Universidade de Lisboa

Faculdade de Farmácia

Triamcinolone acetonide (TA) PLGA drug delivery systems (DDS)

Influence of surfactant addition

Mariana Filipa dos Santos Fernandes Dias

Trabalho de campo orientado pelo Professor Doutor Roland Bodmeier e pela aluna de doutoramento Neele Dietrich, da Freie Universität Berlin - Institut für Pharmazie, e coorientado pela Professora Doutora Helena Florindo, Professora Catedrática, da Faculdade de Farmácia da Universidade de Lisboa.

Mestrado Integrado em Ciências Farmacêuticas

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Trabalho Final de Mestrado Integrado em Ciências Farmacêuticas apresentado à Universidade de Lisboa através da Faculdade de Farmácia

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Abstract

Over the years, controlled drug delivery systems, including microparticles, have attracted considerable attention in the field of pharmaceutical research and development. This increased interest is due to their many advantages over conventional drug delivery systems, such as the ability to modulate drug release kinetics to meet the needs of a specific application, protection of drugs from rapid metabolism and elimination in the body, improved efficacy, and reduced toxicity. As these systems are released over time, they are particularly important in the treatment of chronic diseases, as the reduced frequency of injections will improve patient compliance.

The aim of this study was to evaluate the effect of surfactant incorporation (Tween 20, Tween 80 and Span 80) in triamcinolone acetonide poly(lactic-co-glycolic acid)-based microparticles and its impact on the encapsulation efficiency and drug release profile.

The microparticles (10% w/w and 30% w/w theoretical drug loading) were prepared using a solvent evaporation method (S/O/W emulsion technique) with dichloromethane as the dispersed phase. The microparticles were evaluated according to their macroscopic and microscopic appearance. Drug loading, encapsulation efficiency (%) and release rates were determined by UV-Vis analysis.

Our research findings suggest that the diameter of the 30% drug-loaded microparticles was larger compared to the one measured for 10% drug-loaded microparticles. Microparticles with 10% surfactants and without surfactants presented higher encapsulation efficiency in the case of Tween 80, followed by microparticles without surfactant, Span 80 and finally Tween 20. Microparticles with 10% drug loading presented higher encapsulation efficiency (lower initial unencapsulated drug) compared to those with 30% drug loading. For both 10% and 30% theoretical drug-loaded microparticles, the initial burst was higher for the non-additive microparticles and decreased with the addition of surfactants, resulting in the non-additive microparticles having the highest cumulative drug release.

In conclusion, the addition of surfactants does not appear to have a significant impact on the encapsulation efficiency of triamcinolone acetonide. The addition of surfactants resulted in a reduction in the burst effect and a decrease in the drug release rate, with no significant differences between the different surfactants studied.

Limitations should be considered when interpreting the results of this study, including the instability of triamcinolone acetonide in the release medium (phosphate-buffered saline, pH=7.4) and the fact that the drug release data were not corrected for its degradation products.

Keywords: PLGA Microparticles; Triamcinolone Acetonide; Surfactant; Controlled Parenteral Drug Delivery System.

Resumo

Ao longo dos anos, os sistemas de libertação controlada de fármacos, incluindo as micropartículas, têm atraído substancial atenção no âmbito da investigação e desenvolvimento farmacêutico. Este crescente interesse deve-se às suas inúmeras vantagens comparativamente aos sistemas convencionais utilizados na administração de fármacos, tais como a capacidade de ajustar a cinética de libertação de fármacos para atender requisitos clínicos específicos, a proteção dos fármacos contra o rápido metabolismo e eliminação no organismo, a melhoria da eficácia terapêutica e a redução da toxicidade. Como estes sistemas são libertados ao longo do tempo, são particularmente importantes no tratamento de doenças crónicas, uma vez que a frequência reduzida de injeções leva a uma melhor adesão à terapêutica por parte dos doentes.

Esta investigação teve como principal objetivo avaliar o impacto da adição de tensioativos (Tween 20, Tween 80 e Span 80) em micropartículas de poli(ácido lático-co-ácido glicólico) destinadas à libertação controlada de triamcinolona acetonida, bem como determinar o seu efeito na eficiência de encapsulação e no perfil de libertação do fármaco.

As micropartículas (com 10% e 30% de carga teórica de fármaco) foram preparadas utilizando um método de evaporação do solvente (emulsão S/O/W), no qual o diclorometano foi utilizado como fase dispersa. As micropartículas foram avaliadas de acordo com o seu aspeto macroscópico e microscópico, bem como a carga de fármaco, a eficiência de encapsulação e as taxas de libertação foram determinadas por análise UV-Vis.

Os resultados obtidos sugerem que o diâmetro das micropartículas com 30% de fármaco é maior comparativamente às micropartículas com 10% de fármaco. As micropartículas com 10% de tensioativos e sem tensioativos apresentam uma maior eficiência de encapsulação no caso do Tween 80, seguido das micropartículas sem tensioativos, Span 80 e, finalmente, Tween 20. As micropartículas com 10% de fármaco apresentam uma eficiência de encapsulação mais elevada (menos fármaco inicial não encapsulado) em comparação com as micropartículas com 30% de fármaco. Para as micropartículas com 10% e 30% de carga teórica de fármaco, a libertação inicial foi mais elevada para as micropartículas preparadas sem tensioativos, tendo sido menor na presença de tensioativos, o que faz com que as micropartículas sem estes excipientes tenham uma libertação cumulativa de fármaco final mais elevada.

Em conclusão, a adição de tensioativos não parece ter um impacto significativo na eficiência de encapsulação da triamcinolona acetonida. A adição de tensioativos resultou numa

diminuição da taxa de libertação do fármaco, sem diferenças significativas entre os diferentes tensioativos estudados.

Devem ser consideradas limitações na interpretação dos resultados deste estudo, incluindo a instabilidade da triamcinolona acetonida no meio de libertação (tampão fosfato salino, pH=7,4) e o facto de os dados de libertação do fármaco não terem sido corrigidos para os seus produtos de degradação.

Palavras-chave: Micropartículas de PLGA; Triamcinolona acetonida; Tensioativo; Sistemas de liberação controlada de fármacos.

Acknowledgements

I'm reaching the end of another chapter in my academic journey, which has been marked by many challenges that I overcome thanks to the support and guidance of many people. I would therefore like to thank all those who, in one way or another, have contributed to the completion of this final work.

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Abreviations

A/W – Acetonitrile:Water (1:1)

API – Active Pharmaceutical Ingredient

DCM – Dichloromethane

DDS – Drug delivery systems

DL – Drug loading

DR – Drug release

EE – Encapsulation efficiency

EMA – European Medicines Agency

FDA – Food and Drug Administration

HLB – Hydrolytic-lipophilic balance

HSP – Hansen Solubility Parameter

IUPAC – International Union of Pure and Applied Chemistry

MP – Microparticle(s)

Mw – Molecular weight

PBS – Phosphate-buffered saline

PCL – Polycaprolactone

PEG – Poly(ethylene glycol)

PGA – Poly(glycolic acid)

Ph.Eur – European Pharmacopoeia

PLA – Poly(lactic acid)

PLGA – Poly(lactide-co-glycolide)

PVA – Polyvinyl alcohol

TA – Triamcinolone acetonide

TEG – Tri(ethylene glycol)

USP – United States Pharmacopoeia

δd – Dispersion forces

δp – Polar interactions

δh – Hydrogen bonding forces

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1. Introduction

1.1 Controlled drug delivery systems for parenteral drug delivery

Conventional drug delivery systems (tablets, capsules, ointments, etc.) have some disadvantages, such as rapid metabolism and elimination of the drug in the body, poor bioavailability, fluctuations in plasma drug levels, and the need for multiple administrations. To overcome these problems and, above all, to achieve a constant release of the drug, controlled drug delivery systems have been developed. (1)

Biodegradable polymers have been used in a variety of controlled drug delivery systems (DDS). Examples of marketed controlled release DDS approved by the European and Medicines Agency (EMA) include Ozurdex®, used in eye disorders, Risperdal Consta® for the treatment of schizophrenia, Decapeptyl® indicated for female infertility, Bydureon® used in type 2 diabetes, Relistor® for the treatment of opioid-induced constipation, and cancer therapies such as Zoladex®, Lutrate Depot®, Sandostatin LAR®, Decapeptyl®, among others. (2) These medicines are formulated in microparticles (MP) or implants and provide controlled release for up to 6 months. As these systems are released over time, they are particularly important in the treatment of chronic diseases, as the reduced frequency of injections leads to improved patient compliance. (3)

Biodegradable polymers used for solid controlled DDS include poly(glycolic acid) (PGA), poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), polycaprolactone (PCL), tri(ethylene glycol) (TEG), poly(lactic acid)/poly(ethylene glycol) (PLA/PEG) and others, all of which are synthetic polymers. Synthetic biodegradable polymers have predictable and controlled degradation kinetics and can be precisely modified to exhibit a wide range of properties. (4,5)

The choice of the polymer depends on the desired mechanical properties and the intended degradation rate required in the application. There is also the possibility of using hybrid systems or polymer blends, e.g. PCL/PLGA, PEG/PLGA, among others. (6–9)

Unlike non-degradable polymers such as cellulose derivatives, silicones and others, biodegradable polymers degrade in the body, eliminating the need for surgical removal. (4,10)

1.2 Microparticles for controlled parenteral drug delivery

According to the recommendations of the International Union of Pure and Applied Chemistry (IUPAC), MP are particles with dimensions ranging from 0.1-100 μm. (11)

Depending on their structure, MP can be divided into two main groups: microcapsules, when there is a solid shell surrounding a core that can be solid, semisolid or liquid, or microspheres, when the drug is homogeneously distributed, as shown in Figure 1.1. (12,13)

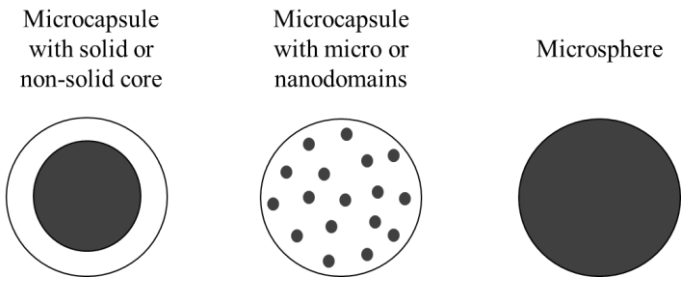


Figure 1.1 Different microparticle morphologies. [adapted from(14)]

Implants have the disadvantage that most manufacturing processes (hot-melt extrusion, injection moulding and melt compression) involve the application of high temperature and pressure conditions, which can lead to thermooxidative and thermomechanical degradation of some polymers and drugs, requiring particular care. (15) In addition, solid implants require minor surgery for administration, which can be associated with lower patient compliance. Non-biodegradable implants require two invasive procedures to insert and remove the implant. The advantage compared to MP is that the implant can be removed if the treatment must be stopped early, for example due to adverse effects. (16,17)

MP solve these problems as they can be injected with small syringes (e.g., 20-gauge needle) without the need for invasive procedures, increasing patient compliance. However, they have other disadvantages, such as the use of organic solvents in their manufacture and the care required to ensure that there are no residues in the final product. (18,19)

1.2.1 Poly(lactide-co-glycolide) as a biodegradable matrix

The Food and Drug Administration (FDA) has approved PLGA, a biocompatible and biodegradable copolymer, as a component of products used for several clinical applications,

including drug delivery systems such as MP, for diagnostics, tissue engineering and others. PLGA is a copolymer composed of two monomers, lactide and glycolide, and can be used to deliver a wide range of active ingredients, including small molecule drugs, proteins, peptides and nucleic acids. (20–22)

The degradation process of PLGA occurs upon contact with aqueous media through an autocatalytic phenomenon based on hydrolytic reactions of the ester linkages present in the copolymer backbone, resulting in a decrease in polymer molecular weight (Mw) but maintaining the size and shape of the matrix. This process causes a decrease in pH and may increase the osmotic pressure in the matrix. When the PLGA Mw reaches a threshold (1100 Da), it becomes water soluble and begins to diffuse, causing the pH of the surrounding medium to decrease and the matrix to collapse, known as bulk erosion, which is discussed in more detail in section 1.2.2. (23,24)

Polymer degradation and thus drug release rate from PLGA MP can be controlled by the polymer composition, Mw, drug type, medium pH, drug loading (DL), particle size, surface morphology, porosity and drug distribution. (25)

Regarding the polymer composition, an increase in the glycolic content leads to enhanced hydrophilicity of the polymer and therefore to a faster degradation rate. However, the copolymer with an equal ratio of lactide and glycolide monomers (50:50) is an exception as it shows the faster degradation. (26)

The size of the polymer chain is directly related to its Mw. In this sense, polymers with higher Mw take longer to degrade. (27)

The rate of drug release also depends on the type of drug and DL. In most cases, hydrophilic drugs are associated with greater water penetration and more porous matrices, leading to increased matrix degradation, whereas lipophilic drugs are more associated with delayed polymer degradation. (28,29)

In terms of medium pH, *in vitro* experiments suggest that polymer degradation is accelerated in both highly acidic and highly alkaline environments. (30)

1.2.2 Drug release from PLGA microparticles

The degradable polymers are characterised by controlled drug release through erosion processes. This erosion can be classified as surface erosion (heterogeneous) or bulk erosion (homogeneous). Surface erosion is characterised by a faster rate of polymer surface degradation

than water penetration into the matrix. The erosion therefore affects the surface layers without affecting the inner part of the polymer, hence the term heterogeneous. In the case of bulk erosion, the opposite is true, i.e., water penetration is faster than polymer degradation and erosion is therefore homogeneous, as shown in Figure 1.2. PLGA matrices are degraded via bulk erosion. (31,32)

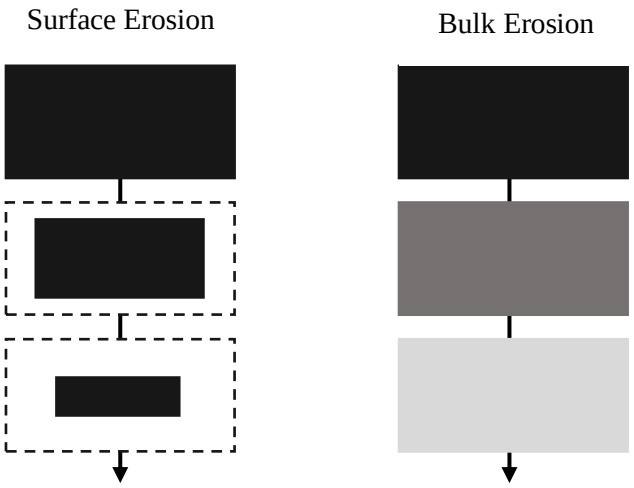


Figure 1.2 Schematic illustration of the surface and bulk eroding process. (31)

Although zero order release is the most desirable, drug release from PLGA MP is usually biphasic or triphasic due to heterogeneous degradation of the particle. In a typical triphasic release, phase I corresponds to the burst effect, an initial rapid release that occurs mainly due to the dissolution of drug particles with direct access to the microparticle surface or unencapsulated drug, phase II (or lag-phase) corresponds to a slow release phase mainly due to drug entrapped, solubility and diffusion through the polymeric systems , and finally phase III (or fast release) corresponds to a rapid release of the drug, that is usually associated with the onset of the erosion process. Figure 1.3 shows examples of different release profiles. (24,33,34)

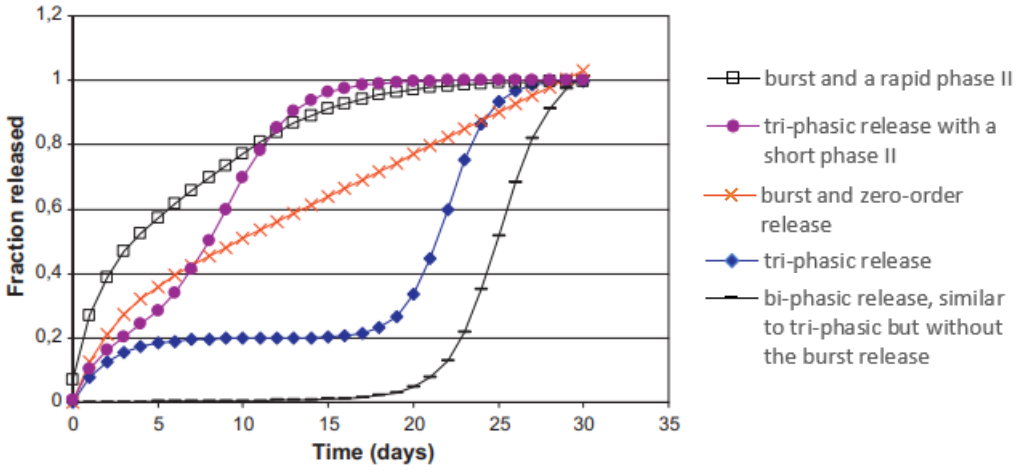


Figure 1.3 Representative graph of different release profiles with different phases. (24)

The encapsulation efficiency (EE) and drug release of hydrophobic drugs can be influenced by their solubility in polymers. The Hansen Solubility Parameter (HSP) is a widely used mechanism in drug delivery systems' research to evaluate the solubility of materials. This method assumes that "like dissolves like" and aims to measure the proximity between 2 substances defined by 3 parameters: dispersion forces (δd), polar interactions (δp) and hydrogen bonding forces (δh). It can be concluded that the closer the δ values, the greater the miscibility. (35)

Panyam J. *et al.* found that the solid-state solubility of dexamethasone in the polymer increased with higher lactide content in the polymer (however, 50:50 and 75:25 lactide:glycolide ratios showed almost similar solubilities) and with lower molecular weight. The solubility of the drug decreased in the presence of free acid end groups in the polymer. In general, polymers with higher solid-state solubility resulted in higher DL and lower cumulative *in vitro* drug release. (36)

1.2.3 Common preparation methods for PLGA microparticles

MP can be prepared by bottom-up methods (conventional synthesis), the most common being emulsion solvent evaporation, organic phase separation (coacervation) and spray drying, or by top-down methods, such as lithography, micro-moulding, and imprinting. This research will focus on bottom-up methods, which are the most widely studied and thus reported in the literature. (37,38)

Emulsification is the most used method to produce microspheres on a laboratory scale due to its simplicity and low cost. This process can involve a single emulsification method [water -

in-oil (W/O), oil-in-oil (O/O), oil-in-water (O/W)], or a double emulsification method [water-in-oil-in-water (W/O/W), water-in-oil-in-oil (W/O/O), oil-in-oil-in water (O/O/W), solid-in-oil-in-water (S/O/W), solid-in-oil-oil (S/O/O)].

In the case of this work, we have used a S/O/W process, which is widely discussed in the literature as being useful for the incorporation of drugs with low water solubility, such as in the case of TA. (39)

Solvent evaporation can be divided into 4 main phases. First, the active pharmaceutical ingredient (API) is dissolved, dispersed or emulsified in an organic solvent (e.g. dichloromethane, ethyl acetate or ethyl formate) containing the polymer that will constitute the matrix. The organic phase is then emulsified in an external phase called the continuous phase. This phase consists of a surfactant which stabilises the emulsion by reducing the surface tension of the continuous phase and preventing droplet coalescence. The surfactant can be anionic, cationic, amphoteric, or non-ionic. PVA (non-ionic) is one of the most used as it is known to produce smaller MP. The size of MP is also influenced by the stirring speed of this step. This is followed by solvent extraction/evaporation from the dispersed phase where the solidified MP are separated from the continuous phase by filtration or centrifugation. Finally, the MP are subjected to a drying process, which can be carried out either at room temperature, with heat, at low pressure or by lyophilisation. (38,40)

The method of organic phase separation (coacervation) can be simple or complex. Simple coacervation involves dissolving, dispersing, or emulsifying the API in an organic solvent containing the polymer and a suitable solvent. A non-solvent, also known as a coacervate (e.g. polydimethylsiloxane), is then added to induce liquid-liquid phase separation by electrostatic interactions, resulting in a polymer-rich, also known as the dispersed phase, and a polymer-poor, also known as the continuous phase. Finally, the coacervate droplets are hardened, dried, and sieved. Complex coacervation differs in that it involves the separation of a macromolecular solution in the presence of two oppositely charged macroions into two immiscible liquid phases. (41,42)

Spray drying is suitable for drying solutions, suspensions, or emulsions. This method can produce MP in a continuous one-step process, typically involving a sequence of 4 steps: atomisation through a nozzle, droplet mixing with dry gas, solvent volatilisation, and product separation. This method has the advantage of being a fast, reproducible, and scalable technology and is therefore widely used on an industrial scale. (43)

1.3 Influence of surfactant addition

Dinarvand R *et al.* prepared PLA-naltrexone HCl MP by W/O/W emulsification (solvent evaporation process) and found that the presence of emulsifier in W1 resulted in smaller particles with a denser and more uniform internal structure. The EE depends on the hydrolytic-lipophilic balance (HLB) of the surfactant. The highest EE was obtained with the addition of Span 80 because it has more lipophilic properties (lower HLB = 4.3) and therefore presents a greater affinity for stabilising W/O emulsions. Increasing the HLB value to 8 or 11 (Span 20 or Tween 85), and thus increasing the hydrophilic properties, resulted in a decrease in EE. While HLB values above 15 (Tween 80 or Tween 20) unexpectedly increased the EE, which could be attributed to the migration of these emulsifiers to the O/W2 interface and the modification of the surface properties of the MP. (44)

This study aims to characterise different release profiles by adding different surfactants (Tween 20, Tween 80 and Span 80) at different concentrations (1% w/w and 10% w/w).

Tween is the trade name for a group of polysorbate-based compounds. Tweens are non-ionic surfactants with amphiphilic properties, meaning that they have both hydrophilic (due to the PEG chain) and hydrophobic (due to the fatty acid chain) components in their molecular structure. The differences in chemical structure will influence the different levels of lipophilicity and hydrophilicity of each polysorbate, but all polysorbates are classified as hydrophilic emulsifiers and stabilisers. (45,46)

Tween-20 and Tween-80 are the most used biopharmaceuticals stabilisers for polymeric nanoparticles. These are recognised by the FDA and EMA as safe for parenteral administration when used within established limits and are known to be rapidly degraded by plasma esterases after administration. (47)

Tween 20 has lauric acid as its major fatty acid, making it a more hydrophilic molecule than Tween 80, which has oleic acid, a larger fatty acid. Tween 80 has a longer monounsaturated chain, making it more surface active with a lower CMC. This property makes Tween-80 the most widely used polysorbate for nanoparticle systems. (45,46)

Span 80 (sorbitan monooleate) is also a non-ionic surfactant with amphiphilic properties, but unlike polysorbates, it does not have the PEG chain. In this case, the surfactant is formed by the reaction of sorbitol with fatty acids, usually oleic acid, at high temperatures. Compared to Tween 20 and Tween 80, Span 80 has more hydrophobic properties. (48–50)

1.4 Triamcinolone Acetonide (TA)

Triamcinolone acetonide (TA) was used as a model drug in this study. TA ($C_{24}H_{31}FO_6$) is a synthetic glucocorticoid with a molecular weight of 434.5 g/mol, which has anti-inflammatory and immunosuppressive properties. (19,51)

According to the United States Pharmacopoeia (USP) and the European Pharmacopoeia (Ph.Eur), TA is practically insoluble in water and sparingly soluble in ethanol (96%), dehydrated alcohol, chloroform, methanol and dichloromethane. (52) TA presents a melting point temperature ranging between 292° and 294°C.

The solubility of TA in water is reported to be 0.0423 mg/mL in the DrugBank. (53) The solubility of TA in PBS (pH=7.4) was reported by Thakur A. et al. to be 0.02612 mg/mL. (54)

The solubility of TA in the polymer PLGA is not found in the literature. Given the structural similarity between TA and dexamethasone (similar corticosteroid backbone with a fluorine atom at position 9, but differ in that TA has an acetonide group, a cyclic ketal, and dexamethasone has a hydroxyl group -OH, as shown in Figure 1.4), it can be assumed that the behaviour of these substances may be similar.

The solubility of dexamethasone in the PLGA 503H polymer was calculated by solvent casting, a simple screening method, using polarised light micrography and was found to be < 2% (w/w). (55)

The mechanism of action of TA involves binding and activation of the glucocorticoid receptor (agonist activity) on DNA, leading to changes in gene expression. This results in the activation of the transcription of anti-inflammatory factors such as lipocortins, and inhibition of inflammatory transduction pathways by blocking the release of arachidonic acid and preventing the synthesis of prostaglandins and leukotrienes. (19,51)

It is most used topically to treat various skin conditions, but can also be administered by intralesional, intramuscular or intra-articular injection. (51)

1.5 Stability of triamcinolone acetonide in aqueous medium

In long-term drug release studies, drug degradation may occur in the release medium and such degradation may be a source of error in drug release quantification. 21-Hydroxycorticosteroids (e.g., dexamethasone, hydrocortisone, TA, etc.) are capable of degrading in aqueous solutions. Degradation (both oxidative and non-oxidative) is mainly due to reactions occurring in the C17-dihydroxyacetone side chain. (56)

Using an LC-MS/MS analytical method, Matter B. *et al.* found that dexamethasone can be degraded into 13 major degradation products in phosphate buffered saline (PBS) (pH 7.4) as a function of time, temperature, and exposure to light. This study showed that dexamethasone concentrations decreased as a function of time and temperature. (57) As TA and dexamethasone have similar structures (similar corticosteroid backbone with a fluorine atom at position 9, but differ in that TA has an acetonide group, a cyclic ketal, and dexamethasone has a hydroxyl group -OH, as shown in Figure 1.4), it can be assumed that similar degradation reactions may occur with TA in PBS, as suggested by some studies. (58,59)

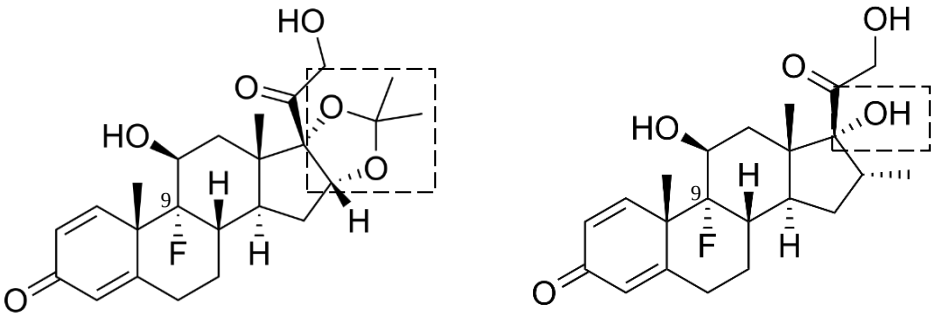


Figure 1.4 Comparison of the structure of triamcinolone acetonide and dexamethasone.

It should be noted that, even under similar incubation conditions, the distribution of degradation products differed between dexamethasone dissolved directly in PBS and dexamethasone formulated in PLGA implants as follows: degradation was lower in the case of dexamethasone-PLGA implants, indicating a protective effect of the drug by the implant. This suggests that the drug remains stable in the solid state prior to release and that most degradation occurs in solution following its release into the bulk medium. Despite the differences between PLGA microspheres and implant geometry, a similar effect would be expected when dexamethasone is formulated in MP. (57)

Regarding the experiments discussed so far, it would have been advisable to change the release medium to one that ensures the stability of the drug (simpler resolution). An alternative approach could have involved the identification of the degradation products through an appropriate method (e.g., LC–MS/MS), subsequently adjusting the *in vitro* drug release test to account for these degradation products, in accordance with best practices documented in the literature. (57,60)

1.6 Clinical impact of corticosteroids for intra-articular (IA) administration

The drug Zilretta®, approved by the FDA in October 2017, is the only PLGA microparticle formulation for extended release (ER) of TA currently on the market. In addition to TA, the FDA has approved the following corticosteroids for IA administration: methylprednisolone acetate, triamcinolone hexacetonide, betamethasone acetate, betamethasone sodium phosphate and dexamethasone sodium phosphate. (61)

The therapeutic effect of IA corticosteroids usually wanes within four weeks of administration. This suggests a relatively short-lived clinical benefit. (62) This short-term benefit is thought to be related to the rapid diffusion of the drug from the knee joint into the systemic circulation, leading to corticosteroid-related adverse effects, such as the transient increase in blood glucose levels that persists for 2.5-4 days postinjection in patients with type 2 diabetes mellitus. Zilretta® was approved to overcome this problem associated with conventional formulations of IA corticosteroids. (63,64)

1.7 Research Objectives

Considering the information described above, this work aims to discuss the formulation process and manufacturing technique of biodegradable drug-loaded MP, their characterisation, and associated challenges. Special attention is given to the influence of the addition of different surfactants (Tween 20, Tween 80 and Span 80) and their impact on the EE and drug release profile.

2. Materials and Methods

2.1 Materials

Triamcinolone Acetonide (TA); PLGA Resomer® 503H (acid terminated; lactide:glycolide 50:50; Mw 24,000-38,000); Tween 20 (Mw =1 227,54 g/mol); Tween 80 (Mw =1 310 g/mol); Span 80 (Mw = 428,6 g/mol); Dichloromethane (DCM); 0,25% Polyvinyl alcohol (PVA); Acetonitrile; pH 7.4 PBS.

2.2Methods

2.2.1 Preparation of microparticles by solvent evaporation – S/O/W method

Batches with 10% and 30% theoretical DL were prepared as described in Table 2.1. The amount of surfactants (%) was calculated considering the proportion with the polymer. The amount of drug (%) was adjusted based on the total weight of the MP.

Table 2.1 Quantitative and qualitative description of the batches prepared.

DL	Additive	Quantity	PLGA (mg)	Additive (mg)	TA (mg)	DCM (mL)
10% w/w	**No surfactant**		400	-	44.44	3
	Tween 20	**1% w/w**		4.04	44.89	
		10% w/w		44.44	49.38	
	Tween 80	**1% w/w**		4.04	44.89	
		10% w/w		44.44	49.38	
	Span 80	**1% w/w**		4.04	44.89	
		10% w/w		44.44	49.38	
30% w/w	**No surfactant**			-	171.42	
	Tween 80	**10% w/w**		44.44	190.48	
	Span 80	**10% w/w**		44.44	190.48	

DL = drug loading; TA = triamcinolone acetonide; DCM = Dichloromethane

The amount of TA and surfactant of each batch (Table 2.1) was dispersed in 400 mg of PLGA in 3 mL of DCM to form the dispersed phase (vortexed for 10 seconds). 2.5 mL of the dispersed phase was then emulsified at room temperature in an aqueous outer phase composed of 500 mL of 0.25% w/w PVA solution. The organic phase (DCM) was evaporated under stirring overnight (24 hours). Stirring was performed with a laboratory stirrer (Eurostar 20 digital IKA – WERKE) with three blades to create a turbulent flow at 500 rpm. The MP were separated by vacuum filtration, washed 4 times with 200 mL of deionised water and then dried under vacuum using a desiccator (24 hours; Room temperature).

2.2.2 Microscopic and macroscopic appearance

After 24 hours of drying, the dry samples were subjected to macroscopic and microscopic observation (Axioscope, Carl Zeiss Microcopy GmbH, Jena, Germany) at 10x magnification.

2.2.3 Determination of drug loading and encapsulation efficiency by UV-Vis

The actual DL was determined by dissolving 5 mg of MP (0.5 mg TA 10% DL; 1.5 mg TA 30% DL) in 75 mL and 200 mL of pure acetonitrile, respectively, followed by 15 minutes in an ultrasonic bath and the addition of 75 mL and 200 mL of water, respectively, to obtain acetonitrile:water (A/W) (1:1) – total 150 mL 10% DL MP and 400 mL 30% DL MP. The absorbance of the solution was then measured by UV-Vis spectroscopy at 243 nm. Concentrations were calculated using previously established standard curves (Appendix A2).

The actual DL was calculated using the following equation:

$$DL_{act}\ (\%) = \frac{c*V}{m_{MP}} * 100 \qquad (1)$$

With DL_{act} = actual drug loading; c = concentration of TA in the solution; V = volume of the MP dilution; m_{MP}= weight of MP under test.

The theoretical DL was calculated by the following equation:

$$DL_{theo}(\%) = \frac{m_{TA}}{m_{TA}+m_{PLGA}+m_{additive}} * 100 \quad (2)$$

With DL_{theo}= theoretical drug loading; m_{TA}= weight of TA; m_{PLGA}= weight of PLGA; $m_{additive}$= weight of surfactant.

The EE was calculated by the following equation:

$$EE(\%) = \frac{DL_{act}}{DL_{theo}} * 100 \quad (3)$$

With EE = encapsulation efficiency; DL_{act} = actual drug loading; DL_{theo} = theoretical drug loading.

2.2.4 Determination of drug release from microparticles

Approximately, 10 mg of MP were accurately weighed and placed in amber vials containing 100 mL (10% DL) and 250 mL (30% DL) of dissolution medium (PBS pH 7.4) to obtain sink conditions (concentration less than 30 % of the saturation solubility in the release medium). Vials were incubated on a horizontal shaker at 37°C and 80 rpm. At pre-determined time-points (3 hours; 24 hours; 48 hours…), 10 mL of releasing medium was removed using a needle filter and replaced with an equal volume of receiving medium. Quantification of the drug concentration was performed by measuring the UV-Vis absorption at 243 nm, using previously established standard curves (Appendix A1). Determination of drug release was conducted in triplicate.

3. Results and Discussion

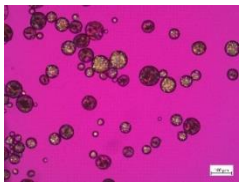
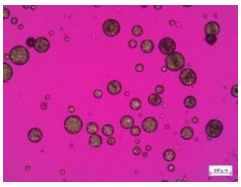
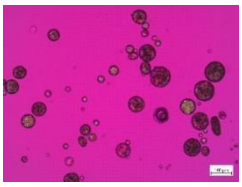
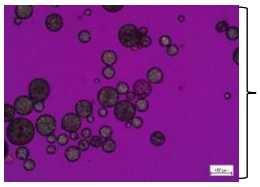
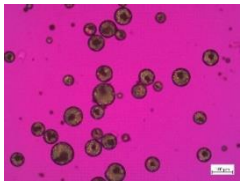
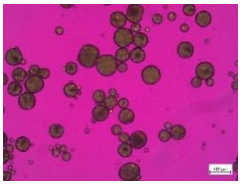
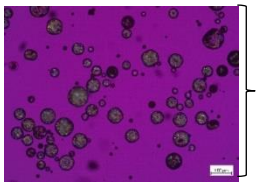
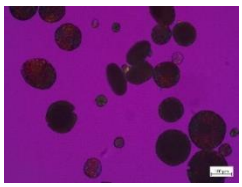
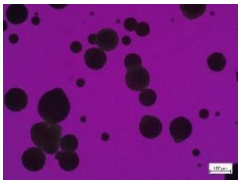
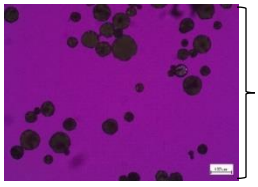
It should be noted that the addition of 1% surfactants corresponds to a very small amount of TA (approximately 4.04 mg TA in 448.93 mg total MP produced), which is more likely to be related to errors in the weighing process, losses in the microparticle manufacturing process and consequently in the final results. Therefore, results related to MP with 1% surfactants are associated with a higher probability of error.

3.1 Surface morphology and particle size distribution by optical microscopy

After 24 hours of drying, the dry samples were subjected to macroscopic observation, where MP appeared as a white powder with a uniform appearance, with no apparent differences between them.

Microscopic analysis is described in Table 3.1, samples were analysed using a 10x objective with each scale unit equal to 100 μm.

Table 3.1 Morphology analysis of the microparticles after drying (10x magnification).

DL	No surfactant	Tween 20	Tween 80	Span 80
10%				 1%
				 10%
30%				 10%

As expected, the diameter of the 30% drug-loaded MP was greater, with average values of 125 μm, compared to the 10% DL, with average values of 75 μm (Table 3.1).

The variation noted in the background colour of MP with 10% DL and surfactants Tween 20 and Tween 80 (appearing lighter) compared to MP with 10% DL and Span 80 and

30% DL (appearing darker) may be potentially attributed to discrepancies in the microscope parameters, including adjustments to illumination intensity and contrast settings (Table 3.1).

3.2 Drug loading and encapsulation efficiency

MP with 10% surfactants and without surfactants presented higher encapsulation efficiency in the case of Tween 80 (10% DL MP, EE (%) = 114%; 30% DL MP, EE (%) = 92%), followed by MP without surfactant (10% DL MP, EE (%) = 95%; 30% DL MP, EE (%) = 83%), Span 80 (10% DL MP, EE(%) = 93%; 30% DL MP, EE (%) = 79%) and finally Tween 20 (10% DL MP, EE(%) = 90%) (Table 3.2).

The EE (%) of MP with 10% DL is higher compared to those with 30% DL (No surfactant: 95 > 83; Tween 80: 114 > 92; Span 80: 93 > 79) (Table 3.2).

Table 3.2 Drug loading and encapsulation efficiency values of all batches produced.

DL	Additive		DLtheo (%)	DLact (%)	EE (%)
10% w/w	No surfactant		9.96	9.49	95.25
	Tween 20	1% w/w	9.86	9.56	96.90
		10% w/w	10.05	9.04	89.88
	Tween 80	1% w/w	9.57	9.42	98.48
		10% w/w	10.00	11.42	114.21
	Span 80	1% w/w	9.99	8.96	89.70
		10% w/w	9.99	9.28	92.93
30% w/w	No surfactant		29.97	24.77	82.65
	Tween 80	10% w/w	29.88	27.36	91.55
	Span 80	10% w/w	29.89	23.56	78.82

The high DL (30%) typically results in a lower EE due to a high concentration gradient causing the drug to diffuse out of the polymer into the external medium, limited solubility of the drug in the polymer, drug aggregation or loss of stability.

The EE depends on the hydrolytic-lipophilic properties of the surfactant. Tween 80 seems to be the one with the highest EE (%) value, which could be attributed to the to the migration of the surfactant to the O/W2 interface, modification of the surface properties of the

MP, and to the fact that it is the surfactant more surface active with a lower critical micelle concentration.

By preparing solutions of the surfactants in A/W (1:1) at the same concentration as the surfactants in the MP analysed in the DL and EE test (as discussed in section 2.2.3), it was confirmed by UV-Vis spectroscopy at 243 nm that Tween 20 and 80 are not absorbed in A/W, and therefore the values given in Table 3.2 concern the encapsulation of the active substance without interference from the surfactants. It remains to be verified whether Span 80 absorbs in the same solvent and at the same wavelength, since this test was not carried out due to lack of time and no information on this subject was found in the literature.

Since the EE (%) of the MP (10% DL; 10% Tween 80) exceeded 100% (114%), the test should be repeated to identify potential sources of error that may have been made. These may include errors in the sample preparation and handling, as well as in the measurements made.

3.3 Drug Release

3.3.1 Drug release profile of 10% TA MP (RG 503H)

Drug release from the 10% TA MP was quantified at specific time intervals over a period of 21 days, focusing on the period up to 7 days to facilitate the analysis of the initial drug release (burst). The study could not be extended further due to time constraints.

After 7 days of testing, for MP with 1% surfactants, the drug release (DR) was higher in MP prepared using Tween 80 (DR (%) = 24%; DLact (%) = 9%) followed by those with Tween 20 (DR (%) = 20%; DLact (%) = 10%) and finally Span 80 (DR (%) = 18%; DLact (%) = 9%). However, at 10% of surfactants, the release was still higher with Tween 80 (DR (%) = 32%; DLact (%) = 11%), followed by Span 80 (DR (%) = 26%; DLact (%) = 9%) and only then Tween 20 (DR (%) = 16%; DLact (%) = 9%), (Figure 3.1, b).

After 21 days of testing, the cumulative drug release was 87% for MP without surfactant (DLact (%) = 9%), 60% for MP with Tween 80 (10%), 45% for MP with Tween 80 (1%), 43% for MP with Tween 20 (1%), and 40% for MP with Tween 20 (10%) (Figure 3.1, a).

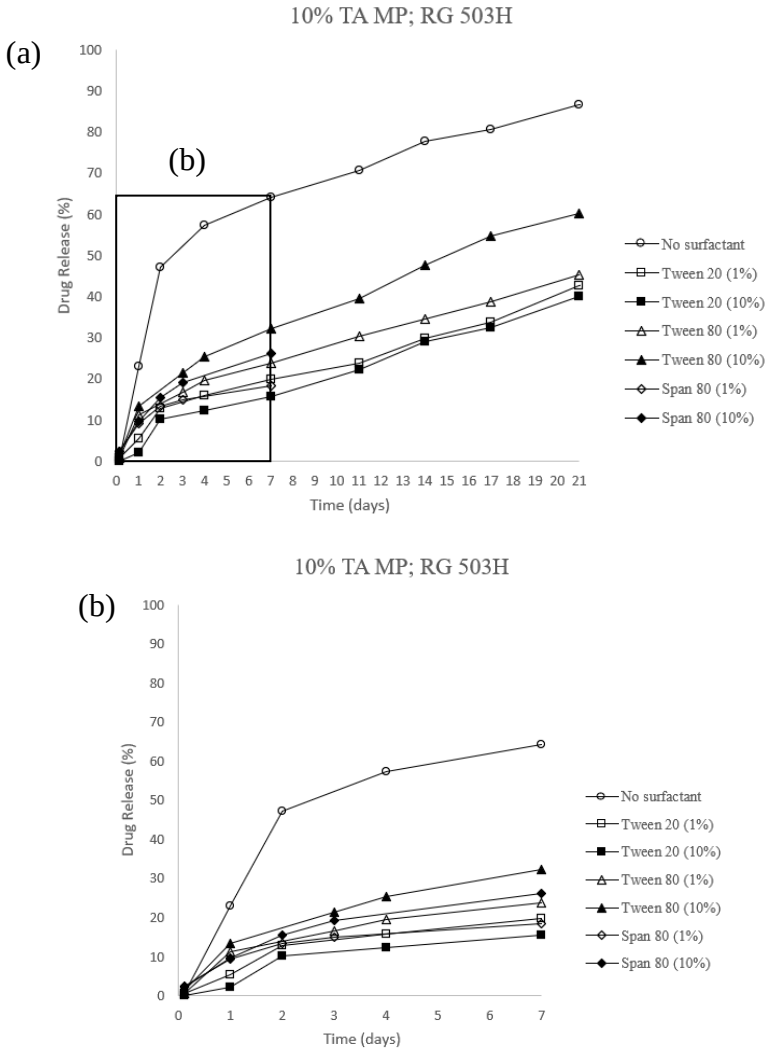


Figure 3.1 Comparison of the drug release profile of 10% TA MP, RG 503H between microparticles without surfactant, and with surfactants Tween 80, Tween 20 and Span 80. Release over: (a) 21 days and (b) 7 days.

Drug release and initial burst was higher in MP without surfactant and decreased with the addition of Tween 20, Tween 80 and Span 80 (Figure 3.1).

The initial burst release is inversely related to the water solubility of the drug. This can be explained by the fact that in the microparticle manufacturing process, during the solvent evaporation step to form solid drug/PLGA particles, drug molecules migrate with the solvent

to the surface, and the extent of drug migration is expected to be higher for more hydrophobic drugs (MP without surfactant), resulting in higher surface accumulation, which in turn results in higher burst release. The addition of surfactant increases the water solubility of TA and consequently less drug migrates to the surface resulting in a delayed release compared to MP without surfactant as shown in Figure 3.1.

The experimental value of the solubility of TA in the release medium PBS (pH= 7.4) was calculated at the Institute, but it was not possible to determine a value as TA was found to be unstable in the release medium (data not shown).

3.3.2 Drug release profile of 30% TA MP (RG 503H)

Drug release from the 30% TA MP was quantified at specific time intervals over a period of 7 days. The study could not be extended further due to time constraints.

After 7 days of testing, the cumulative drug release was higher in the case of MP without surfactant (DR (%) = 131%; DLact (%) = 25%) followed by MP with Span 80 (10%) (DR (%) = 112%; DLact (%) =24%) and finally MP with Tween 80 (10%) (DR (%) = 92%; DLact (%) = 27%) (Figure 3.2).

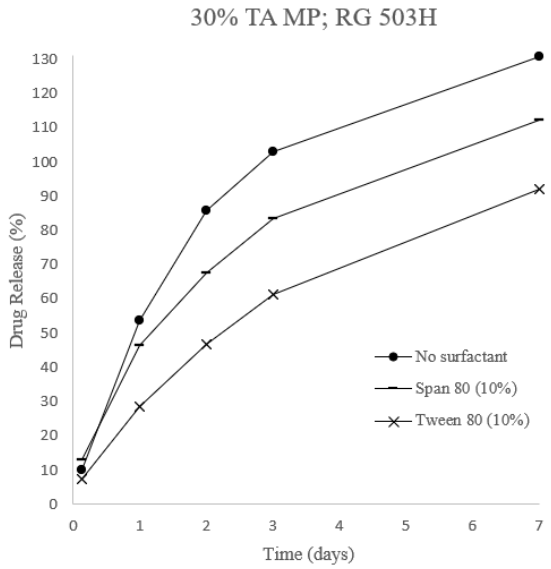


Figure 3.2 Comparison of the drug release profile of 30% TA MP (RG 503H) without surfactant, and with surfactants Tween 80 and Span 80.

MP without surfactant and with Span 80 (10%) showed a cumulative drug release higher than 100% and are therefore associated with an error. The test should be repeated to identify

potential sources of error that may have been made. These may include errors in the sample preparation and handling, as well as in the measurements made.

All the 30% drug-loaded batches produced (No surfactant, Span 80 (10%) and Tween 80 (10%)) presented similar release rates as the release is parallel (similar slopes) (Figure 3.2).

The differences observed in the cumulative drug release obtained for MP prepared with surfactants can be explained by the fact that the MP with Span 80 (10%) have more initial unencapsulated drug (lower EE%) compared to the ones with Tween 80 (10%), as evidenced by the fact that at time 0, a higher percentage of drug was released in the case of the Span 80 MP, corresponding to a greater cumulative drug release (Figure 3.2).

The MP without surfactant presented the highest burst effect, since the initial burst release is inversely related to the water solubility of the drug, as explained in the previous section (3.3.1).

3.3.3 Effect of drug loading on drug release - 10% DL vs. 30% DL

Figure 3.3 shows comparative graphs of the drug release profile for the 10% DL MP, also described in the Figure 3.1, and the 30 % DL MP, also described in the Figure 3.2.

The 30% drug-loaded formulations (DLact (%): No surfactant = 25%; Tween 80 = 27%; Span 80 = 24%) have more initial unencapsulated drug (lower EE%) compared to those with 10% DL (DLact (%): No surfactant = 9%; Tween 80 = 11%; Span 80 = 9%), as evidenced by the fact that when time equals 0, a higher percentage of drug was released in the case of MP with 30% DL compared to those with 10% DL (Figure 6; a, b, c).

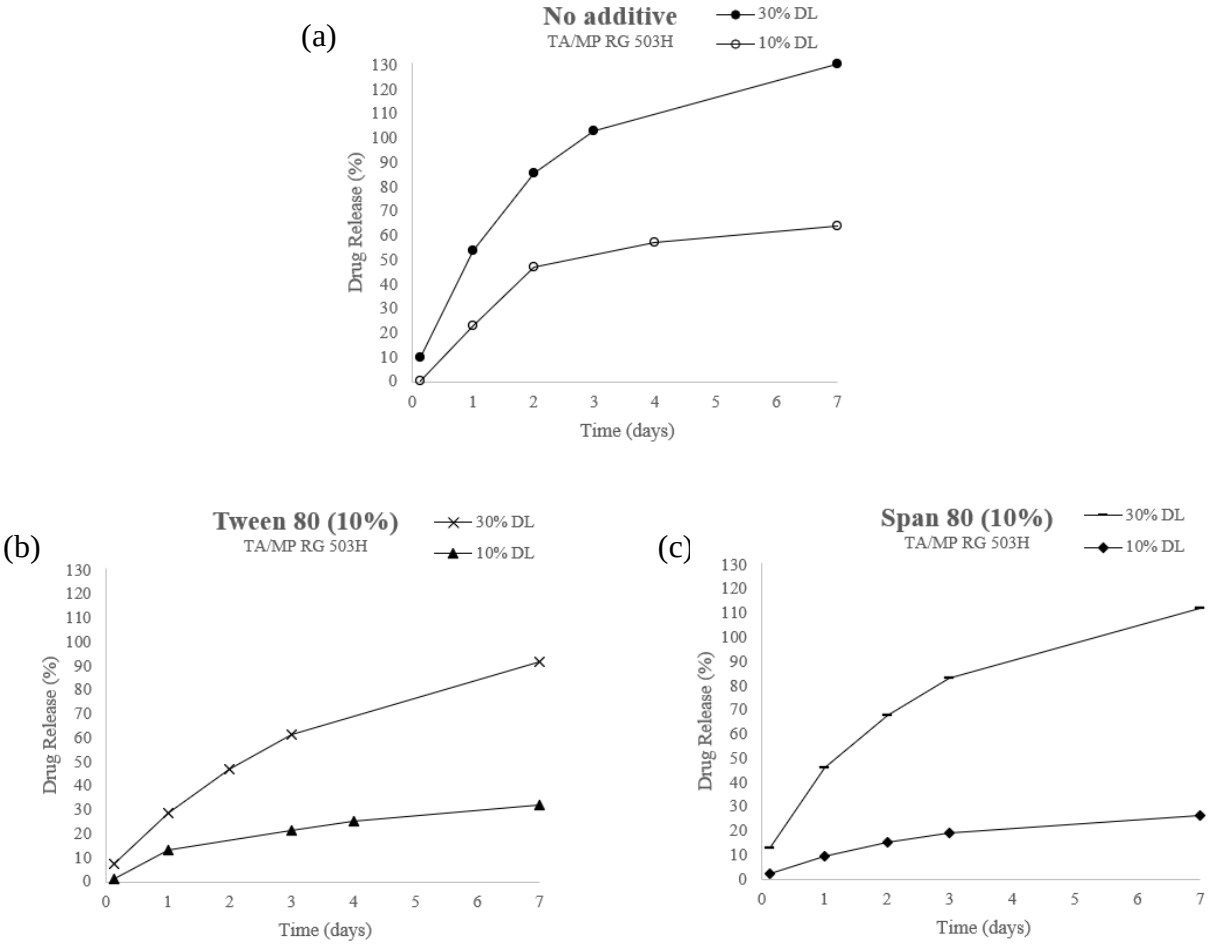


Figure 3.3 Comparison of drug release profile between 10% and 30% TA/MP (RG 503H) in the case of microparticles with: (a) No surfactant, (b) Tween 80 (10%) and (c) Span 80 (10%).

In all batches produced, the 30% DL MP showed a higher release rate (greater slope) compared to the 10% DL MP, as it was expected, since a higher DL would lead to accumulation of the drug near the surface and consequently leaving more empty pores in the polymer, thereby resulting in faster drug release.

4. Conclusion

This study allowed us to evaluate the influence of surfactant addition to PLGA MP. Our research results indicate that the diameter of the 30% drug loaded MP was larger than the ones presented by MP prepared with the 10% DL. MP with 10% surfactants and without surfactants presented higher encapsulation efficiency in the case of Tween 80, followed by MP without surfactant, Span 80 and finally Tween 20. MP with 10% DL presented higher EE(%) (lower initial unencapsulated drug) compared to those with 30% DL. For both 10% and 30% theoretical DL MP, the initial burst was higher for MP without surfactant and decreased with the addition of surfactants since the initial burst is inversely related to the water solubility of the drug. In this sense, the MPs without surfactant are the ones with the highest cumulative drug release.

In all batches produced, the 30% DL MP showed a higher release rate compared to the 10% DL MP, as it was expected since higher DL favours the accumulation of the drug close to the surface and consequently more drug would be released.

TA was found to be unstable in the release medium, which is a limitation of this study. To overcome this limitation, it would have been interesting to change the release medium to one that ensures the stability of the drug or identify the degradation products of TA in the drug release test to make a correction of the drug release considering the degradation products, thereby obtaining more accurate and reliable data.

Due to the short duration of this internship, it was not possible to carry out all the desired tests. During the microparticle production process, it was not possible to quantify TA in the washing solution and adjust the calculations for the loss of the drug during the production process. In addition, it would have been interesting to perform the drug recovery test and to perform the calibration curves in triplicate to obtain more reliable data. All these tests should be performed whenever possible.

It remains to be seen whether there is a relationship between the addition of the different surfactants and the rate of drug release, since it was not possible to establish any corroboration in this respect, as the results obtained for MP with different DL (10% w/w or 30% w/w) and different amounts of surfactants (1% w/w or 10% w/w) were contradictory.

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6. Appendix

A1. Calibration Curve TA/PBS

Table A1 Dilution steps conducted to establish the calibration curve solutions.

Solubility TA in water = 0,0423 mg/mL m(TA) = 15,75 mg V(PBS) =1000 mL Concentration stock solution (ss) = 15,75 µg/mL			
	Dilution steps	ug/mL	Absorb.
B	-	0	0
V	0,5 mL ss + 4,5 mL PBS	1,575	0,049
IV	1 mL ss + 4 mL PBS	3,15	0,099
III	2,5 mL ss + 2,5 mL PBS	7,875	0,243
II	4 mL ss + 1 mL PBS	12,6	0,364
I	ss	15,75	0,432

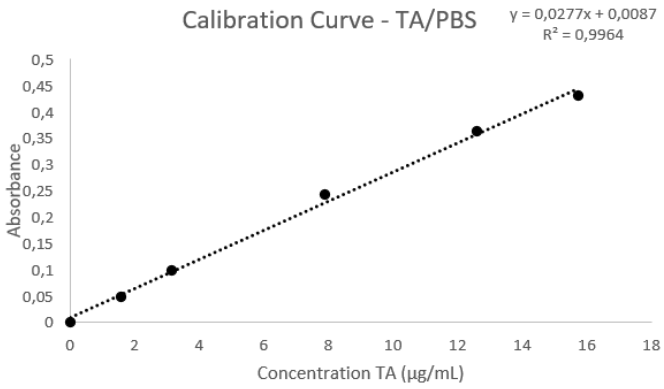

Figure A1 Calibration Curve of TA in PBS.

A2. Calibration Curve TA/(A/W)

Table A2 Dilution steps conducted to establish the calibration curve solutions.

Solubility TA in acetonitrile = No info m(TA) = 15,38 mg A/W = 500 mL Concentration stock solution (ss) = 30,76 µg/mL			
	Dilution steps	µg/mL	Absorb.
B	-	0	0
V	0,5 mL (I) + 4,5 mL A/W	1,845	0,075
IV	1 mL (I) + 4 mL A/W	3,691	0,139
III	2,5 mL (I) + 2,5 mL A/W	9,228	0,367
II	4 mL (I) + 1 mL A/W	14,764	0,63
I	30 mL ss + 20 mL A/W	18,456	0,747

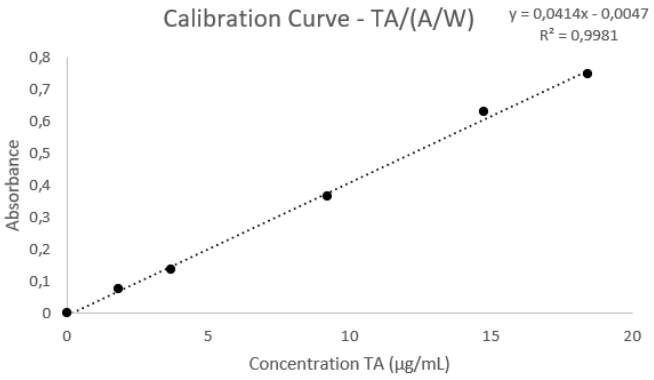

Figure A2 Calibration Curve of TA in A/W.