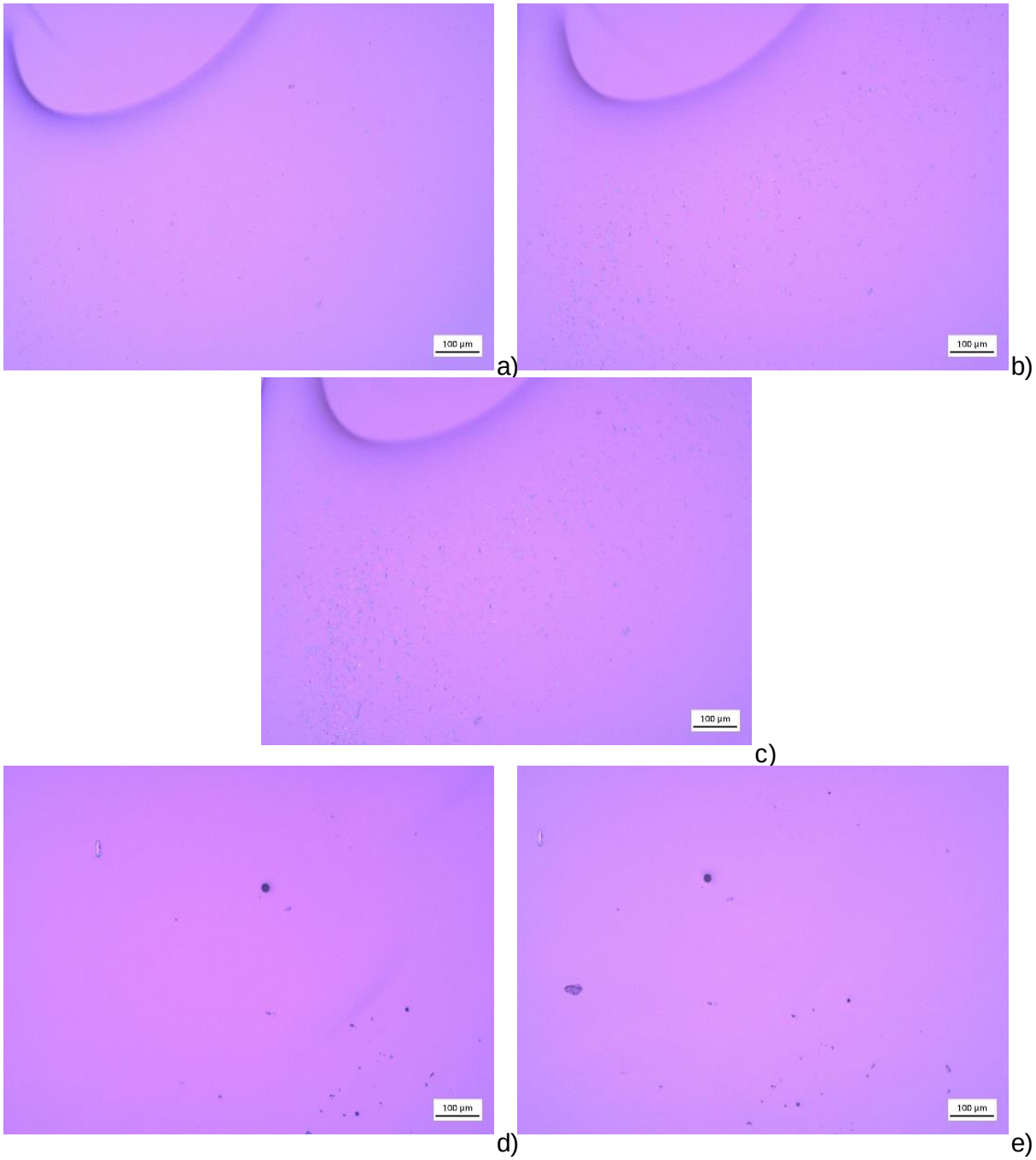
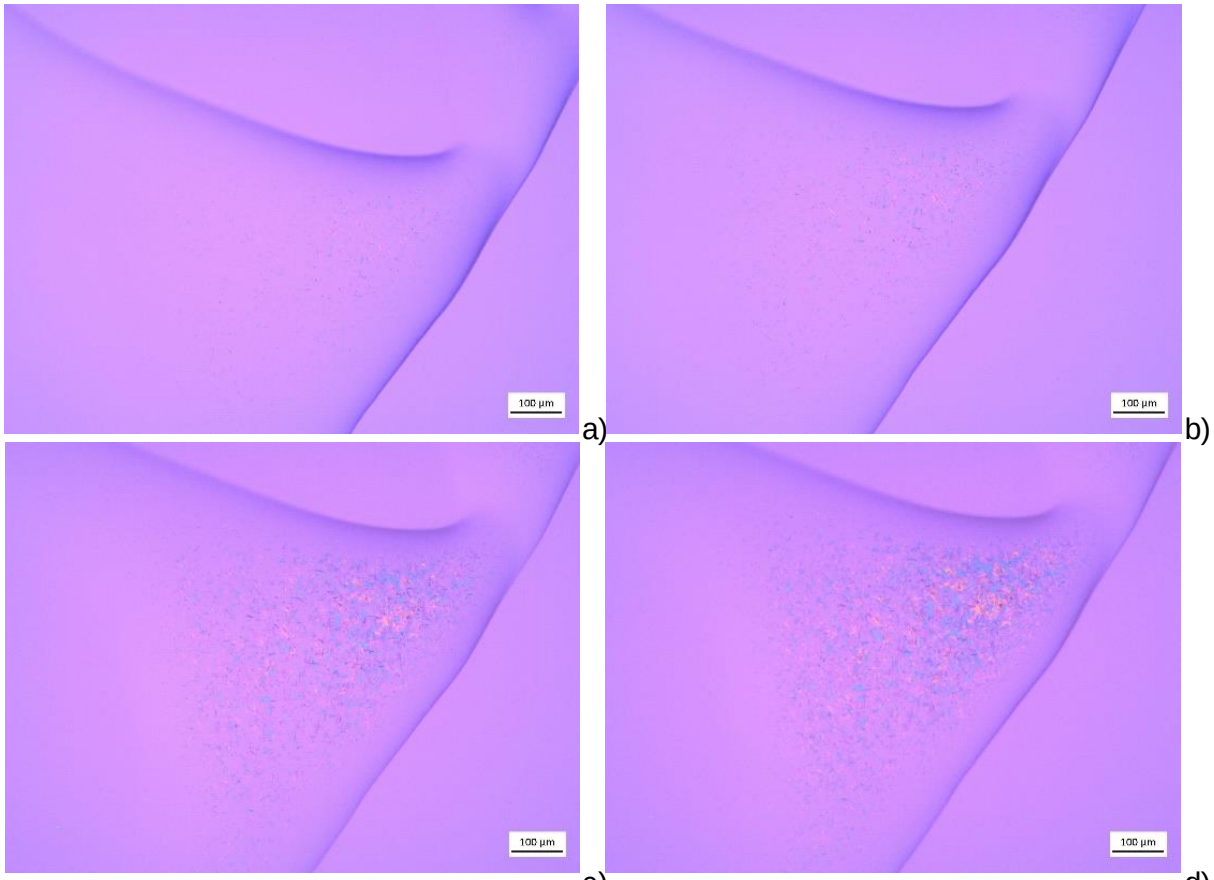
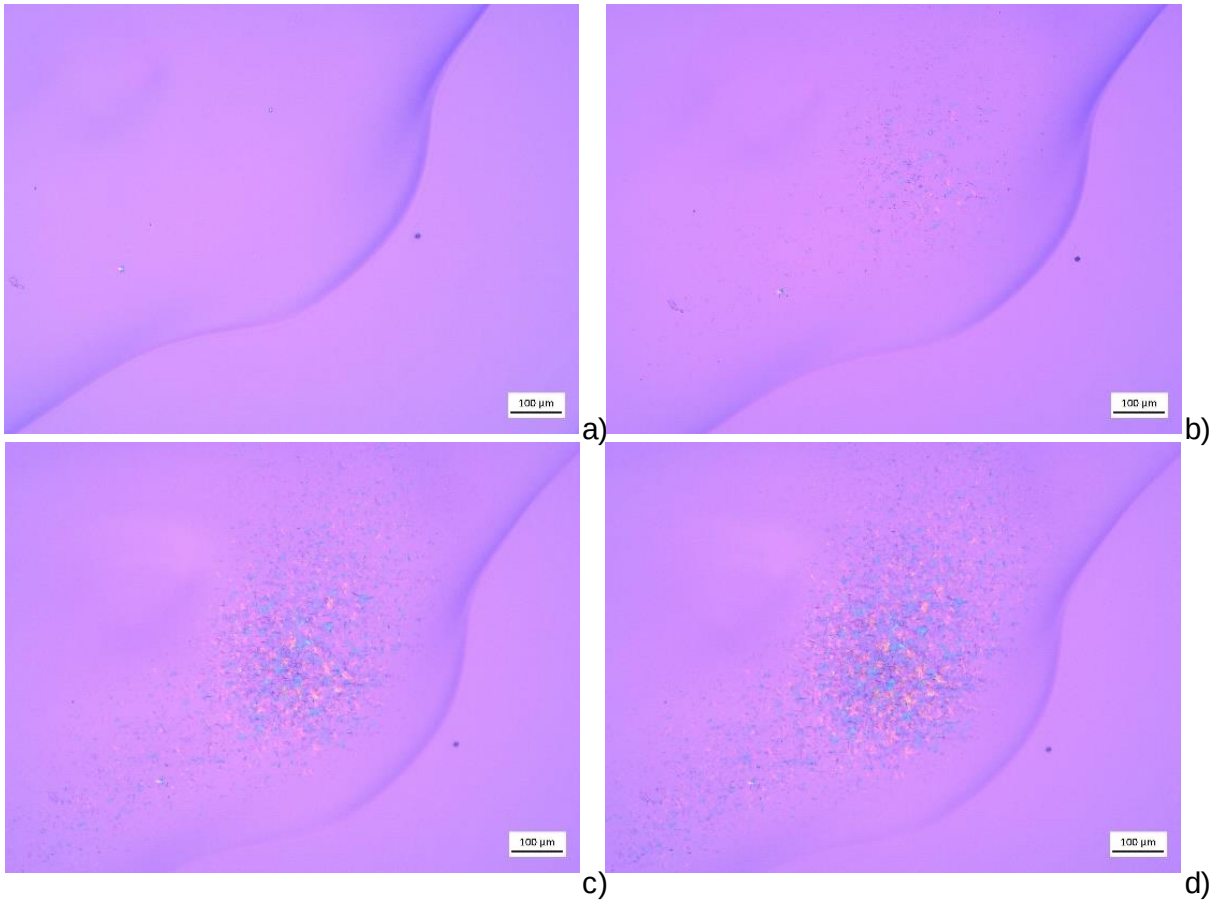


Figure 4.6. Microscopical observation of 6% DM (w/w) loaded films after a) ~1h, b) ~7h and c) ~24h and in the centre of the films, showing no changes, after d) ~15 min. and e) ~7h.



a) b) c) d)

Figure 4.7. Microscopical observation of 7% DM (w/w) loaded films after a) ~40 min., b) ~1h, c) ~7h and d) ~24h.



a) b) c) d)

Figure 4.8. Microscopical observation of 8% DM (w/w) loaded films after a) ~5 min., b) ~1h, c) ~7h and d) ~24h.

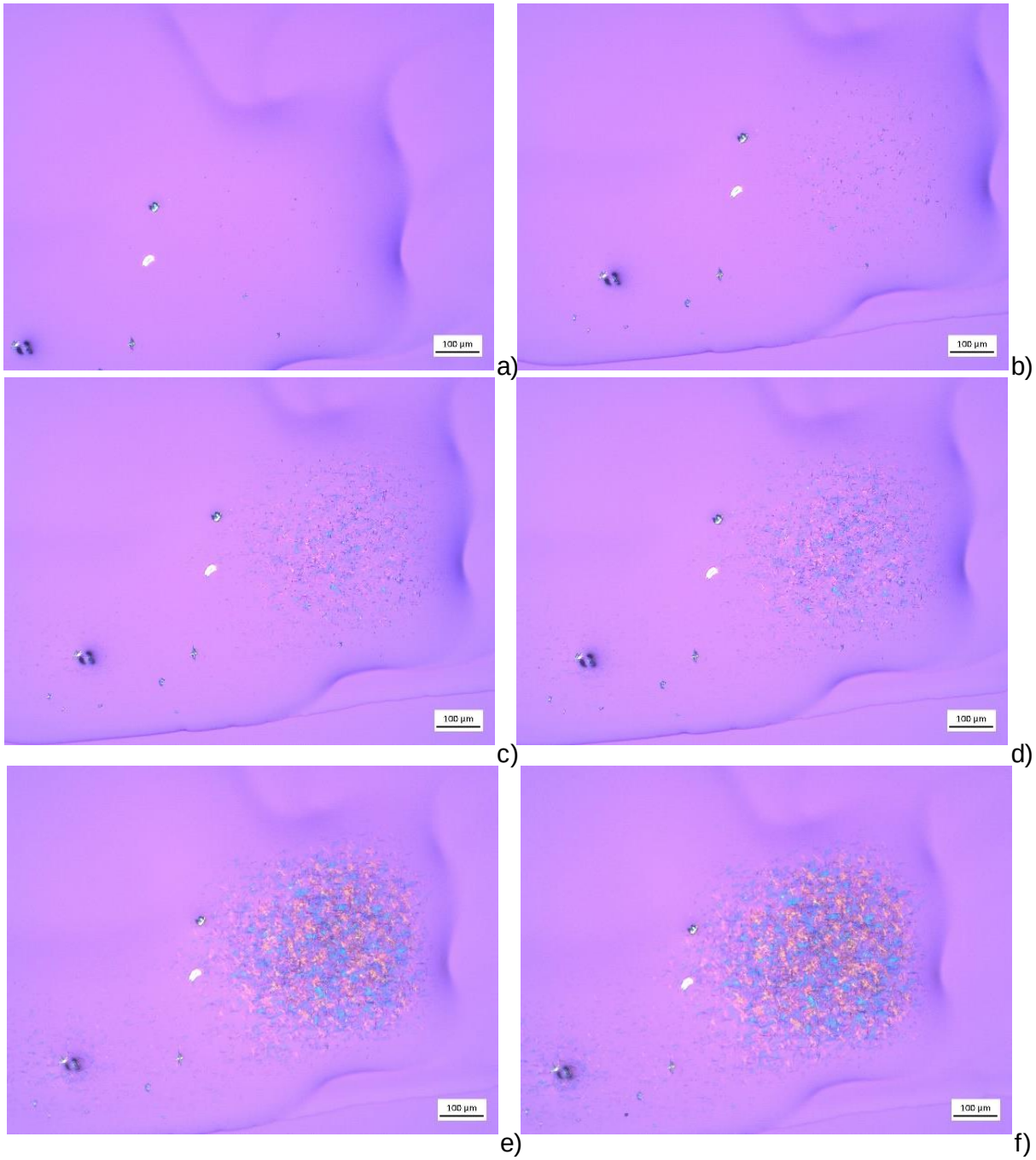


Figure 4.9. Microscopical observation of 9% DM (w/w) loaded films after a) ~5 min., b) ~20 min., c) ~40 min., d) ~1h, e) ~7h and f) ~24h.

4.1.4. Effect of drying on film solid state

In comparison to previous results, of films that dried under the hood overnight (Figure 4.10.a), those from the films that dried for 1 hour under vacuum (Figure 4.10.b), present a significant decrease in the amount of crystals that are observed under the microscope (Table 4.1). Only films with 9% and 10% of drug loading had a white streak in the periphery. In the first day, both 6% and 5% (w/w) of drug loading samples had almost no crystals.

Using the same mixtures as the ones that are mentioned in Tables 3.1 and 4.1, films were vacuum dried at 50ºC (Figure 4.10.d), which led to an increase of the amount of crystals in every drug load. The ones with the lowest drug load (5:95 DM:PLGA ratio) still showed a low amount of crystals, even so higher than when the vacuum oven was at room temperature. Facing these results, there was still another attempt at heating the films, placing them at 35ºC during vacuum drying, but once more the results indicated a dramatic increase of crystals' size and amount, not only under the microscope but also macroscopically, presenting more opaque streaks than previously. Figure 4.11. shows macroscopic results after 3 days of manufacturing vacuum dried films, with a clear increase of opacity, thus increase of crystal amount, when temperature was raised.

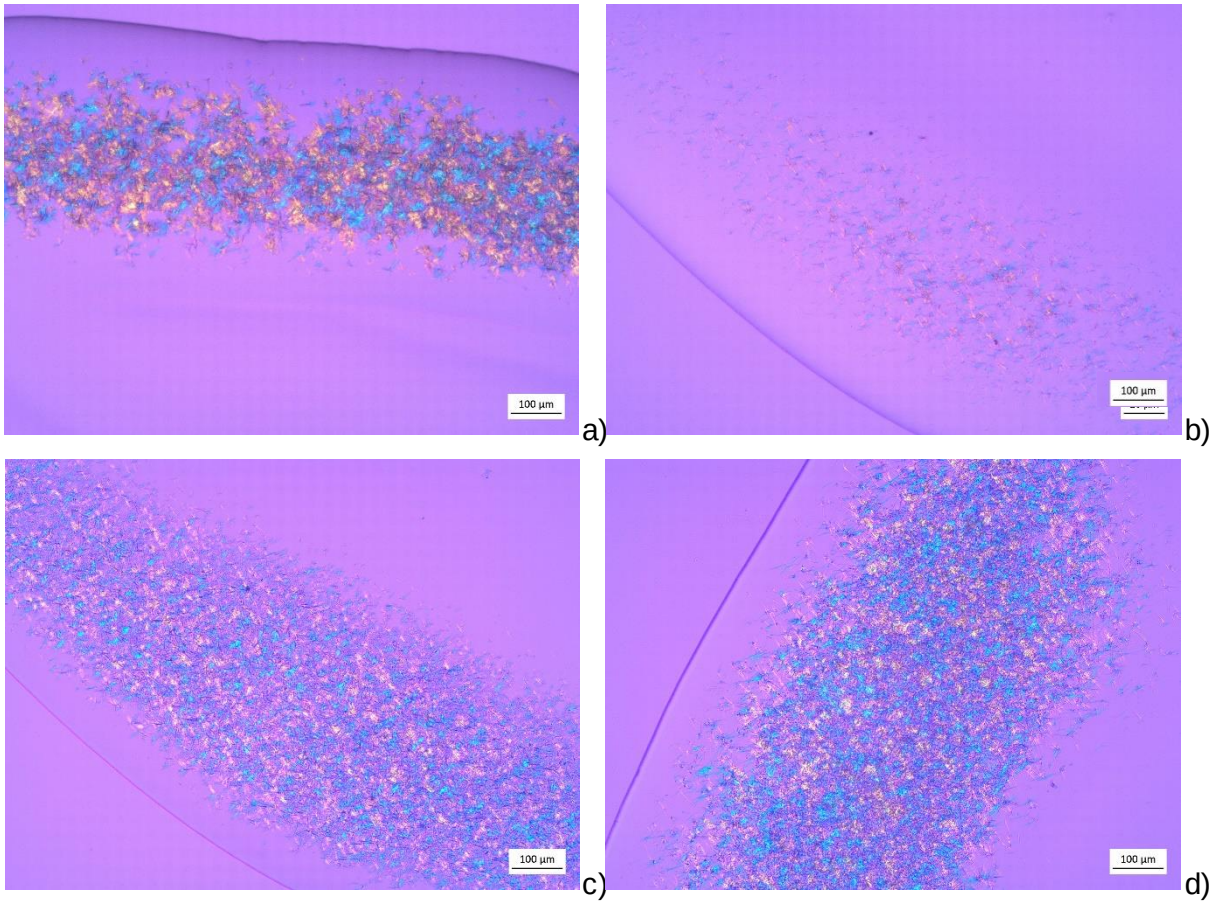


Figure 4.10. Worst case microscopical pictures of 10% DM (w/w) loaded films, a) dried under the hood overnight, b) vacuum dried for 1h, c) 3 days after vacuum drying and d) vacuum dried at 50ºC, for 1h.

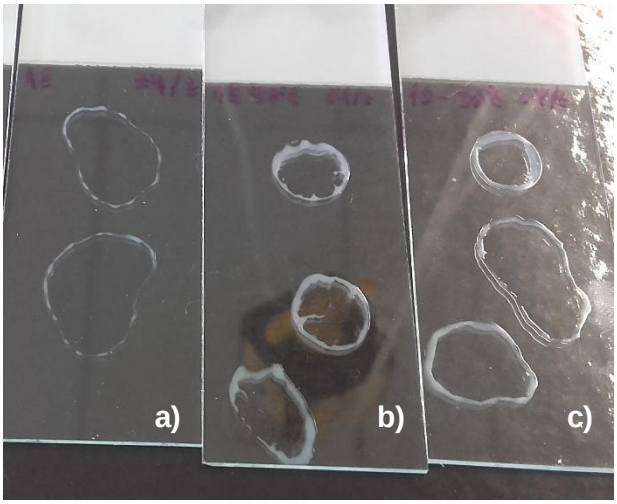


Figure 4.11. 10% DM (w/w) loaded films after 3 days of being casted that were vacuum dried for 1h at a) room temperature, b) 50ºC and c) 35ºC.

The fact that under vacuum, for 1 hour, crystal amount decreases immensely, could be explained by the fact that drug didn't have enough time to rearrange into a crystal lattice, due to the higher evaporation rate. Also, viscosity of the solvent is more homogenous, leading to a homogenous amorphous solidification of drug. (101) The increase of temperature during vacuum drying, as an attempt to reduce even more the drying time, didn't work, since crystals were bigger and more numerous. Although lower temperatures usually favour nucleation, higher temperatures provided for better conditions for crystal growth. (101) Besides, 50ºC, being above the Tg of PLGA, (103) led to higher molecular mobility of the polymer, thus facilitating nucleation. (94) However, most of the drug was dissolved in PLGA, since crystals only appear in very small amounts of the films. If the drug wasn't dissolved at all, crystals would be visible throughout the whole extension of the film.

4.1.5. Effect of solvent volume on film solid state

When the volume of solvents was increased, a 10% (w/w) of dexamethasone and 90% (w/w) of PLGA formulation of films was finally possible to obtain with almost no crystals. It should be taken into consideration that the amount of drug in the film was lower than the previous formulation, but the drug/PLGA ratio was the same, which was intended for the release tests. So, the masses of drug and PLGA in the final mixture were maintained constant, but the solvents' volumes were varied (Table 4.2), by altering the concentrations of the initial solutions. No crystals were observed, except for very rare isolated ones, when both the volume of methanol (5 mg DM/mL MeOH) and the volume of chloroform (6mg PLGA/mL CLF) were higher in the initial solutions and, thus, in the final mixture that was used to cast the films. Since there's more volume of solvent and less drug amount in each film, less crystals were visible. But also, the mixture is less saturated, so the drug stays more easily dissolved. Furthermore,

very low amount of crystals was also observed when only the volume of chloroform was increased, i.e. when only PLGA's initial solution was less concentrated (6mg PLGA/mL CLF). When only dexamethasone's solution was less concentrated (5 mg DM/mL MeOH), it resulted in completely opaque white films, that transposed to a very dark colour and amorphous content, under the microscope. This was due to the precipitation of PLGA, which has low solubility in methanol, the co-solvent. (104)

Table 4.2. Qualitative analysis of crystal amount in vacuum dried films, when solvents' volumes are changed.

Drug loading/%	Drug final mix/mg	PLGA/mg	Drug solvent/mL	PLGA solvent/mL	Crystals
10	5	45	0.2	1.5	1
10	5	45	0.2	7.5	0
10	5	45	1	1.5	1
10	5	45	1	7.5	0

4.1.6. Effect of casting technique on film solid state

Since crystals were observed in the edges of the films when these were casted using a micropipette, it was attempted to make films, which are as plane as possible, meaning they would fill the entire microscope slide. This was accomplished by means of a film casting knife, which spreads the solution uniformly throughout the casting surface, in a way that allows to obtain thinner films. Drug load was 10% (w/w), with 5 mg DM/1 mL MeOH and 45 mg PLGA/ 7.5 mL CLF, just like the formulation that showed almost no crystals in Table 4.2. The initial films were air dried, under the hood. Macroscopically, all films looked uniform and transparent, but, using the microscope, some crystals were observed in the edges of the films, which corresponded to the edges of the microscope slides. These crystals had different shapes, sizes and colours (Figure 4.12), when compared to the films that were produced using a micropipette. By vacuum drying such films, no crystals were observed, after being removed from the vacuum oven, nor did they have any crystals, one day later. This higher uniformity and better spreading of the casting mixture, resulted in a higher distribution of the drug throughout the film, thus lower supersaturation degree in a higher evaporation rate, all of which have contributed to a lower nucleation rate. (101) The better stability after 1 day of these vacuum dried films is due to the fact that there was a lower volume of casted mixture, because this casting knife allowed for thinner films, which led to a lower probability of occurring heterogenous nucleation, which is catalysed by the surface of impurities that, serving as

substrates, decrease the activation energy for the formation of nuclei that can grow into mature crystals. (94)

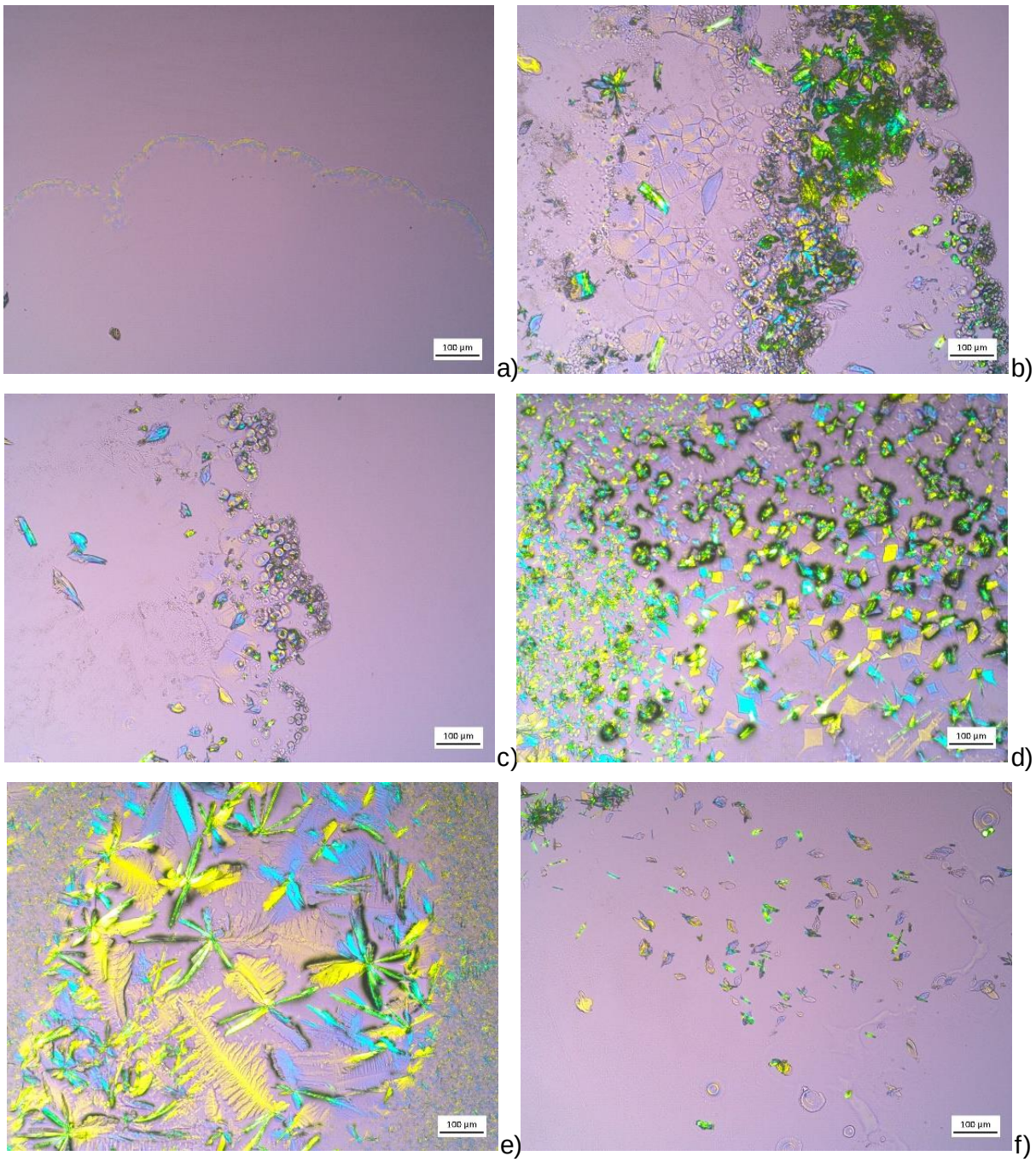


Figure 4.12. Microscopical observation of a variety of crystals (a, b, c, d, e, f) of 10% DM (w/w) loaded PLGA films, using a casting knife, dried under the hood overnight.

4.1.7. Stability of films

It has already been stated that vacuum drying (Figure 4.10.b) results in significantly lower amount of crystals, in comparison to drying the films under the hood overnight (Figure 4.10.a). However, after 3 days, the vacuum dried films (Figure 4.10.c) presented yet again the appearance of more crystals, with larger dimensions. Regarding the macroscopic observation,

only films with 9% of drug load had opaque streaks on the first day and 7% and 8% of drug load only showed this result on the third day.

Despite leading to a lower amount of crystals after 1 hour, increasing evaporation rate didn't assure the stability of this amorphous state, because it doesn't inhibit crystal growth, as much as inhibits nucleation. After the formation of nuclei, even in a dry matrix, there is potential for these small crystalline regions to start to grow into each other, which explains why crystals reappeared after 3 days, even at vacuum dried films. (101) Therefore, it was possible to create temporarily supersaturated systems, from which the drug then recrystallized over time, coming back to a 6% solubility, that existed in the films that were dried under the hood. For the intended release tests, this short-term stability would have been enough.

4.2. Assay

Based on the above-mentioned results, the formulation that originated less crystals, thus came closer to approaching a solid solution, was the one where both volumes of solvents were increased, maintaining a 10% drug/polymer ratio. Thus, new films were produced using 5 mg DM/1mL MeOH and 45 mg PLGA/7.5 mL CLF and dried under vacuum for 1 hour. To cast each film with a micropipette, 100 µL of the final mixture were used, resulting in a theoretical mass in each film of 5.882×10^{-2} mg DM. Since each sample was dissolved in 2.5 mL acetonitrile, the theoretical concentration of DM is 2.353×10^{-2} mg/mL. Therefore, upon obtained absorbance values, the mean concentration of dexamethasone in the acetonitrile solution, using Equation 4, was 2.279×10^{-2} mg/mL ± 2.222×10^{-3} (SD), which makes for a mass of dexamethasone per film of 5.698×10^{-2} mg (Equation 5). Its theoretical mass is 5.882×10^{-2} mg DM, so the relative error is 3.128×10^{-2}.

5. Conclusions

Dexamethasone is a drug with several therapeutic indications, namely inflammatory conditions. Associated with this variety of indications are various dosages and routes of administration. To optimize patient compliance and treatment effectiveness, there are several possible approaches, among them, DDS and more precisely, polymeric matrices, which have the advantage of controlling release rate and the location where this drug release happens.

Moreover, there are many different polymers to form these matrices, which can be considered, according to the desired characteristics, for each formulation. However, these polymers can sometimes be associated with disadvantages, such as toxicity, need for surgical removal, or even lack of clinical studies.

PLGA is a copolymer that is advantageous due to its biodegradability, whose non-toxic degradation products are naturally eliminated from the body, through metabolic routes, but also due to its extensive use, for several decades. (23) This copolymer also has the advantage of being very versatile, since it can be changed, according to what is intended, e.g. in terms of release rate. The release profile can be studied from dexamethasone loaded PLGA films, particularly the difference between the release profiles of solid solutions and solid non-molecular dispersions.

To achieve these solid solutions, several parameters can be modified in the preparation of the films. Although only drug loading of 5% (w/w) has been documented, (34) 10% (w/w) drug load has been achieved, in this work, for the short period of 1 day. This short-term stability would have been enough for the intended release tests. Although small amounts of crystals were usually observed, this didn't correspond to the most part of the drug, which wasn't visible by the microscope and was assumed to be dissolved. However, this observation was only supported by microscopical pictures, without the use of the described methods to characterise solid dispersions, like DSC or X-ray diffraction, thus remaining the question if there were any crystals that weren't visible with the microscope, in which case the classification of molecular dispersion wouldn't be correct. However, none of the previous methods are for sure better than microscopic observation, because X-ray diffraction method has low sensitivity and DSC resorts to high temperatures, which lead to uncontrolled changes in the samples during heating.

Among the changed parameters was drug/polymer ratio, which, being decreased, decreases the formation of crystals, ergo approaches the state of solid solution. The solubility of the drug in the co-solvent is also a factor that enhances the solid solution, since when using a co-solvent that dissolves the drug better, less crystals are observed, because while the main solvent evaporated and PLGA started to solidify, the drug would more easily stay in a dissolved state.

The drying process of films also has an impact on the amount of observed crystals. In fact, films dry from the centre to the periphery, where crystals are observed. Therefore, the higher the evaporation rate of the solvent, the lower the crystallization, i.e. the greater the probability of obtaining a solid solution. This was confirmed, when the evaporation rate was increased, by drying the films under vacuum, resulting in a minimization of the amount and size of crystals. However, it doesn't have a fundamental part in improving solid solution's stability, since it doesn't inhibit crystal growth, as much as it decreases nucleation rate. (101)

If the casted mixture is less saturated, by increasing solvent volume, there is a high decrease in crystals observed under the microscope, since nucleation rate increases with the degree of supersaturation. (94) This decrease is also due to the presence of a lower absolute drug amount in the films.

When the films were thinner and more plane, after resorting to a casting knife, due to the better distribution of the drug and a more uniform evaporation rate, no crystals were found, in vacuum dried films. After 1 day, there were no changes in these films due to the lower absolute amount of drug in each film, but also because crystal growth is reduced with the lower volume used, due to the presence of less impurities.

This stability is short term, because the amorphous state, having higher energy, tends to change to a crystalline state, reducing free energy. (94) Over time, crystals grow, by aggregating drug particles to the nuclei, formed during nucleation.

The development of the assay showed that acetonitrile is the best solvent of those analysed, since it allows for a more linear calibration curve. The films that were analysed had a drug amount close to the theoretical amount.

6. Recommendations for future work

The study of release tests from solid solutions consisting dexamethasone and PLGA was left undone, as well as the development of its method. Therefore, comparing release profiles of solid solutions with other dispersions might be interesting. Since this drug is practically insoluble in water, with a solid solution, it can be released already in a molecular state, upon erosion of polymer, which increases its release rate. However, if it has higher affinity in the polymer than in the release medium, drug release may be inhibited. This could be compared against a solid solution with another drug. Varying film thickness may affect polymer erosion rate, thus release rate.

References

1. Center for Drug Evaluation and Research (FDA). Quality Assessment [Internet]. Product Quality Review(s). 2017 [cited 2020 Sep 20]. p. 1–7. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2019/211379Orig1s000ChemR.pdf

2. Lipinski CA. Drug-like properties and the causes of poor solubility and poor permeability. J Pharmacol Toxicol Methods. 2000;44(1):235–49.

3. Villar J, Belda J, Añón JM, Blanco J, Pérez-Méndez L, Ferrando C, et al. Evaluating the efficacy of dexamethasone in the treatment of patients with persistent acute respiratory distress syndrome: Study protocol for a randomized controlled trial. Trials [Internet]. 2016;17(342):1–10. Available from: https://doi.org/10.1186/s13063-016-1456-4

4. National Center for Biotechnology Information. PubChem [Internet]. National Library of Medicine. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/5743

5. Johnson D, Lopez M, Kelley B. Dexamethasone [Internet]. Treasure Island (FL): StatPearls Publishing. 2020. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482130/

6. Nieman LK, Biller BMK, Findling JW, Newell-Price J, Savage MO, Stewart PM, et al. The diagnosis of Cushing's syndrome: An endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2008;93(5):1526–40.

7. L'espérance S, Vincent F, Gaudreault M, Ouellet JA, Li M, Tosikyan A, et al. Treatment of metastatic spinal cord compression: CEPO review and clinical recommendations. Curr Oncol. 2012;19(6):e478–90.

8. Drug Bank: Dexamethasone [Internet]. [cited 2020 Sep 20]. Available from: https://go.drugbank.com/drugs/DB01234

9. Low-cost dexamethasone reduces death by up to one third in hospitalised patients with severe respiratory complications of COVID-19 [Internet]. Oxford University News Release. 2020 [cited 2020 Sep 23]. p. 1–2. Available from: https://www.recoverytrial.net/files/recovery_dexamethasone_statement_160620_v2final.pdf

10. Food and Drug Administration (FDA). Highlights of Prescribing Information. Hemady [Internet]. [cited 2020 Sep 20]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/211379s000lbl.pdf

11. Food and Drug Administration (FDA). Dexamethasone Sodium Phosphate Injection,

USP [Internet]. 2014 [cited 2020 Sep 20]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/40572s002lbledt.pdf

12. Food and Drug Administration (FDA). Highlights of Prescribing Information. Ciprodex [Internet]. [cited 2020 Sep 20]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/021537s017lbl.pdf

13. Karti O, Saatci AO. Intravitreal Dexamethasone Implant in the Treatment of Non-Infectious Uveitic Macular Edema. Med hypothesis, Discov Innov Ophthalmol J [Internet]. 2018;7(4):169–75. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6229676/

14. London NJS, Chiang A, Haller JA. The dexamethasone drug delivery system: Indications and evidence. Adv Ther [Internet]. 2011;28:351–66. Available from: https://doi.org/10.1007/s12325-011-0019-z

15. Sinha VR, Trehan A. Biodegradable microspheres for protein delivery. J Control Release [Internet]. 2003;90(3):261–80. Available from: https://www.sciencedirect.com/science/article/pii/S0168365903001949?via%3Dihub

16. Huang CL, Steele TWJ, Widjaja E, Boey FYC, Venkatraman SS, Loo JSC. The influence of additives in modulating drug delivery and degradation of PLGA thin films. NPG Asia Mater [Internet]. 2013;5. Available from: https://doi.org/10.1038/am.2013.26

17. Manome Y, Kobayashi T, Mori M, Suzuki R, Funamizu N, Akiyama N, et al. Local delivery of doxorubicin for malignant glioma by a biodegradable PLGA polymer sheet. Anticancer Res [Internet]. 2006;26(5A):3317–26. Available from: https://pubmed.ncbi.nlm.nih.gov/17094447/

18. Elvira C, Gallardo A, San Roman J, Cifuentes A. Covalent polymer-drug conjugates. Molecules [Internet]. 2005;10(1):114–25. Available from: https://doi.org/10.3390/10010114

19. Vert M, Doi Y, Hellwich KH, Hess M, Hodge P, Kubisa P, et al. Terminology for biorelated polymers and applications (IUPAC recommendations 2012). Pure Appl Chem [Internet]. 2012;84(2):377–410. Available from: https://doi.org/10.1351/PAC-REC-10-12-04

20. Lopes CM, Costa P. Sistemas matriciais. In: Souto EB, Lopes CM, editors. Novas Formas Farmacêuticas para Administração de Fármacos. Porto: Edições Universidade Fernando Pessoa; 2012. p. 53–103.

21. Gumargalieva KZ, Moiseev Y V., Daurova TT, Voronkova OS, Rozanova IB. Polycaproamide degradation in rabbits and in several model media. Biomaterials

[Internet]. 1980;1(4):214–6. Available from: https://doi.org/10.1016/0142-9612(80)90020-4

22. Yang WW, Pierstorff E. Reservoir-based polymer drug delivery systems. J Lab Autom. 2012;17(1):50–8.

23. Rosen HB, Kohn J, Leong K, Langer R. Bioerodible polymers for controlled release systems. In: Hsieh D, editor. Controlled Release Systems: Fabrication Technology. CRC Press. Florida; 1988. p. 83–110.

24. Ekladious I, Colson YL, Grinstaff MW. Polymer–drug conjugate therapeutics: advances, insights and prospects. Nat Rev Drug Discov [Internet]. 2019;18:273–94. Available from: https://doi.org/10.1038/s41573-018-0005-0

25. Booth C, Gaspar HB. Pegademase bovine (PEG-ADA) for the treatment of infants and children with severe combined immunodeficiency (SCID). Biol Targets Ther [Internet]. 2009;3:349–58. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2726071/

26. Aulton ME, Taylor KMG. Aulton's pharmaceutics : the design and manufacture of medicines. 5th ed. London: Elsevier; 2018.

27. Fadda H, Khalili A, Khaw PT, Brocchini S. Ocular drug delivery. In: Aulton M, Taylor K, editors. Aulton's Pharmaceutics: The Design and Manufacture of Medicines. 5th ed. London: Elsevier; 2018. p. 690–713.

28. Nair LS, Laurencin CT. Biodegradable polymers as biomaterials. Prog Polym Sci. 2007;32(8–9):762–98.

29. Schwendeman SP, Shah RB, Bailey BA, Schwendeman AS. Injectable controlled release depots for large molecules. J Control Release. 2014;190:240–53.

30. Auras R, Harte B, Selke S. An overview of polylactides as packaging materials. Macromol Biosci. 2004;4(9):835–64.

31. Tekade RK. Basic fundamentals of drug delivery. 1st ed. Basic Fundamentals of Drug Delivery. 2018.

32. Lanao RPF, Jonker AM, Wolke JGC, Jansen JA, Van Hest JCM, Leeuwenburgh SCG. Physicochemical properties and applications of poly(lactic-co-glycolic acid) for use in bone regeneration. Tissue Eng - Part B Rev [Internet]. 2013;19(4):380–90. Available from: https://europepmc.org/article/med/23350707

33. Anderson JM, Shive MS. Biodegradation and biocompatibility of PLA and PLGA microspheres. Adv Drug Deliv Rev [Internet]. 1997;28(1):5–24. Available from:

https://doi.org/10.1016/s0169-409x(97)00048-3

34. Panyam J, William D, Dash A, Leslie-Pelecky D, Labhasetwar V. Solid-state solubility influences encapsulation and release of hydrophobic drugs from PLGA/PLA nanoparticles. J Pharm Sci. 2004;93(7):1804–14.

35. Fernández-Carballido A, Herrero-Vanrell R, Molina-Martínez IT, Pastoriza P. Biodegradable ibuprofen-loaded PLGA microspheres for intraarticular administration: Effect of Labrafil addition on release in vitro. Int J Pharm [Internet]. 2004;279(1–2):33–41. Available from: https://doi.org/10.1016/j.ijpharm.2004.04.003

36. Xu Q, Czernuszka JT. Controlled release of amoxicillin from hydroxyapatite-coated poly(lactic-co-glycolic acid) microspheres. J Control Release [Internet]. 2008;127(2):146–53. Available from: https://doi.org/10.1016/j.jconrel.2008.01.017

37. Pang J, Luan Y, Li F, Cai X, Du J, Li Z. Ibuprofen-loaded poly(lactic-co-glycolic acid) films for controlled drug release. Int J Nanomedicine. 2011;6:659–65.

38. Lu L, Garcia CA, Mikos AG. In vitro degradation of thin poly(DL-lactic-co-glycolic acid) films. J Biomed Mater Res. 1999;46(2):236–44.

39. Leelakanok N, Geary S, Salem A, Therapeutics E, City I. Fabrication and Use of PLGA-based Formulations Designed for Modified Release of 5-Fluorouracil. 2019;107(2):513–28.

40. Makadia HK, Siegel SJ. Poly Lactic-co-Glycolic Acid (PLGA) as biodegradable controlled drug delivery carrier. Polymers (Basel) [Internet]. 2011;3(3):1377–97. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3347861/

41. Hutchinson FG, Furr BJA. Biodegradable polymer systems for the sustained release of polypeptides. J Control Release [Internet]. 1990;13(2–3):279–94. Available from: https://doi.org/10.1016/0168-3659(90)90018-O

42. Danhier F, Ansorena E, Silva JM, Coco R, Breton A Le, Préat V. PLGA-based nanoparticles: An overview of biomedical applications. J Control Release [Internet]. 2012;161(2):505–22. Available from: https://doi.org/10.1016/j.jconrel.2012.01.043

43. Shibata A, Yada S, Terakawa M. Biodegradability of poly(lactic-co-glycolic acid) after femtosecond laser irradiation. Sci Rep [Internet]. 2016;6(27884):1–9. Available from: https://doi.org/10.1038/srep27884

44. Lu L, Mikos AG. The importance of new processing techniques in tissue engineering. MRS Bull [Internet]. 1996;21(11):28–32. Available from: https://doi.org/10.1557/s088376940003181x

45. Acharya G, Shin CS, Vedantham K, McDermott M, Rish T, Hansen K, et al. A study of drug release from homogeneous PLGA microstructures. J Control Release [Internet]. 2010;146(2):201–6. Available from: https://doi.org/10.1016/j.jconrel.2010.03.024

46. Shin M, Kim HK, Lee H. Dopamine-loaded poly(d,l-lactic-co-glycolic acid) microspheres: New strategy for encapsulating small hydrophilic drugs with high efficiency. Biotechnol Prog [Internet]. 2014;30(1):215–23. Available from: https://doi.org/10.1002/btpr.1835

47. Shih KS, Beatty CL. Blends of polycarbonate and poly(hexamethylene sebacate): II. Effect of molecular weight on compatibility. Polym Eng Sci [Internet]. 1987;27(20):1530–41. Available from: https://doi.org/10.1002/pen.760272008

48. Hilton JE, Summers MP. The effect of wetting agents on the dissolution of indomethacin solid dispersion systems. Int J Pharm [Internet]. 1986;31(1–2):157–64. Available from: https://doi.org/10.1016/0378-5173(86)90226-7

49. Tantishaiyakul V, Kaewnopparat N, Ingkatawornwong S. Properties of solid dispersions of piroxicam in polyvinylpyrrolidone. Int J Pharm [Internet]. 1999;181(2):143–51. Available from: https://doi.org/10.1016/s0378-5173(99)00070-8

50. Grizzi I, Garreau H, Li S, Vert M. Hydrolytic degradation of devices based on poly(dl-lactic acid) size-dependence. Biomaterials [Internet]. 1995;16(4):305–11. Available from: https://doi.org/10.1016/0142-9612(95)93258-F

51. Ito H, Minami A, Tanino H, Matsuno T. Fixation with poly-L-lactic acid screws in hip osteotomy: 68 hips followed for 18-46 months. Acta Orthop Scand [Internet]. 2002;73(1):60–4. Available from: https://doi.org/10.1080/000164702317281422

52. Lu L, Garcia CA, Mikos AG. Retinal pigment epithelium cell culture on thin biodegradable poly(DL-lactic-co-glycolic acid) films. J Biomater Sci Polym Ed. 1998;

53. Liu H, Lv P, Zhu Y, Wu H, Zhang K, Xu F, et al. Salidroside promotes peripheral nerve regeneration based on tissue engineering strategy using Schwann cells and PLGA: In vitro and in vivo. Sci Rep [Internet]. 2017;7(39869). Available from: https://doi.org/10.1038/srep39869

54. Carlo Reis EC, Borges APB, Araújo MVF, Mendes VC, Guan L, Davies JE. Periodontal regeneration using a bilayered PLGA/calcium phosphate construct. Biomaterials [Internet]. 2011;32(35):9244–53. Available from: https://doi.org/10.1016/j.biomaterials.2011.08.040

55. Ishaug SL, Crane GM, Miller MJ, Yasko AW, Yaszemski MJ, Mikos AG. Bone formation by three-dimensional stromal osteoblast culture in biodegradable polymer scaffolds. J Biomed Mater Res [Internet]. 1997;36(1):17–28. Available from:

https://doi.org/10.1002/(sici)1097-4636(199707)36:1%3C17::aid-jbm3%3E3.0.co;2-o

56. Sun X, Xu C, Wu G, Ye Q, Wang C. Review poly(lactic-co-glycolic acid): Applications and future prospects for periodontal tissue regeneration. Polymers (Basel) [Internet]. 2017;9(6):189. Available from: https://doi.org/10.3390/polym9060189

57. Noda K, Tanikawa R, Sugimura T, Kawasaki K, Kimura T, Izumi N, et al. Use of bioabsorbable plates for cranial fixation - Technical note. Neurol Med Chir (Tokyo) [Internet]. 2009;49(11):559–562. Available from: https://doi.org/10.2176/nmc.49.559

58. Eppley BL, Reilly M. Degradation characteristics of PLLA-PGA bone fixation devices. J Craniofac Surg [Internet]. 1997;8(2):116–20. Available from: https://doi.org/10.1097/00001665-199703000-00010

59. Infarmed. Infomed [Internet]. [cited 2020 Aug 25]. Available from: https://extranet.infarmed.pt/INFOMED-fo/index.xhtml

60. European Medicines Agency (EMA). Leuprorelin depot medicines: PRAC recommends new measures to avoid handling errors [Internet]. 2020 [cited 2020 Sep 17]. Available from: https://www.ema.europa.eu/en/news/leuprorelin-depot-medicines-prac-recommends-new-measures-avoid-handling-errors

61. Bouissou C, Rouse JJ, Price R, Van Der Walle CF. The influence of surfactant on PLGA microsphere glass transition and water sorption: Remodeling the surface morphology to attenuate the burst release. Pharm Res [Internet]. 2006;23(6):1295–305. Available from: https://doi.org/10.1007/s11095-006-0180-2

62. Kim HK, Chung HJ, Park TG. Biodegradable polymeric microspheres with “open/closed” pores for sustained release of human growth hormone. J Control Release [Internet]. 2006;112(2):167–74. Available from: https://doi.org/10.1016/j.jconrel.2006.02.004

63. Shigeru K, Yasuaki O, Minoru Y. Method of producing sustained-release microcapsules. PAT: EP0586238, 1993.

64. Jeyanthi R, Thanoo BC, Metha RC, DeLuca PP. Effect of solvent removal technique on the matrix characteristics of polylactide/glycolide microspheres for peptide delivery. J Control Release [Internet]. 1996;38(2–3):235–44. Available from: https://doi.org/10.1016/0168-3659(95)00125-5

65. Badri Viswanathan N, Thomas PA, Pandit JK, Kulkarni MG, Mashelkar RA. Preparation of non-porous microspheres with high entrapment efficiency of proteins by a (water-in-oil)-in-oil emulsion technique. J Control Release [Internet]. 1999;58(1):9–20. Available from: https://doi.org/10.1016/s0168-3659(98)00140-0

66. Dorta MJ, Oliva A, Munguía O, Llabrés M, Fariña JB. In-vitro release of fluoropyrimidines from PLGA film implants. J Pharm Pharmacol. 2002;54(6):757–63.

67. Göpferich A, Alonso MJ, Langer R. Development and Characterization of Microencapsulated Microspheres. Pharm Res An Off J Am Assoc Pharm Sci. 1994;II(11):1568–74.

68. Hinds KD, Campbell KM, Holland KM, Lewis DH, Piché CA, Schmidt PG. PEGylated insulin in PLGA microparticles. In vivo and in vitro analysis. J Control Release [Internet]. 2005;104(3):447–60. Available from: https://doi.org/10.1016/j.jconrel.2005.02.020

69. Haggag Y, Abdel-Wahab Y, Ojo O, Osman M, El-Gizawy S, El-Tanani M, et al. Preparation and in vivo evaluation of insulin-loaded biodegradable nanoparticles prepared from diblock copolymers of PLGA and PEG. Int J Pharm [Internet]. 2016;499(1–2):236–46. Available from: https://doi.org/10.1016/j.ijpharm.2015.12.063

70. Ramchandani M, Robinson D. In vitro and in vivo release of ciprofloxacin from PLGA 50:50 implants. J Control Release. 1998;54(2):167–75.

71. Boisdron-Celle M, Menei P, Benoit JP. Preparation and Characterization of 5-Fluorouracil-loaded Microparticles as Biodegradable Anticancer Drug Carriers. J Pharm Pharmacol. 1995;47(2):108–14.

72. Dash AK. Determination of the physical state of drug in microcapsule and microsphere formulations. J Microencapsul. 1997;14(1):101–12.

73. Chu KR, Lee E, Jeong SH, Park ES. Effect of particle size on the dissolution behaviors of poorly water-soluble drugs. Arch Pharm Res [Internet]. 2012;35(7):1187–95. Available from: https://doi.org/10.1007/s12272-012-0709-3

74. Shibata Y, Fujii M, Sugamura Y, Yoshikawa R, Fujimoto S, Nakanishi S, et al. The preparation of a solid dispersion powder of indomethacin with crospovidone using a twin-screw extruder or kneader. Int J Pharm. 2009;365(1–2):53–60.

75. Tran P, Pyo YC, Kim DH, Lee SE, Kim JK, Park JS. Overview of the manufacturing methods of solid dispersion technology for improving the solubility of poorly water-soluble drugs and application to anticancer drugs. Pharmaceutics [Internet]. 2019;11(3):132. Available from: https://doi.org/10.3390/pharmaceutics11030132

76. Chiou WL, Riegelman S. Pharmaceutical applications of solid dispersion systems. J Pharm Sci. 1971;60(9):1281–302.

77. Dhirendra K, Lewis S, Udupa N, Atin K. Solid dispersions: a review. Pak J Pharm Sci. 2009;22(2):234–46.

78. Ellenberger DJ, Miller DA, Williams RO. Expanding the Application and Formulation Space of Amorphous Solid Dispersions with KinetiSol®: a Review. AAPS PharmSciTech. 2018;19(5):1933–56.

79. Janssens S, Van den Mooter G. Review: physical chemistry of solid dispersions. J Pharm Pharmacol. 2009;(61):1571–86.

80. Vasconcelos T, Sarmento B, Costa P. Solid dispersions as strategy to improve oral bioavailability of poor water soluble drugs. Drug Discov Today [Internet]. 2007;12(23–24):1068–75. Available from: https://doi.org/10.1016/j.drudis.2007.09.005

81. Leuner C, Dressman J. Improving drug solubility for oral delivery using solid dispersions. Eur J Pharm Biopharm. 2000;50(1):47–60.

82. Lee T, Boersen N, Hui HW, Chow SF, Wan KY, Chow A. Delivery of Poorly Soluble Compounds by Amorphous Solid Dispersions. Curr Pharm Des. 2014;20(3):303–24.

83. Sridhar I, Doshi A, Joshi B, Wankhede V, Doshi J. Solid Dispersions: an Approach to Enhance Solubility of poorly Water Soluble Drug. J Sci Innov Res JSIR. 2013;2(3):685–94.

84. Theeuwes F, Hussain A, Higuchi T. Quantitative analytical method for determination of drugs dispersed in polymers using differential scanning calorimetry. J Pharm Sci [Internet]. 1974;63(3):427–9. Available from: https://doi.org/10.1002/jps.2600630325

85. Liu P, Yu L, Liu H, Chen L, Li L. Glass transition temperature of starch studied by a high-speed DSC. Carbohydr Polym. 2009;77(2):250–3.

86. Lipp R, Laurent H, Günther C, Riedl J, Esperling P, Täuber U. Prodrugs of gestodene for matrix-type transdermal drug delivery systems. Pharm Res [Internet]. 1998;15(9):1419–24. Available from: https://doi.org/10.1023/a:1011957822961

87. Lipp R. Selection and Use of Crystallization Inhibitors for Matrix-type Transdermal Drug-delivery Systems Containing Sex Steroids. J Pharm Pharmacol. 1998;(50):1343–9.

88. D'Addio SM, Saad W, Ansell SM, Squiers JJ, Adamson DH, Herrera-Alonso M, et al. Effects of block copolymer properties on nanocarrier protection from in vivo clearance. J Control Release. 2012;162(1):208–17.

89. Mendonsa N, Almutairy B, Kallakunta VR, Sarabu S, Thipsay P, Bandari S, et al. Manufacturing strategies to develop amorphous solid dispersions: An overview. J Drug Deliv Sci Technol [Internet]. 2020;55. Available from: https://doi.org/10.1016/j.jddst.2019.101459

90. Crowley MM, Zhang F, Repka MA, Thumma S, Upadhye SB, Battu SK, et al.

Pharmaceutical applications of hot-melt extrusion: Part I. Drug Dev Ind Pharm [Internet]. 2007;33(9):909–26. Available from: https://doi.org/10.1080/03639040701498759

91. De Mohac LM, Raimi-Abraham B, Caruana R, Gaetano G, Licciardi M. Multicomponent solid dispersion a new generation of solid dispersion produced by spray-drying. J Drug Deliv Sci Technol. 2020;57:1–6.

92. Fitriani L, Haqi A, Zaini E. Preparation and characterization of solid dispersion freeze-dried efavirenz - Polyvinylpyrrolidone K-30. J Adv Pharm Technol Res [Internet]. 2016;7(3):105–9. Available from: https://doi.org/10.4103/2231-4040.184592

93. Gránásy L, Pusztai T, Börzsönyi T, Tóth G, Tegze G, Warren JA, et al. Phase field theory of crystal nucleation and polycrystalline growth: A review. J Mater Res. 2006;21(2):309–19.

94. Rodríguez-Hornedo N, Murphy D. Significance of controlling crystallization mechanisms and kinetics in pharmaceutical systems. J Pharm Sci. 1999;88(7):651–60.

95. Baghel S, Cathcart H, O'Reilly NJ. Polymeric Amorphous Solid Dispersions: A Review of Amorphization, Crystallization, Stabilization, Solid-State Characterization, and Aqueous Solubilization of Biopharmaceutical Classification System Class II Drugs. J Pharm Sci. 2016;105(9):2527–44.

96. Maniruzzaman M, Boateng JS, Snowden MJ, Douroumis D. A Review of Hot-Melt Extrusion: Process Technology to Pharmaceutical Products. ISRN Pharm. 2012;

97. Karki S, Kim H, Na SJ, Shin D, Jo K, Lee J. Thin films as an emerging platform for drug delivery. Asian J Pharm Sci [Internet]. 2016;11(5):559–74. Available from: http://dx.doi.org/10.1016/j.ajps.2016.05.004

98. Dorta MJ, Santoveña A, Llabrés M, Fariña JB. Potential applications of PLGA film-implants in modulating in vitro drugs release. Int J Pharm. 2002;248(1–2):149–56.

99. Knill CJ, Kennedy JF, Mistry J, Miraftab M, Smart G, Groocock MR, et al. Alginate fibres modified with unhydrolysed and hydrolysed chitosans for wound dressings. Carbohydr Polym [Internet]. 2004;55(1):65–76. Available from: https://doi.org/10.1016/j.carbpol.2003.08.004

100. Zilberman M. Dexamethasone loaded bioresorbable films used in medical support devices: Structure, degradation, crystallinity and drug release. Acta Biomater. 2005;1(6):615–24.

101. Wu JX, Yang M, Berg F Van Den, Pajander J, Rades T, Rantanen J. Influence of solvent evaporation rate and formulation factors on solid dispersion physical stability. Eur J

Pharm Sci. 2011;44(5):610–20.

102. Brunacci N, Neffe AT, Wischke C, Naolou T, Nöchel U, Lendlein A. Oligodepsipeptide (nano)carriers: Computational design and analysis of enhanced drug loading. J Control Release. 2019;301:146–56.

103. Hausberger AG, DeLuca PP. Characterization of biodegradable poly(d,l-lactide-co-glycolide) polymers and microspheres. J Pharm Biomed Anal. 1995;13(6):747–60.

104. Huang W, Zhang C. Tuning the Size of Poly(lactic-co-glycolic Acid) (PLGA) Nanoparticles Fabricated by Nanoprecipitation. Biotechnol J [Internet]. 2018;13(1). Available from: https://doi.org/10.1002/biot.201700203