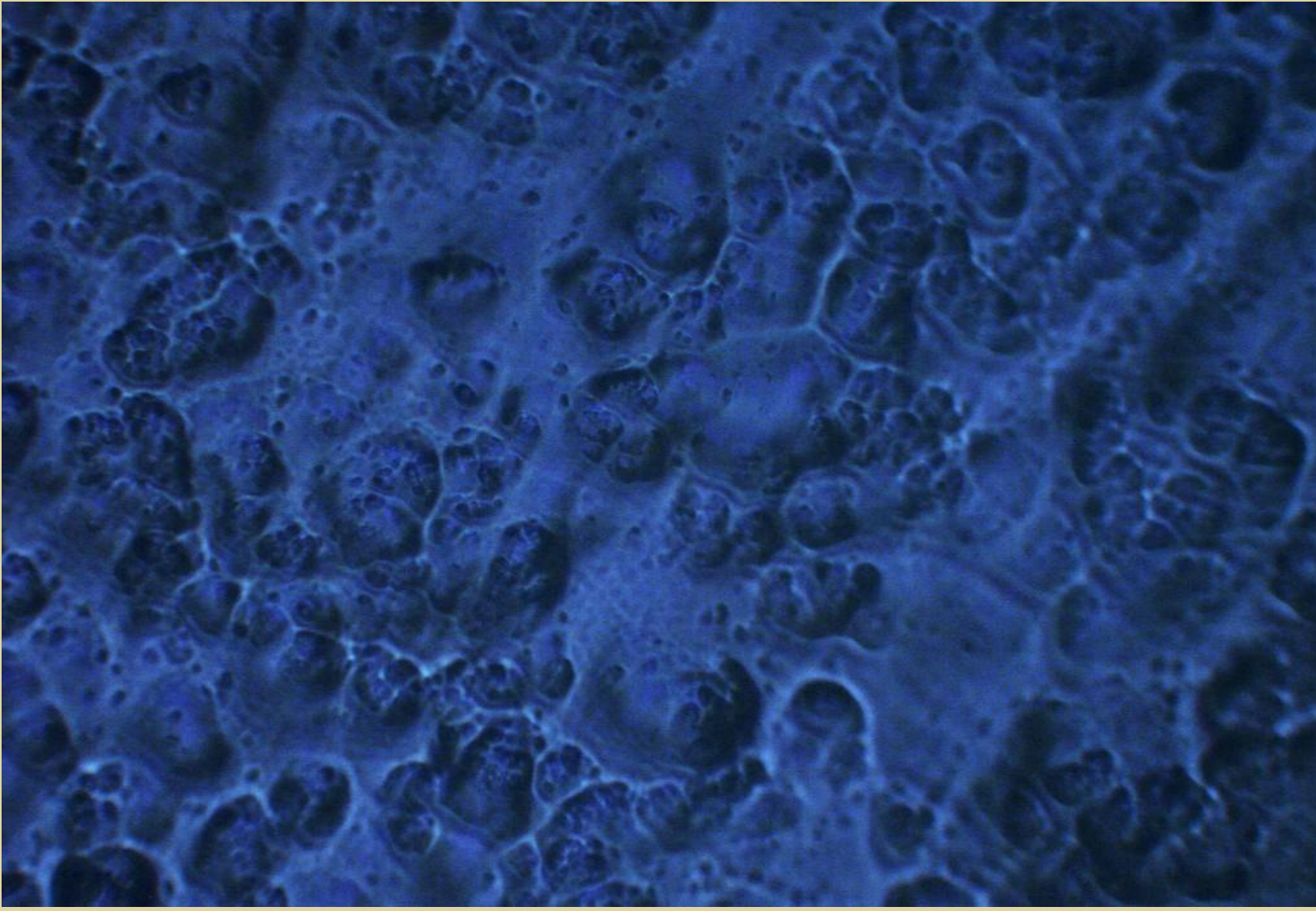

Branca Margarida Almeida Silva

Oral films: exploring formulation tailoring and clinical proof of concept

Tese de doutoramento em Ciências Farmacêuticas, ramo de Tecnologia Farmacêutica, orientada pelo Professor Doutor Sérgio Simões e pelo Professor Doutor Jorge Coelho e apresentada à Faculdade de Farmácia da Universidade de Coimbra

Setembro de 2017

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Universidade de Coimbra

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Oral films: exploring formulation tailoring and clinical proof of concept

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Faculty of Pharmacy, University of Coimbra

Doctoral thesis in Pharmaceutical Sciences, branch of Pharmaceutical Technology presented to the Faculty of Pharmacy of the University of Coimbra

Tese de doutoramento em Ciências Farmacêuticas, ramo de Tecnologia Farmacêutica apresentada à Faculdade de Farmácia da Universidade de Coimbra

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Table of Contents

List of Abbreviations

λ_z – Apparent terminal elimination rate constant

A – Average

AEs – Adverse Events

ANOVA – Analysis of Variance

AUC_{0-72} – Area under the blood concentration versus time curve from time zero to 72 h

AV – Acceptance Value

BcFs – Buccal Films

BMI – Body Mass Index

BUP - Buprenorphine

CEIC – Ethics Committee for Clinical Research

CI – Confidence Interval

CMAs – Critical Material Attributes

C_{max} – maximum observed blood concentration post dose

CNPD – National Data Protection Committee

CPPs – Critical Process Parameters

CQAs – Critical Quality Attributes

DoE – Design of Experiments

DS – Drug Substances

EMA – European Medicines Agency

EP – European Pharmacopeia

FDA – United States Food and Drug Administration

FMEA – Failure Mode and Effect Analysis

FTIR – Fourier transform infrared

GI – Gastrointestinal

GMP – Good Manufacturing Practice

GMR – Geometric Means Ratio

GxP – Good Practice

HPLC – High Performance Liquid Chromatography

HS – High Strength

ICH – International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

KF – Karl Fischer

LC-MS – Liquid Chromatography – Mass Spectrometry

LSmeans – Least Squares means

LS – Low Strength

MSD - Multivariate Statistical Difference

NaCMC – Carboxymethylcellulose sodium

NF – Normalization Factor

NLT – Not Less Than

NLX - Naloxone

NMT – Not More Than

ODFs – Orodispersible Films

OF - Oral Films

OFAT – One Factor at a Time

PDA – Photodiode Array Detector

PT – Preferred Term

PVP - Polyvinylpyrrolidone

QbD – Quality by Design

QTPP – Quality Target Product Profile

R&D – Research and Development

REM – Risk Estimation Matrix

RH – Relative Humidity

RP – Reference Product

rQbD – Retrospective Quality by Design

SD – Standard Deviation

SlFs – Sublingual Films

SLS – Sodium Lauryl Sulfate

SOC – System Organ Class

$t_{1/2}$ – Apparent terminal elimination half-life

TAMC – Total Aerobic Microbial Count

TEAEs – Treatment Emergent Adverse Events

t_{max} – Time to maximum observed blood concentration post dose

TPGS – Vitamin E Polyethylene Glycol Succinate

TYMC – Total Yeast/ Mould Count

USA – United States of America

USP – United States Pharmacopeia

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List of Publications

Papers published from the research work presented in this thesis

B.M.A. Silva, S. Vicente, S. Cunha, J.F.J. Coelho, C. Silva, M.S. Reis, S. Simões, Retrospective Quality by Design (rQbD) applied to the optimization of orodispersible films, Int. J. Pharm. 528 (2017) 655–663. doi:10.1016/j.ijpharm.2017.06.054.

B.M.A. Silva, A.F. Borges, C. Silva, J.F.J. Coelho, S. Simões, Mucoadhesive oral films: The potential for unmet needs, Int. J. Pharm. 494 (2015) 537–551. doi:10.1016/j.ijpharm.2015.08.038

Papers published from the collaboration in other projects during the PhD program

A.F. Borges, B.M.A. Silva, C. Silva, J.F.J. Coelho, S. Simões, Hydrophobic polymers for orodispersible films: a quality by design approach, Expert Opin. Drug Deliv. 13 (2016) 1357–1374. doi:10.1080/17425247.2016.1218458

Patent application

A.F. Borges, B.M.A. Silva, C. Silva, J.F.J. Coelho, S. Simões, Oral Dispersible Films, US 9603035 B2, 2017.

Contributions to meetings

B.M.A. Silva, S. Vicente, S. Cunha, C. Silva, J.F.J. Coelho, S. Simões. Critical Process Parameters of Orodispersible Films (ODFs). In: 8TH Conference of the European Paediatric Formulation Initiative, 2016. Lisbon.

B.M.A. Silva, S. Vicente, C. Cunha, G. Silva C. Silva, J.F.J. Coelho, S. Simões. Effect of process parameters on oral thin films quality attributes. In: 10th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, 2016. Glasgow.

B.M.A. Silva, A.F. Borges, C. Silva, J.F.J. Coelho, S. Simões. Oral Films: Adhesive properties. In: 9th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, 2014. Lisbon.

Abstract

Oral drug delivery is the most common route of drug administration but some limitations of conventional oral solid dosage forms (e.g. capsules, tablets) require the development of novel and more advanced drug delivery systems. Such limitations include poor patient compliance and low oral bioavailability, among others.

Oral films are promising dosage forms that are placed in the oral cavity and may be designed in order to allow gastrointestinal or oromucosal absorption of the drug, which present several advantages such as: increased patient compliance because it is easy to swallow, and does not need water for the intake; avoidance of the first-pass hepatic metabolism; fast onset of action; ease transportation and handling. The use of such dosage form is particularly beneficial for drugs that have poor oral bioavailability, drugs that need to be rapidly absorbed (e.g. emergency situations), and drugs to treat diseases where the patients have difficulty in swallowing, namely in patients with dysphagia. In spite of the known benefits, the knowledge around this dosage form and the number of marketed products is still quite limited.

In this thesis, scientific work related with the research and development of two types of oral films, orodispersible films (ODFs) and sublingual films (SIFs), is presented. The manufacturing process of orodispersible films was investigated and optimized using retrospective Quality by Design (rQbD). In particular, the root-cause of a decrease in the drug release rate of orodispersible films upon storage was investigated through the use of rQbD. Risk assessment tools were used to identify parameters affecting ODFs critical quality attributes (CQAs), namely the percentage of drug release and residual water content. The identified critical process parameters (room temperature, room relative humidity, drying temperature and the type of mixing equipment) were used in the statistical modeling of the available data. The estimated models were then used to define the design space. Statistical modeling suggested that initial residual water content of the ODFs is mainly affected by 2^{nd} order interactions of room temperature, room relative humidity and drying temperature. The stability of drug release profile upon storage is mostly influenced by room temperature and an interaction between room relative humidity and drying temperature. Depending on the drying temperature employed the effect of room temperature and room relative humidity can change significantly. This research work also demonstrated the potential of the application of rQbD to gain

a deeper understanding of the manufacturing process of pharmaceutical formulations in general, and ODFs in particular, as well as to define a proper design space.

These results allowed advancing to the production of a GMP batch of this new formulation of ODFs to treat a neurodegenerative disorder, where a high number of patients suffer from dysphagia. After that, a proof of concept clinical trial was conducted. The main aim of the clinical trial was to validate, in the clinical setting, the performance and acceptability of the investigational ODFs. The pharmacokinetic parameters of the investigational ODFs and the Reference Product (RP) (capsules) as well as the acceptability of the ODFs, as assessed by the taste evaluation, and the disintegration time, were determined in healthy volunteers. Twenty-four healthy subjects were enrolled in this open-label, randomized, parallel-group study. Half of the subjects received a single dose of the RP and the other half received a single dose of ODFs. Blood samples were collected at specified time points for pharmacokinetics evaluation. The 90% confidence intervals values of body weight-adjusted C_{max} and area under the curve (AUC_{0-72}) of the two tested formulations were within the acceptable range for bioequivalence (80,00-125,00%). Both ODFs and RP were well tolerated as no discontinuations or serious adverse events occurred. ODFs had a favorable taste acceptability and fast disintegration time in the mouth. In conclusion, ODFs and RP exhibited comparable pharmacokinetics, safety and tolerability, proving the concept in humans.

To demonstrate the versatility of oral films technology, Sublingual Films (SlFs) to treat opioid dependence were also developed. This study followed a QbD approach, starting with the definition of the quality target product profile (QTPP) and the CQAs. The patents evaluation of other innovative products and some preliminary experiments on formulation development were crucial to identify the critical material attributes (CMAs). A D-Optimal Design of Experiments (DoE) was used in order to understand the relationship between CMAs ranges and drug product CQAs assay, drug release and pH. The DoE results allowed to define two formulations with the potential to meet the QTPP. Their characterization and pre-stability studies demonstrated promising results: drug release profiles similar to those observed for other products already available in the market.

In this thesis, research work from the initial R&D of a formulation until the clinical proof of concept is presented. QbD principles such as risk assessment tools and DoE were used for the development, characterization and optimization of two types of oral films. This approach contributes to the enrichment of the state of the art through the establishment of reliable methodologies for formulation development and process optimization. Therefore, robust formulations and

manufacturing processes are obtained, which provides products that meet the desired performance and facilitates the upscaling. Also, the success of the clinical studies validates the assumptions that this technology can fulfill specific unmet patients' needs.

Resumo

A administração oral é a forma mais comum de administração de fármacos. No entanto, as formas farmacêuticas convencionais (p. ex. cápsulas e comprimidos) apresentam algumas limitações, como a baixa adesão à terapêutica e a baixa biodisponibilidade oral. Para ultrapassar estas limitações é fundamental a investigação e desenvolvimento de sistemas de entrega de medicamentos mais avançados.

As películas orais são formas farmacêuticas promissoras que são colocadas na cavidade oral e podem ser formuladas de forma a permitir tanto a absorção de fármacos a nível gastrointestinal ou através da mucosa oral, apresentando diversas vantagens: aumentam a adesão à terapêutica devido à facilidade de deglutição e à não necessidade de ingestão de água; evitam o metabolismo hepático de primeira passagem; permitem um início de ação mais rápido e são fáceis de transportar e manusear. O uso desta forma farmacêutica é útil para fármacos com baixa biodisponibilidade oral, para fármacos em que se pretende um rápido início da ação terapêutica (p. ex. situações de emergência) e para fármacos utilizados no tratamento de doentes com disfagia. Apesar dos benefícios conhecidos, o conhecimento científico em torno desta forma farmacêutica e número de produtos comercializados ainda é bastante limitado.

Nesta tese, é apresentado trabalho científico relativo à investigação e desenvolvimento de dois tipos de películas orais, películas orodispersíveis (ODFs) e películas sublinguais (SlFs). O *Quality by Design* retrospectivo (rQbD) foi utilizado para a investigação e otimização do processo de fabrico de ODFs. Mais especificamente, é investigada a causa para a libertação mais lenta do fármaco observada durante o armazenamento dos ODFs. Os parâmetros que afetam os atributos de qualidade críticos (CQAs) dos ODFs foram identificados através de ferramentas de avaliação de risco. Para a modelação estatística dos dados foram usados os parâmetros de processo críticos (temperatura ambiente, humidade relativa da sala, temperatura de secagem e tipo de equipamento de mistura) sendo que os modelos obtidos foram usados para definir o *design space*. De acordo com a modelação estatística, o conteúdo inicial de água residual dos ODFs é afetado principalmente por interações de segunda ordem da temperatura ambiente, humidade relativa da sala e temperatura de secagem. A estabilidade do perfil de liberação do fármaco é maioritariamente influenciada pela temperatura ambiente e por uma interação entre a humidade relativa da sala e a temperatura de

secagem. Dependendo da temperatura de secagem definida, o efeito da temperatura ambiente e da humidade relativa da sala altera significativamente. Este trabalho demonstra que é possível aplicar rQbD para obter uma maior compreensão do processo de fabrico de novas formas farmacêuticas de uma forma geral, dos ODFs em particular e permite definir um *design space* apropriado.

Os resultados obtidos permitiram que se avançasse para a produção de um lote GMP de ODFs para tratar uma doença neurodegenerativa em que um elevado número de doentes sofre de disfagia. Depois disto, um ensaio clínico de prova de conceito foi realizado de forma a validar a performance e aceitação, em ambiente clínico, dos ODFs investigacionais. Os parâmetros farmacocinéticos dos ODFs e do produto de referência (RP) (cápsulas), bem como a aceitação dos ODFs (sabor, tolerabilidade e tempo de desagregação) foram avaliados em voluntários saudáveis. O ensaio clínico foi um ensaio aberto, randomizado com grupos paralelos e incluiu 24 indivíduos saudáveis em que metade recebeu uma dose única do RP e a outra metade recebeu uma dose única de ODFs. Os valores dos intervalos de confiança a 90% para C_{max} ajustada ao peso corporal e da área sob a curva (AUC_{0-72}) das duas formulações testadas estavam dentro do intervalo aceitável para bioequivalência (80,00-125,00%). Tanto os ODFs como o RP foram bem tolerados, uma vez que não ocorreram interrupções ou eventos adversos graves. Os ODFs demonstraram boa aceitação e um rápido tempo de desintegração na boca. Em conclusão, os ODFs e o RP apresentaram uma farmacocinética, segurança e tolerabilidade comparáveis, provando o conceito em humanos.

Para demonstrar a versatilidade da tecnologia de películas orais, desenvolveram-se Películas Sublinguais (SlFs) para tratar a dependência de opióides. Este desenvolvimento seguiu uma abordagem QbD, começando com a definição do perfil de qualidade alvo do produto (QTPP) e os CQAs. O estudo de patentes de outros produtos inovadores e algumas experiências preliminares de desenvolvimento da formulação foram cruciais para identificar os atributos críticos das matérias-primas (CMAs). De forma a entender a relação entre CMAs e os CQAs conteúdo, libertação do fármaco e pH, foi realizado um desenho de experiências (DoE). Os resultados de DoE permitiram definir duas formulações com potencial para responder ao QTPP. A caracterização e os dados de pré-estabilidade demonstraram resultados promissores: perfis de libertação semelhantes aos de outros produtos disponíveis no mercado.

Nesta tese é apresentado o trabalho de investigação desde os passos iniciais de I&D até à prova de conceito clínica. Dois tipos de películas orais foram desenvolvidos e caracterizados com base nas

ferramentas de QbD de avaliação de risco e desenho de experiências. Esta abordagem permite estabelecer metodologias fiáveis para o desenvolvimento de novas formulações e otimização de processos, contribuindo assim para o enriquecimento do estado da arte. Desta forma é possível obter formulações e processos de fabrico robustos, que facultam produtos que cumprem com a performance desejada e facilitam o aumento de escala. Adicionalmente, o sucesso do ensaio clínico valida os pressupostos de que esta tecnologia pode dar resposta a necessidades médicas não atendidas dos doentes.

Outline of the thesis

The present thesis is organized in seven chapters:

I- Introduction

II- Orodispersible films to treat a neurodegenerative disorder- manufacturing process optimization

III- Orodispersible films to treat a neurodegenerative disorder- clinical trial

IV and V- Sublingual films to treat opioid dependence- formulation development

VI- General Conclusions

VII- Final remarks and future perspectives

The first chapter aims to contextualize the reader with the topic of the thesis, development and optimization of oral films through the application of quality by design (QbD) principles, providing concepts required to understand the subsequent chapters. Chapters II through IV present the results of the scientific work undertaken. On Chapter II, retrospective QbD was used to investigate the root-cause of a decrease in the drug release rate of orodispersible films upon storage. Based on this investigation, it was possible to define the manufacturing process design space that allowed a process adjustment to ensure a stable drug release profile over time and to proceed to a clinical trial. The clinical study described on chapter III was conducted in order to compare the oral bioavailability of a drug when administered in the form of ODFs with a commercially available reference product. The safety, tolerability and acceptability of these orodispersible films by the healthy volunteers was also investigated. To evaluate the versatility of the BlueOS® technology, sublingual films to treat opioid dependence were developed by applying QbD. This part of the work is described in Chapter IV and V.

The main conclusions of this thesis are presented in Chapter VI and, in Chapter VII the perspectives for future development are discussed.

I. General Introduction

The majority of the drug substances are administered through the oral route because it is safe, cost-effective and is still the most preferred by patients. Limitations such as enzymatic degradation in the gastrointestinal (GI) tract and low oral bioavailability of the drug substance due to the first pass hepatic metabolism are important challenges that remain to be overcome [1–3]. As such, oral mucosa has gained relevance in the past years as an alternative route of drug administration. The oral cavity has some important features, such as: low enzymatic activity; it is easily accessed facilitating the administration and the acceptance by patients; and it is highly vascularized and permeable, allowing drug substances to enter directly into the systemic circulation [1–3]. Also, the oral cavity play an important role in dosage forms that dissolve or disintegrate prior to swallowing when in contact with saliva [4]. Several pharmaceutical dosage forms have been developed for the intraoral administration, namely oral disintegrating tablets (ODT), lozenges, chewing gums, sprays, buccal solutions and gels, and oral films [1,5].

1.1 Oral mucosa

The oral cavity includes the lips, tongue, cheek (buccal), hard palate, soft palate and floor of the mouth. Oral mucosa is the common term to identify the lining of the oral cavity and includes the buccal, sublingual, gingival, palatal and labial mucosa [1,6,7].

It is possible to find different layers in the oral mucosa: the epithelium that represents a barrier to penetration; the basement membrane; the lamina propria and submucosa which contains nerves and blood vessels (Figure I.1). The epithelium may contain keratinized or non-keratinized cells. Three different areas are identified: the lining mucosa with non-keratinized epithelium covering the sublingual and buccal tissues; the masticatory mucosa, of hard palate and gums, containing keratinized epithelium and the specialized mucosa of the dorsal surface of the tongue [1–3]. Buccal and sublingual routes are the most common oral transmucosal routes of administration [1,6].

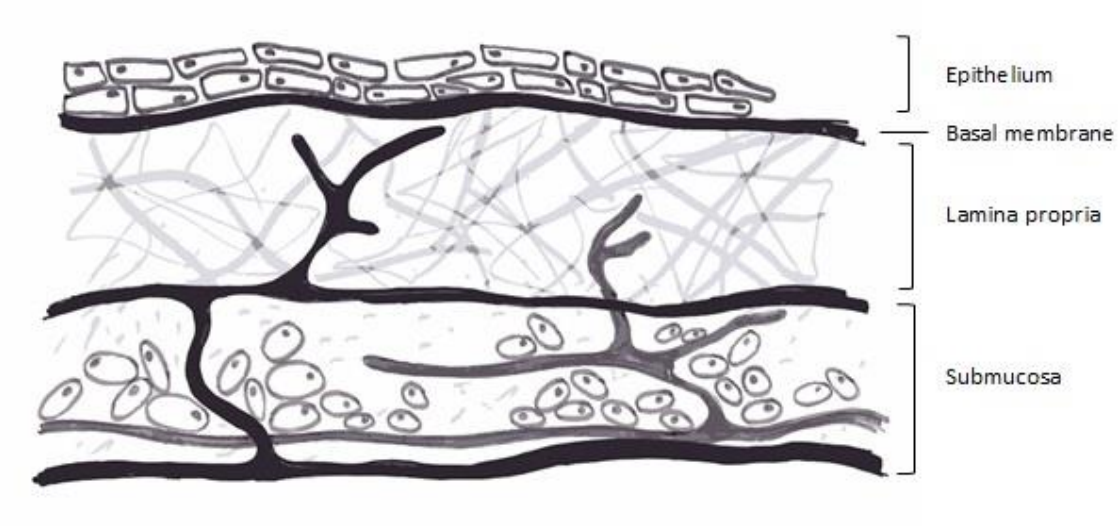


Figure I.1. Schematic representation of the oral mucosa structure.

The drug delivery through the oral mucosa is conditioned by saliva volume and flow rate, pH, enzyme activity and permeability of oral mucosa. On the other hand, the permeability is influenced by mucosal thickness, different epithelial cell composition and vascularization. The non-keratinized epithelium of buccal and sublingual mucosa has small amounts of neutral and polar lipids such as cholesterol sulfate and glucosyl ceramides; only small amounts of ceramides are present and acylceramides are absent. Therefore, it has greater permeability than the keratinized epithelium with its non-polar lipids like ceramides and acylceramides [6,7]. The sublingual mucosa (thickness 100-200 µm) is relatively thinner and more vascularized than the buccal mucosa (thickness 500-800 µm) and has demonstrated to be more permeable [1,6,8]. Overall, the sublingual mucosa is mainly used for a rapid onset of drug action while the buccal mucosa is suitable for local and systemic drug delivery.

Understanding the barrier features of the oral mucosa is crucial for the appropriate selection of the local of administration and the drug delivery system. The limitations of the drug delivery through buccal and sublingual mucosa have been reviewed by several authors, we list here some examples: saliva wash-out effect; displacement of the dosage form and consequent involuntary swallowing; challenging device placement, especially in the sublingual mucosa due to the smaller surface area; difficult retention and great effort of keeping dosage form in contact with the mucosa, which

requires formulations with mucoadhesive polymers, and physicochemical properties of the drug candidates [1,6,7].

1.2 Oral films

Oral films (OFs) are stamp-sized thin polymeric matrices intended to disintegrate /dissolve in the oral cavity. OFs are normally composed by film forming polymers, plasticizers, stabilizers, colorants, sweeteners and flavors for taste masking or improved palatability [9,10]. From the components mentioned film forming polymers and plasticizers can be considered the most critical components [9–11]. The polymer selection should be carefully performed since it directly influences the disintegration time, drug loading capacity, mechanical strength, elasticity, mucoadhesion, mouthfeel and handling properties. Different polymers can be combined to tailor the desired properties [9–11]. Hydrophilic film forming polymers such as cellulose derivatives, starch derivatives, polyvinyl alcohol or polyethylene oxide, are widely used in OFs development and in the products currently marketed [9,11–14]. Hydrophilic polymers promote the water retention and water absorption in OFs which can beneficial but also damaging. A proper water content is essential to ensure OFs flexibility, but an water uptake above an ideal level may compromise the stability of the drug substances and result in sticky OFs [14–16]. Plasticizers are frequently incorporated in OFs because most of the film forming polymers alone produce hard or brittle OFs. Plasticizers work by decreasing the glass transition temperature of the polymer, thereby promoting the polymer chains mobility and consequently the plasticity and elasticity of the resulting OFs [4,9,11,17]. Typical plasticizers include glycerol, propylene glycol, low molecular weight polyethylene glycols, and citrate derivatives such as tributyl, triethyl and acetyl citrate. The choice of these excipients is dependent on the compatibility with the film forming polymer, the drug substance as well as other excipients [4,9,11,17].

Regarding the design, OFs can be composed by one layer (single-layer) or several layers (multi-layer) considering the purpose of the formulation (Figure I.2). With the single-layer there is a multidirectional release of the drug, while multi-layer formulations ensure the unidirectional release of the drug towards the oral mucosa. This effect is due to the presence of a backing layer that reduces the diffusion of saliva into the following layers. Double-layer OFs consist of a mucoadhesive

layer and a backing layer, while triple-layer OFs have an additional intermediate layer that works as a drug deposit and ensures its prolonged or sustained release [18,19].

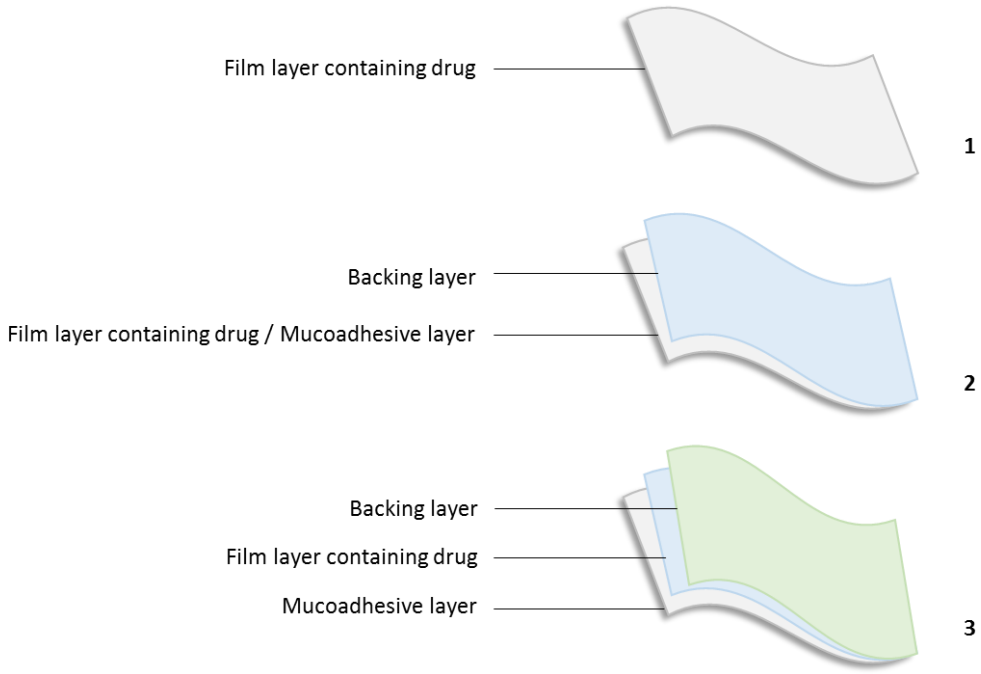


Figure I.2. Schematic representation of the different oral films designs: single-layer film (1), double-layer film (2) and triple-layer (3) film.

1.2.1 Regulatory framework

The United States Pharmacopeia (USP) 40th edition describes "films" as thin *sheets that are placed in the oral cavity. They contain one or more layers. A layer may or may not contain the drug substance. Typically, these thin sheets are formed by casting or extrusion which results in a dispersion of the components through the film* [20]. Additionally, "films" are classified according to the application site: oral films are intended to deliver the medication to the mouth or to the GI tract; sublingual and buccal films are designed to promote the absorption through the oral mucosa [20]. The European Pharmacopeia (EP) 9.1 has a monograph entitled "Oromucosal Preparations" with several sections including Mucoadhesive preparations and Orodispersible films [21]. *Mucoadhesive preparations contain one or more active substances intended for systemic absorption through the buccal mucosa over a prolonged period of time*. Buccal films are referred as mucoadhesive preparations that are *single- or multilayer sheets of suitable materials* and *may dissolve*.

Orodispersible films are single- or multilayer sheets of suitable materials, to be placed in the mouth where they disperse rapidly [21]. These differences help to understand the diversity of terms that are found in the literature to designate this dosage form [4,10].

In this work, it was adopted the terminology proposed by Borges et al. [10]. The OFs are divided in two classes according to the absorption site of the drug substances: orodispersible films (ODFs) are intended for gastrointestinal absorption while sublingual (SlFs) and buccal films (BcFs) are designed for oral mucosa absorption (Figure I.3).

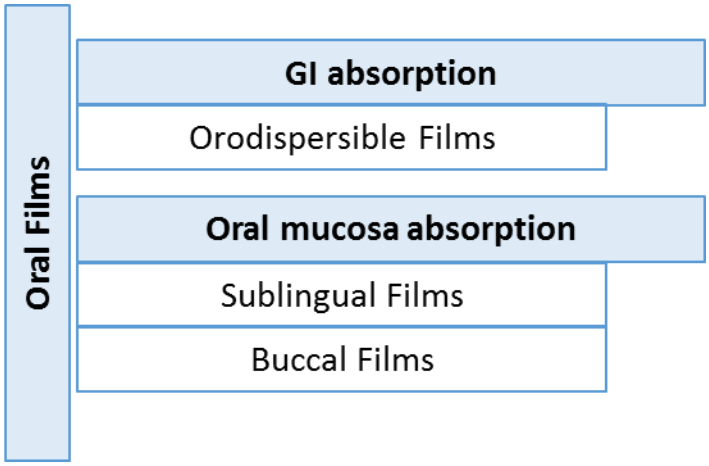


Figure I.3. Schematic representation of oral films classification (Adapted from Borges et al. [10]*).*

As mentioned in section 1.1 the oral mucosa has differences in permeability that determine the application site of the OFs and their names (Figure I.4). ODFs are placed on top of the tongue while SlFs are applied under the tongue. BcFs are placed on the inner cheek of the oral cavity or in the space between the cheek and the gum [1,10].

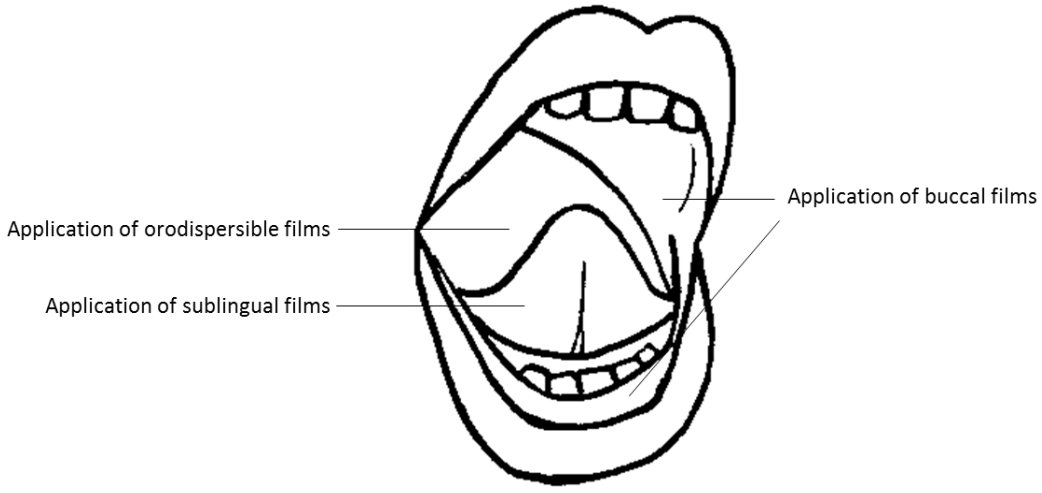


Figure I.4. Schematic representation of the different application sites of the oral films. Orodispersible films are placed on the tongue while buccal and sublingual films are placed on the buccal and sublingual mucosa respectively (Adapted from Borges et al. [10] *and Lam et al.* [1]*).*

1.2.2 Advantages, limitations and challenges

OFs offer several advantages over conventional dosage forms (e.g. capsules and tablets): no need of water intake and fast disintegration which makes them suitable for patients with dysphagia; dose flexibility; ease of transportation, handling and storage; dose accuracy when compared with syrups and drops; improved oral drug bioavailability by avoiding first-pass hepatic metabolism; dose reduction due to the improved oral bioavailability and consequently a decrease of side effects; fast onset of action achieved by the direct absorption of the drug through the oral mucosa and entrance into the systemic circulation [4,9,10,22].

Despite the many advantages, OFs have a limited drug loading. Therefore, OFs are restricted to low dose and high potency drug substances. In turn, uniformity of dose may be challenging. Also, suitable mouthfeel and taste masking may be challenging.

The most common manufacturing technique is solvent based which poses some challenges in terms of microbiological stability and chemical stability of the drug substances. The patent landscape is also very competitive and the requirement of specific equipment and special packaging can discourage the investment in this novel dosage form by other companies [4,23,24].

1.2.3 Manufacturing process

The manufacture of OFs is generally based on established technologies such as solvent casting technique and hot-melt extrusion, although new techniques such as printing technology are being developed and evaluated [9,25].

1.2.3.1 Solvent casting

The majority of commercially available OFs are manufactured by solvent casting [13]. A liquid mixture (solution, suspension or emulsion) is prepared by dissolving the film forming polymers in water or organic solvents, followed by the addition of the other excipients and the drug substance. The liquid mixture is then casted and dried to form the film (laminate) as illustrated in Figure I.5. The laminate is cut to the desired size and the OFs are normally individually packed [11,12,14]. During manufacturing several aspects should be taken in consideration: mixture rheology because it influences the drying rate, the uniformity of drug content and the appearance of the OFs; air bubbles entrapment in the liquid mixture because it may compromise the uniformity of drug content and OFs appearance; residual solvents content due to safety issues; drug substances (DS) particle size that impacts content uniformity in the case of liquid suspensions; drying temperature and air flow rate during drying that can promote OFs uneven surface and compromise the complete film formation [3,4,9,11,13].

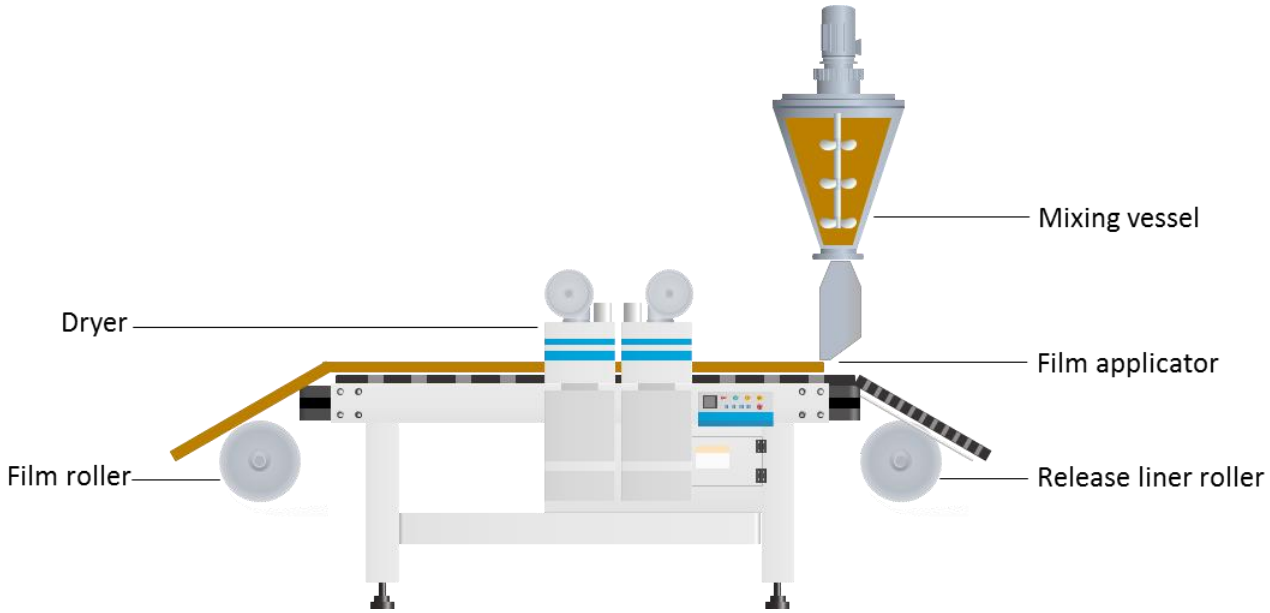


Figure I.5. Schematic representation of a typical solvent casting machine.

1.2.3.2 Hot-melt extrusion

Hot-melt extrusion has been widely used in the development of tablets and granules for improved oral bioavailability or controlled drug release [3]. The film forming polymers, the drug substance (s) and the other excipients are blended in the solid state. The blend is molten and pressed through an orifice (nozzle/ die) to a web that is then cooled down (Figure I.6) and cut to the desired dimensions [3,4,26,27]. The hot-melt extrusion method offers some advantages over the solvent casting method, namely absence of solvents; less energy consumption which is translated into lower operating costs; improved solubility and oral bioavailability of poorly soluble drugs; higher efficiency, a medium-sized equipment can produce 550-700 Kg of OFs for hour while in solvent casting only 15 Kg are produced [4,13,26]. However, all the components of the formulation must have a good thermal stability and it may be necessary to use anti-tacking agents that can compromise the disintegration and mouth feel [4,13,26].

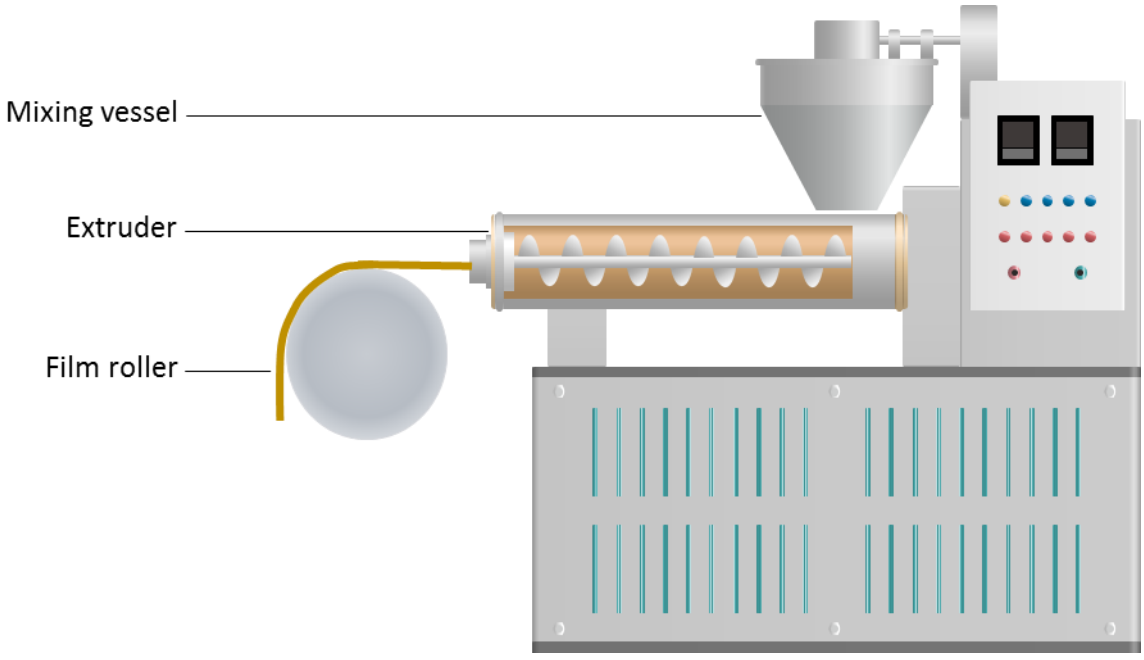


Figure I.6. Schematic representation of a typical hot-melt extrusion machine

1.2.4 Pharmaceutical applications

OFs market remains a niche market with only 23 products (Table I.1) [22,28]. These products are based in different technological platforms namely *Orally disintegrating films* from Kyukyu Pharmaceutical Co Ltd, *Schmelzfilm* from Hexal, *SmartFilm* from Seoul Pharma, *QuickSol* from SK Chemicals, *Rapidfilm* from tesa Labetec, *PharmFilm* from MonoSol Rx, *BEMA* from Biodelivery Sciences International, *LTS Oral Thin Films* from LTS Lohmann and *ARx Oral Thin Films* from Adhesives Research [22,28].

Table I.1. Oral films available in the market organized by type, drug, company, technology and therapeutic indication.

Drug	Product name	Developer Company	Technology	Therapeutic Indication
Orodispersible films				
Olopatadine		Kyukyu Pharmaceutical	Orally disintegrating films	Allergy
Loratadine		Kyukyu Pharmaceutical	Orally disintegrating films	Allergy
Donepezil	Donepezil-HCl Hexal® SF	Hexal	Schmelzfilm	Alzheimer's disease
Donepezil		Kyukyu Pharmaceutical	Orally disintegrating films	Alzheimer's disease
Montelukast	Monte ODF	CHA Bio & Diostech		Asthma
Voglibose		Kyukyu Pharmaceutical	Orally disintegrating films	Diabetes
Sildenafil	SildeHexal SF	Hexal	Schmelzfilm	Erectile dysfunction
Sildenafil		Seoul pharma	SmartFilm	Erectile dysfunction
Mirodenafil	MVix-S	SK Chemicals	Quicksol	Erectile dysfunction
Tadalafil		Seoul pharma	SmartFilm	Erectile dysfunction
Simethicone	Gas-X	Adhesives Research	ARx Oral Thin Films	Flatulence
Zolpidem		Kyukyu Pharmaceutical	Orally disintegrating films	Insomnia
Amlodipine		Kyukyu Pharmaceutical	Orally disintegrating films	High blood pressure
Zolmitriptan	Zolmitriptan ODF	tesa Labtec	Rapidfilm	Migraines
Ondansetron	Zuplenz	MonoSol Rx	PharmFilm	Nausea and vomiting
Ondansetron	Setofilm®	tesa Labtec	Rapidfilm	Nausea and vomiting
Nicotine	Niquitin Strips	LTS Lohmann	LTS Oral Thin Films	Nicotine dependence
Triamcinolone acetonide		Kyukyu Pharmaceutical	Orally disintegrating films	Oral mucositis
Risperidone	Risperidone Hexal® SF	Hexal	Schmelzfilm	Schizophrenia
Olanzapine	Olanzapine Hexal® SF	Hexal	Schmelzfilm	Schizophrenia
Sublingual films				
Buprenorphine/ naloxone	Suboxone®	MonoSol Rx	PharmFilm	Opioid dependence
Buccal films				
Fentanyl	Onsolis®/ Breakyl	BioDelivery Sciences International	BEMA™	Breakthrough cancer pain
Buprenorphine/ naloxone	Bunavail®	BioDelivery Sciences International	BEMA™	Opioid dependence
Buprenorphine	Belbuca®	BioDelivery Sciences International	BEMA™	Severe Pain

In Table I.2, the characteristics of the technologies used in the products available in the market are described. The Japanese company Kyukyu Pharmaceutical Co Ltd owns a technology that provides ODFs with fast disintegration, 10 to 30 seconds, and another technology to obtain mucoadhesive films that have disintegration times ranging from 30 minutes to 8 hours [28]. Schmelzfilm technology is based on cellulose derivatives and the ODFs have a fast disintegration [28]. The orodispersible *Rapidfilm* technology contains water soluble polymers such as starch and polyvinyl alcohol, and the design can vary from single to multilayer [28,29]. The portfolio of tesa Labtec includes now a mucoadhesive film technology, *Mucofilm*, which offers buccal and sublingual films [30]. *PharmFilm* technology was developed by MonoSol RX and enables buccal, sublingual, enteral and vaginal delivery [31]. This company has several patents to produce film compositions as single or multi-layer. The polymers used include polyethylene oxide and hydroxypropylmethyl cellulose, both mucoadhesive polymers [3,32,33]. The *BEMA* technology consists of a double-layer bioerodible film for application in the buccal mucosa [34]. Both layers have the mucoadhesive polymers hydroxypropyl cellulose and hydroxyethyl cellulose and, the active layer has the additional polymers polycarbophil and carboxymethylcellulose sodium [35]. Both the LTS Lohmann and the Adhesives Research technology are very flexible enabling the production of single or multi-layer oral films with fast or sustained disintegration [36–38].

Table I.2. Characteristics of the oral films technologies used in the manufacturing of the commercially available products.

Technology	Design	Polymers	Application zone	Disintegration time
Orally disintegrating films	Not disclosed	Not disclosed	On the tongue	10 seconds to 30 seconds
Schmelzfilm	Single layer	Ethylcellulose and hydroxypropylmethyl cellulose	On the tongue	Fast disintegration
SmartFilm	Single layer	Not disclosed	On the tongue	Fast disintegration
QuickSol	Single layer	Not disclosed	On the tongue	Fast disintegration
RapidFilm	Single-layer; multi-layer	Starch and polyvinyl alcohol	On the tongue	Fast disintegration
PharmFilm	Single-layer; multi-layer	Polyethylene oxide and hydroxypropylmethyl cellulose	Buccal, sublingual, enteral and vaginal delivery	1 to 3 min
BEMA	Double-layer	Backing layer: hydroxypropyl cellulose, hydroxyethyl cellulose Active layer: polycarbophil and carboxymethylcellulose sodium	Buccal	15 to 30 min
LTS Oral Thin Films	Single-layer; multi-layer	Methacrylic Acid - Ethyl Acrylate Copolymer Pullulan	On the tongue, buccal and sublingual	Few seconds to hours
ARx Oral Thin Films	Single-layer; multi-layer	Polyvinyl alcohol	On the tongue, buccal and sublingual	Few seconds to hours

The therapeutic indication with more marketed products is erectile dysfunction (4 OFs) followed by allergies, Alzheimer's disease, nausea and vomiting, opioid dependence and schizophrenia with two marketed OFs for each (Table I.1 and Figure I.7). The remaining therapeutic indications (asthma, breakthrough cancer pain, diabetes, flatulence, high blood pressure, insomnia, migraines, nicotine dependence, oral mucositis and severe pain have one product commercialized (Table I.1 and Figure I.7).

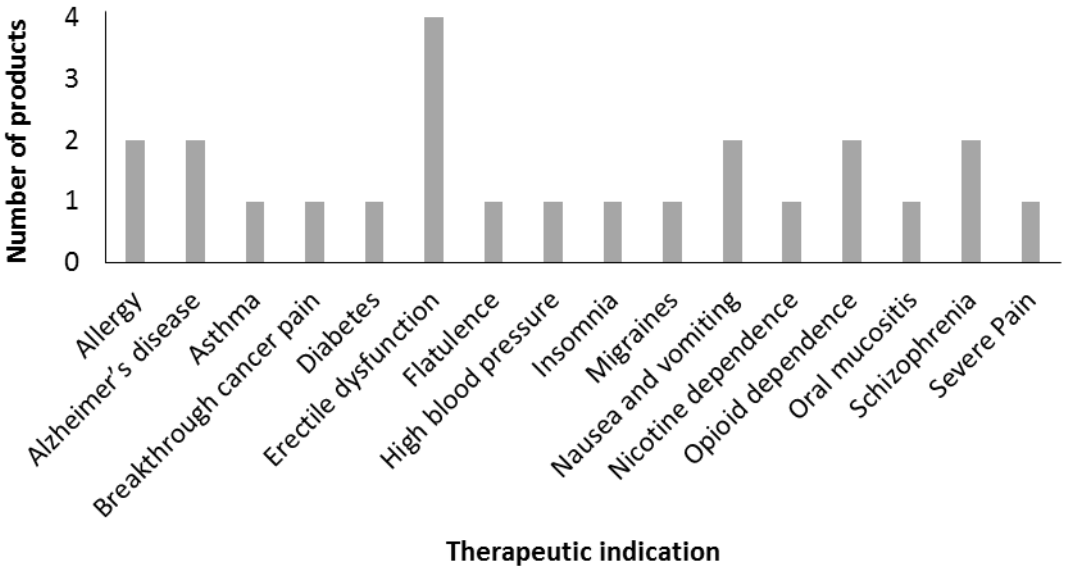


Figure I.7. Number of marketed oral films by therapeutic indication.

Erectile dysfunction is a condition that negatively impacts men's self-esteem and interpersonal relationships [39,40]. The patient satisfaction with the treatments is dependent not only in the erectile response and safety of the treatment, but also in how well the patient needs are fulfilled [39,40]. OFs present a convenient and discrete method of administration with added patient acceptability which can explain the number of products launched to treat erectile dysfunction [39,40].

Dysphagia is the term employed to describe the swallowing difficulties either by aging or disease, such as Parkinson's disease, Alzheimer's disease, stroke, multiple sclerosis, nausea and vomiting, xerostomia among others [41–43]. The dysphagia has a significant impact on patients' health by affecting ingestion not only of medicines but also of food. The inability to swallow medicines is one of the major causes for patients' non-compliance with the therapeutic regimen which may lead to uncontrolled symptoms and diseases [43,44]. Several strategies have been adopted to guarantee that individuals receive their medicines, namely crushing tablets or opening capsules, and selection of alternative routes for drug administration or switching the dosage form [43,44]. It is clear the need for new dosage forms for patients suffering from dysphagia. OFs are an attractive option, since

there is no need for swallowing the dosage form neither for water intake, and the film is easily placed in the mouth by the patient or the caregivers.

Opioid dependence is characterized by the inability of an individual to stop using opioids, both prescription and illicit opioids [45]. Medications to treat opioid dependence are helpful to ease craving and other physical symptoms and include methadone, buprenorphine and naltrexone [46,47]. The use of opioid analgesics carries the possibility of misuse and abuse which is a public health concern with the abuse rates having quadrupled between 1990 and 2000. Illegal users take an excess number of pills orally or crush them for snorting, smoking or injecting in order to get "high". The development of novel dosage forms that are tamper-resistant and abuse-deterrent are one of the strategies pointed to subvert the abuse [48,49]. In fact, FDA has specific guidelines for industries about the studies that should be conducted to demonstrate abuse-deterrent properties of formulations. Abuse deterrent formulations can be categorized in physical/chemical barriers, agonist/antagonist combinations, aversion, delivery system, prodrug or combination of the previous categories [50]. In OFs the drug substance is kept in a polymeric matrix that cannot be crushed for snorting or smoking. In the specific case of buprenorphine and naloxone products, mucoadhesive films (sublingual or buccal) offer the advantage of improving buprenorphine oral bioavailability by avoiding the first-pass hepatic metabolism while avoiding the absorption of naloxone into the bloodstream [51].

1.2.5 BlueOS® technology

As mentioned above, hydrophilic polymers have some constraints namely the higher affinity for water retention that may result in sticky OFs and drug substances and excipients instability (degradation and crystallization) [14–16]. The intense intellectual property associated to OFs technologies and the limitations of hydrophilic polymers motivated the development of a novel technology [14,52]. A quality by design approach was employed to develop OFs based on hydrophobic polymers (polyvinyl acetate, ammonium methacrylate copolymer and shellac) that are known to have less water absorption capacity, while ensuring at the same time the fast disintegration characteristic of OFs. The formulation studies also included stabilizers (polyvinyl alcohol, hydroxypropyl methylcellulose and tween 80); disintegrant (carboxymethylcellulose sodium); plasticizers (triethyl citrate, propylene glycol, glycerol, polyethylene glycol 400, polyethylene glycol 1000 and polyethylene glycol 6000); buffering agents; sweeteners, flavors and colorants [14,52]. In the end, three different formulations with a fast disintegration and four

essential components (hydrophobic polymer, stabilizer, disintegrant and plasticizer) were obtained [14,52]. The work performed resulted in an innovative drug delivery technology, BlueOS®, a proprietary technology from Bluepharma Indústria Farmacêutica S.A. [52,53].

1.3 Pharmaceutical Development

According to the ICH Q8 (R2) the pharmaceutical development aims *to design a quality product and its manufacturing process to consistently deliver the intended performance of the product* [54]. The pharmaceutical development comprises different activities such as drug substance development, formulation development, manufacture of investigational products, delivery system development (if relevant), manufacturing process development and scale-up and, analytical method development [55]. Therefore, the pharmaceutical development to be successful should be seen as an integrated approach where quality cannot be tested into products but rather should be built in by design. The applicants are encouraged to follow a systematic approach to product development, nonetheless an empirical approach or a combination of systematic and empirical methodologies are also acceptable [54,56,57].

1.3.1 Quality by design

Quality by Design (QbD) is a methodology used to build quality into products, by design [12,54]. The QbD approach ensures that the pharmaceutical development is conducted in order to have in the end a scientific understanding of how process parameters affect product performance. This knowledge enables the establishment of a design space, product and process specifications, as well as appropriate manufacturing controls that ensure that all necessary quality targets and product requirements will be achieved consistently [54,56,57]. The QbD implementation occurs mainly during the pharmaceutical development stage with the definition of the Quality Target Product Profile (QTPP), identification of potential Critical Quality Attributes (CQAs), Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs) [54,57]. This information should be integrated with the Quality Risk Management principles in order to assess, control, communicate and review the risks that may impact the quality of the product [54–58]. In this sense, different phases of the pharmaceutical QbD can be identified as illustrated in Figure I.8: define, design, characterize, validate, and monitor and control. As part of the quality management process,

improvement opportunities, identified in the monitor and control phase, can instigate a new cycle of development as demonstrated in Figure I.8 [54–58].

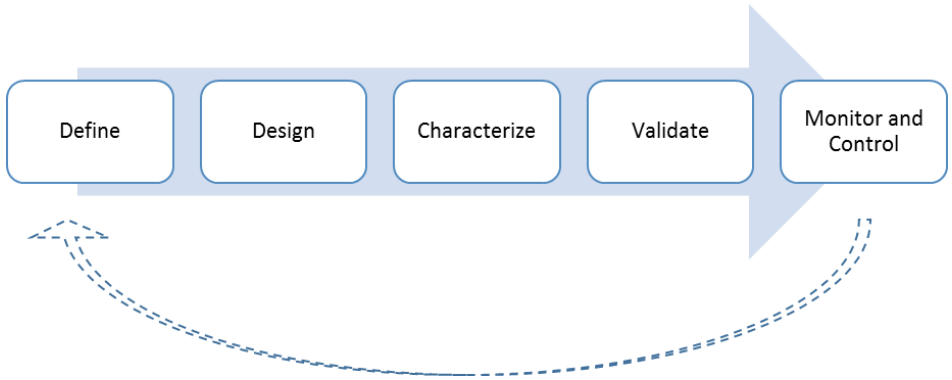


Figure I.8. Different phases of pharmaceutical QbD (adapted from Rathore and Winkle [57]*).*

1.3.1.1 Implementation

The QbD approach (Figure I.9) starts with the definition of the QTPP which is a summary of the quality characteristics of the drug product that are expected to be achieved to ensure the desired quality, taking into account the safety and efficacy. The QTPP usually includes the route of administration, dosage form, dosage strength(s), container closure system, characteristics affecting pharmacokinetic parameters (e. g. dissolution), drug product quality criteria (e. g. assay and uniformity, impurity profile, stability and dissolution) and so on [54,56,57,59].

The next step in the pharmaceutical development is the identification of CQAs that are physical, chemical, biological or microbiological properties or characteristics of the drug product that should be within an appropriate limit, range, or distribution to ensure the desired product quality [54]. The CQAs identification is performed through risk assessment tools taking into consideration the severity of harm to the patient but not the probability of occurrence, detectability or controllability. Prior knowledge, such as laboratory, non-clinical and clinical experience, is essential to perform these risk assessments [54,56–58].

During development it is not possible to study all the material attributes of the drug substance(s) and excipients, and all the process parameters. Therefore, risk assessment tools such as Cause-and-effect diagram, Risk Estimation Matrix (REM) and Failure Mode and Effect Analysis (FMEA) can be

used to prioritize which material attributes should proceed for further studies - CMAs and CPPs identification [54,56–58]. CMAs can be defined as physical, chemical, biological or microbiological property or characteristic of input materials that should be within an appropriate limit, range, or distribution to ensure the desired quality of the drug substance, excipient or in-process material. CMAs refer to input materials including drug substance(s) and excipients and, CQAs concern to output materials such as product intermediates and finished product. CMAs are for example solubility, particle size distribution, moisture content, grade and degree of substitution [54,56,57]. CPPs are process inputs that have an impact on CQAs namely type and geometry of mixer, order of addition, speed, temperature or environment [54,56]. The effect and relationship of process parameters and material attributes on the CQAs is investigated during development, usually through Design of Experiments (DoE) approach, in order to establish the region that ensure the product quality (design space) [54,56,57]. The defined CMAs and CPPs should be re-evaluated based on the knowledge acquired during DoE such that a control strategy can be developed and a continuous monitoring implemented [54–58].

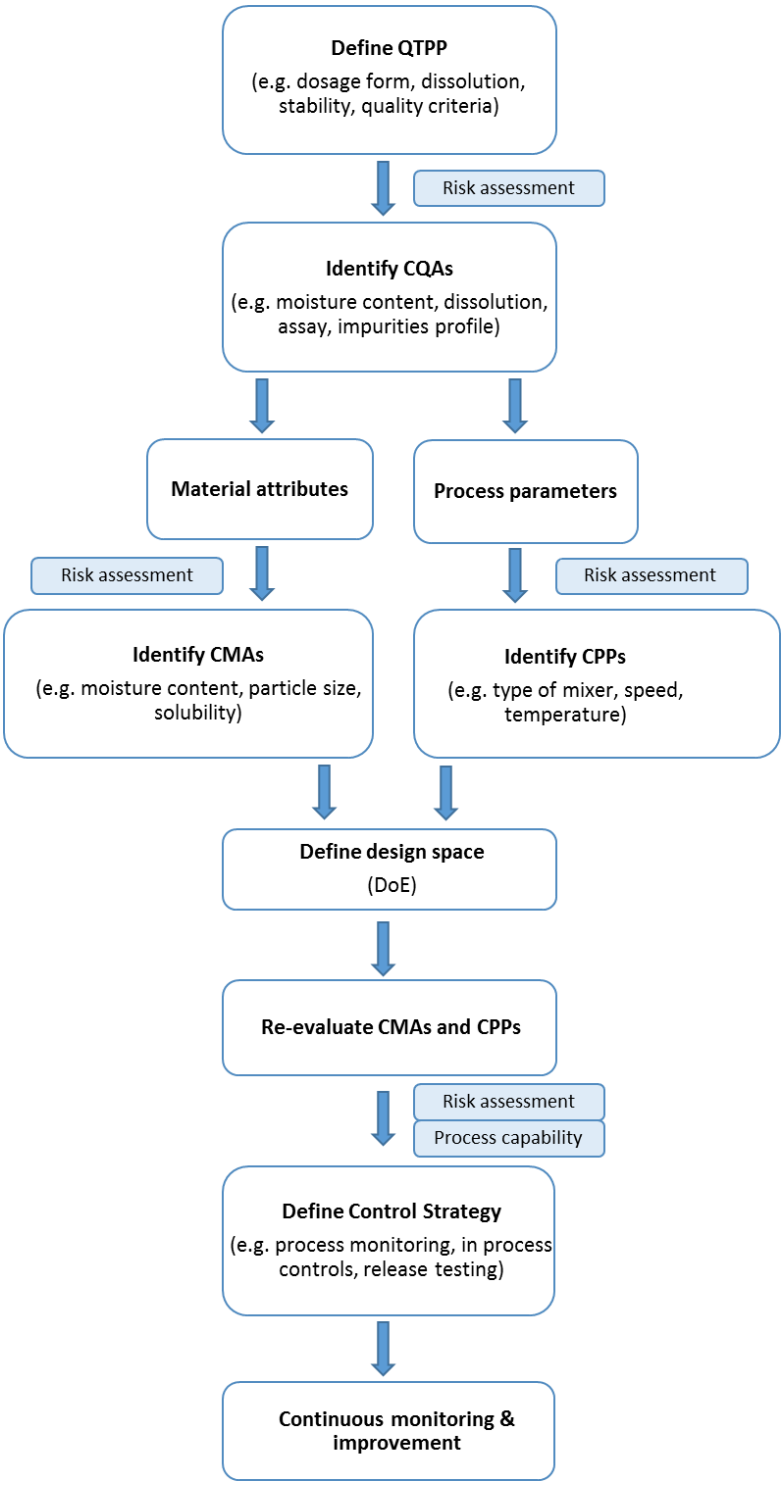
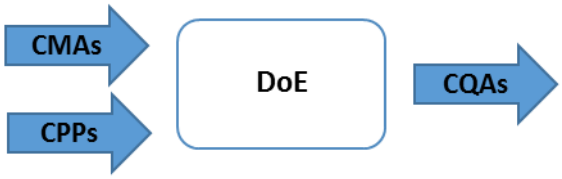


Figure I.9. Quality by Design implementation as defined in the ICH Q8, Q9 and Q10 [54,55,58]. *QTPP- quality target product profile; CQAs- critical quality attributes; CMAs- critical material attributes; CPPs- critical process parameters; DoE- design of experiments.*

1.3.1.2 Design of Experiments

The DoE is the main statistical tool used in the application of QbD and it is important to ensure that the experiments yield the maximum of relevant information [60,61]. The DoE allows to determine how the independent variables being studied/ factors/ parameters (CMAs and CPPs) interact and how its interaction affects the responses/ outputs (CQAs) Figure I.10. These relationships are studied at different factors levels and allow to determine the design space (*the multidimensional combination and interaction of input variables and process parameters that have been demonstrated to provide assurance of quality)* [54,56,60,61].



$$CQAs = f(CMAs_1, CMAs_2, CMAS_i, CPPs_1, CPPs_2 CPPs_i)$$

Figure I.10. Critical quality attributes (CQAs) will be defined as a function of critical material attributes (CMAs) and critical process parameters (CPPs) during Design of Experiments (DoE) (adapted from Yu et al. [56] *and Politis et al.* [61]*).*

The DoE offers several advantages over the traditional approach of changing one factor at a time (OFAT), namely maximize knowledge with less resources than OFAT; robust identification of cause and effect relationships between CPPs, CMAs and CQAs; definition of the relative significance of each factor; construction of prediction models and simultaneous optimization of multiple CQAs [60,61].

The execution of a DoE requires the definition of solid objectives through QTPP, the selection of factors (CMAs and CPPs) and responses (CQAs) to be investigated and their respective levels and ranges, the choice of the type of experimental design (screening, optimization and robustness testing), the execution of the experiments, and the analysis and interpretation of the results. The choice of the experimental design is a critical step in the implementation of the DoE and should be based on the intended objectives of performing such experiments [60–62]. Screening designs are used to determine the most influential factors and their appropriate ranges and require few experiments in relation to the number of factors. Optimization designs involve more experiments

and can be employed as a continuation of the screening designs or when there is previous knowledge of the interactions among factors. The goal of this type of design is to build a better understanding of the interactions and to find the optimum ranges to obtain the specified quality. The sensitivity of CQAs to small changes in the factors is investigated using robustness testing designs [60–62].

1.3.1.3 Design space and Control strategy

The ICH Q8 (R2) defines the design space (Figure I.11) as *the multidimensional combination and interaction of input variables (e. g. material attributes) and process parameters that have been demonstrated to provide assurance of quality* [54]. Therefore, each CQAs is a dimension that is estimated through the statistical modelling of the data obtained in the DoE. The prediction models of each CQA can be combined to find the region where the product meets the QTPP [54,57,63]. During DoE execution, the ranges (characterization range) of the CMAs and CPPs studied are defined as the knowledge space. The region within the knowledge space that generates products with acceptable quality (acceptable range) is considered the design space. Within the design space, it is possible to find the normal operation range to be included in the manufacturing procedures.

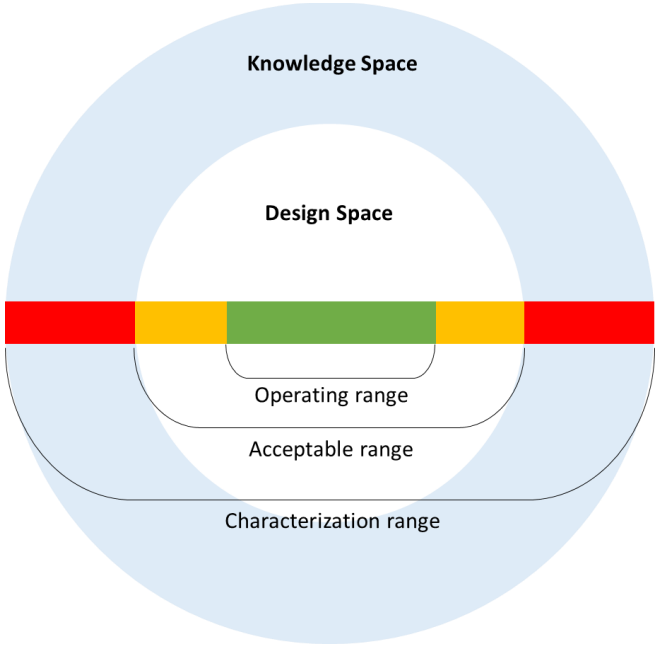


Figure I.11. Schematic representation of the different regions that can be defined during QbD implementation. The characterization range was studied during design of experiments (DoE) and constitute the knowledge space. Within the design space it is possible to find the acceptable range, that is the output of the DoE and, the operating range that constitute the ranges defined in the manufacturing procedures (adapted from Rathore and Winkle [57] *and Boukouvala et al.* [64]*).*

The major advantages of this approach is that working within the design space is not considered a change to the approved dossier of the product and it is ensured that no unacceptable product is generated [54,63,64].

The knowledge and understanding gained during the development and application of the DoE methodology culminates in the establishment of a control strategy that ensures the consistent production a product that meets the QTPP [54–56,58]. A control strategy can include control of input materials attributes (CMAs) and control of unit operations (CPPs) that were found to be critical for the processability and product quality, and in-process or real-time testing of the CQAs. The bottom line of the control strategy is the end-product testing that can be reduced to a minimum when the sources of variability were well characterized and broadly understood in the DoE [54–56,58].

1.3.1.4 Continuous monitoring and improvement

Throughout the product lifecycle additional experience and understanding of the product and process performance is acquired, which brings opportunities for improvement [54–58]. The continuous monitoring and evaluation of the manufacturing process performance can trigger the need to perform adjustments in the operating ranges. As referred before, no review or approval by the regulatory authorities of those changes are required when performed within the design space. In contrast, expansion, reduction or redefinition of the design space is subjected to post-approval submission [54–58].

1.4 Aims of the thesis

The main goals of this thesis were to proceed with the development of the first product using BlueOS® technology until the proof of concept clinical trial and to demonstrate its versatility in terms of potential pharmaceutical applications. Quality by design (QbD) was selected as the most suitable approach to achieve this goal. Therefore, the specific goals of this thesis were:

- To investigate and optimize the manufacturing process of orodispersible films at late development stage using QbD.

- To investigate how critical material attributes and critical process parameters influence oral films critical quality attributes increasing the understanding and the knowledge regarding oral films as pharmaceutical drug products.
- To perform the clinical proof of concept of orodispersible films for the treatment of a neurodegenerative disease.
- To explore the versatility of the BlueOS® technology by developing sublingual films with freely water soluble and poorly water soluble drug substances.

II. Orodispersible films to treat a neurodegenerative disorder- manufacturing process optimization

The goal of the present study was to investigate the root-cause for the observed ODFs drug release shift towards a slower drug release observed over time during storage. The application of QbD tools to the understanding of the manufacturing process of a novel dosage form, based on historical data of a product at a later development stage is demonstrated. This is called “retrospective QbD” (rQbD, i.e., QbD based on historical data), in opposition to the conventional QbD approaches that are oriented towards the development of new products. Based on this project, it was possible to define the manufacturing process design space that allowed a process adjustment to ensure a stable drug release profile over time.

2.1 Introduction

Solvent-casting is the most widely used manufacturing process of ODFs and comprises the preparation of a liquid mixture that is then casted in a planar surface, dried and cut to the desired dimensions [9]. The formation of a thin film occurs due to water evaporation that promotes the contact of polymer chains, and with further water evaporation the coalescence effect occurs resulting in a continuous film [65,66].

The film formation from aqueous polymeric dispersions in solvent-casting processes has been associated with changes in the drug release due to physical aging and further coalescence of the film after drying [65–68]. Several parameters can dictate the complete film formation and physical aging, such as the type and amount of plasticizer, temperature, relative humidity (RH) and drying time [65,66]. Therefore, a number of studies have been conducted to determine the most appropriate process parameters for achieving complete film formation during coating and curing of tablets, pellets or granules, in order to obtain stable drug release [66,67,69]. However, similar studies cannot be found in the literature regarding ODFs manufacturing process despite the similarities between the solvent casting technique and the coating process and, the increasing relevance of this novel dosage form. Additionally, this activity would significantly benefit from the adoption of systematic, evidence-based approaches for product development and process improvement, such as Quality by Design (QbD).

Even though this activity is critical nowadays for pharmaceutical companies, there is still a lack of studies in the literature regarding real manufacturing process applications, that could demonstrate the workflow and benefits of its implementation and the added-value it can bring to the companies [70,71]. Therefore, in this article the application of QbD tools to the understanding of the manufacturing process of a novel dosage form (ODFs), based on historical data of a product at a later development stage is demonstrated. This is called “retrospective QbD” (rQbD, i.e., QbD based on historical data), in opposition to the conventional QbD approaches (cQbD) that are oriented towards the development of new products. Although the application of rQbD can bring high benefits for pharmaceutical companies that have already long manufacturing records of their products (regardless the type of dosage form), almost no references can be found in the literature regarding its application. The present work intends to fill this important gap.

2.2 Problem elicitation

During product development, pre-stability studies have shown a clear decrease on drug release during storage under 25°C / 60% RH. A cause-and-effect diagram and a REM were constructed to identify potential CPPs that could impact ODFs CQAs. The cause-and-effect diagram was constructed based on authors' previous experience [14] as well as on related contributions from other researchers [12,68]. The REM was created through a qualitative analysis where each process parameter was ranked as high, medium or low-risk(s) level considering the probability of the risk and severity of the associated impact in the previous selected CQAs. High risk process parameters have an increased probability of affecting with high impact the CQAs. The CPPs identified as high risk factors were further evaluated with statistical tools.

Figure II.1 summarizes the overall workflow adopted for problem identification, assessment and determination of significant CPPs for ODFs' drug release and initial residual water content, based on historical data analysis.

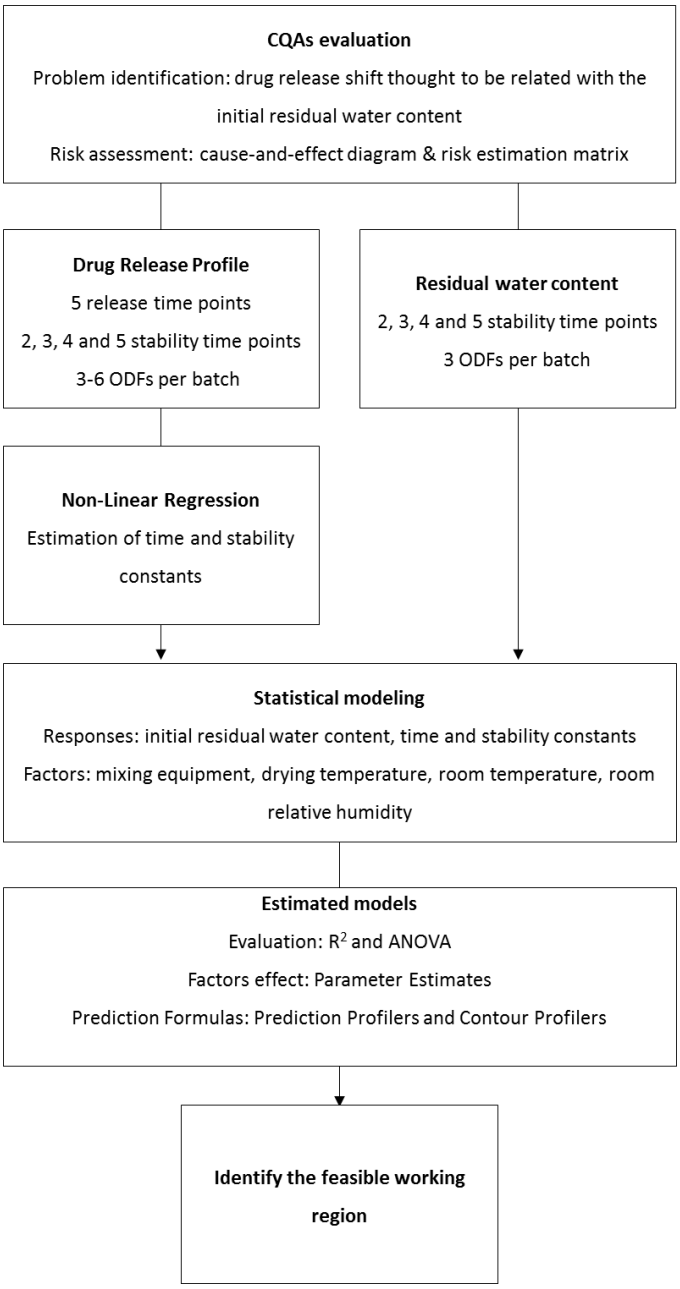


Figure II.1. Flow chart with the main retrospective QbD (rQbD) stages of the methodology followed for data analysis and statistical modeling.

2.3 Materials and Methods

2.3.1 Materials

Polyvinyl acetate dispersion (Kollicoat SR 30D, BASF-SE, Ludwigshafen, Germany); Polyvinyl alcohol 4-88 (Merck KGaA, Darmstadt, Germany); Carboxymethylcellulose Sodium (Blanose 7LF, Aqualon

France BV, Alizay, France); Carbopol 971NF (Lubrizol Advanced Materials, Waalwijk, Netherlands); Tryethyl citrate (Merck KGaA, Darmstadt, Germany); Lemon flavor (IFF, Hilversum, Netherlands); Sucralose (Merck KGaA, Darmstadt, Germany); Sodium Lauryl sulfate (BASF-SE, Ludwigshafen, Germany).

2.3.2 Manufacturing of ODFs

ODFs with a drug loading of 2,51% were prepared by the solvent casting technique as described elsewhere [52]. Briefly, the drug substance was dissolved in purified water and then added to a solution containing the plasticizer and the surfactant. The film-forming polymer and the remaining excipients were added sequentially to the previous solution until a homogenous mixture was obtained. The mixture was prepared with a 4-bladed propeller stirrer mounted in an electronic overhead stirrer (VOS 40D, VWR International, Carnaxide, Portugal) or with a disperser (Polytron PT 2500E, Kinematica, Luzern, Switzerland). The resulting liquid mixture was casted with a film applicator with adjustable gap height (Multicator 411, Erichsen, Hemer, Germany) and dried in a Coatmaster 510 (Erichsen, Hemer, Germany). After drying, the films were cut into pieces of 300 mm2 (Hand operated Press, Tinius Olsen Ltd, Salfords, England), packaged in OPA-ALU-PVC (FORMPACK® Coldform Laminate, Amcor, Singen, Germany) and stored at 25°C / 60% relative humidity (RH).

2.3.3 In vitro drug release

The drug release profile from the ODF samples were determined using a USP apparatus 5 (Agilent 708-DS, Agilent Technologies, Santa Clara, CA, USA) in 500mL of phosphate buffer (pH 6.8, with 0.5 % of tween 20) at 37 ± 0.5 °C with a rotation rate of 100 rpm. At each sampling time interval (2, 5, 10, 20, and 30 min) a sample was collected, filtered and analyzed by HPLC. Separation was achieved on a C-18 column, Luna 100 Å Phenomenex (50 mm x 3mm, 3µm). Mobile phase A (50mM phosphate buffer adjusted to pH 2.8 with 85% ortophosphoric acid) and mobile phase B (acetonitrile) at a flow rate of 1.2 ml/min were used for an isocratic and gradient elution. The gradient program (time (min)/%B) was set at 0/34, 7/34, 9/80, 11/34, 15/34. The column temperature was maintained at 35°C, the detection was monitored at 220nm using a PDA detector and the injection volume was 95 µL.

2.3.4 Residual water content

The residual water content was determined by Karl Fischer (KF) as described elsewhere [14]. The sample was added to the reaction vessel filled with methanol previously dehydrated with the KF reagent. A titration was carried out and water content was determined based on the titration volume.

2.3.5 Data analysis of drug release profiles through nonlinear regression

This study has the interesting feature that one of the response variables of interest is not a scalar entity (i.e., a univariate property, such as residual water content or disintegration time), but a profile – in this case a one-dimensional profile: the drug release profile curve. Situations like this are still uncommon, and lacking standard statistical regression solutions, but will tend to happen more and more in the future with the development of manufacturing processes, analytical technology and metrology [72]. Therefore, in order to handle the tensorial nature of the response variable (in this case a tensor of order 1) a procedure for converting this problem into a classic one, passible of being solved with the available statistical modeling tools, was developed. In this line, each drug release profile was first adjusted with parametric model of the curve that describes its behavior with high fitting quality. The estimated parameters of this model contain all the necessary information to reconstruct the original drug release profile, and were used as the new response variables. Therefore, in this way a drug release profile curve was transformed into a reduced set of parameters that will act as traditional response variables. More specifically, the following model was used:

$$DP^{[t_s]}(t_r) = 100 \times \left(1 - e^{-t_r/\tau(t_s)}\right)$$

[Equation 1]

$$\tau(t_s) = k + \beta \times t_s$$

[Equation 2]

where t_r is the release time, $t_r(min) = \{0, 5, 10, 20, 30\}$, $DP^{[t_s]}(t_r)$ is the drug release profile at the stability time t_s, $t_s(months) = \{0, 0.5, 1, 2, 3, 5, 6\}$ and k is a constant (intercept of the linear regression model); all the remaining parameters and quantities were described before and maintain the same meaning.

The set of parameters that will act as the new response variables is therefore composed by τ and β. These parameters have the following meaning: τ describes the drug release rate for each sub-batch at each stability time, high τ values mean slower drug release rate and, and β is related to the decreasing trend of the drug release rate over time for each sub-batch. The parameter β can be used as a stability trend indicator because for lower β values, the decrease in the drug release rate over time is smaller (i.e., the higher is the stability). Computations and statistical analysis were carried out in the software JMP, release 12 (SAS Institute Inc., NC).

2.3.6 Data modeling and design space definition

The high-risk CPPs identified through the Risk Estimation Matrix (REM) were carefully analyzed in order to identify the parameters that most significantly influence the drug release patterns and to finally define the design space region. The analysis of the influence consist in constructing predictive regression models for the responses of interest (initial residual water content, initial drug release rate (τ) and stability trend (β)), and analyze the parameters whose effect was found to be statistically significant (comparing the p-values associated with their partial regression coefficients with the adopted significance level of 0,05). With the models developed and validated, the design space was finally set through the simultaneous combination of all the individual acceptance regions for each CPP.

2.4 Results and Discussion

Polymeric aqueous dispersions are widely used in film coating of solid dosage forms, namely tablets and pellets. Polymeric organic solutions have been gradually abandoned due to the improved safety as well as higher economic and environmental performance of the aqueous dispersions [66,69,73]. However, the complexity of film formation in water-based dispersions has been associated with a decrease in drug release during storage [66,67,69]. Many studies have been published to study the impact of curing conditions on aqueous polymeric film coatings stability, though such studies, to the best of our knowledge, were not yet performed for ODFs. A slowdown of the drug release rate during pre-stability studies motivated a comprehensive analysis of existing quality data and on process parameters in the scope of a rQbD initiative.

In this section is provided an overview of the historical data used to conduct the rQbD activities (Sections 2.4.1 to 2.4.3), as well as a summary of the preliminary analysis of risk that lead to the

CPPs identified for this study (Section 2.4.4). The models developed are reported in Section 2.4.5 and the design space in Section 2.4.6.

2.4.1 Manufacturing conditions

The set of process parameters contemplated in the historical data used for rQbD are summarized in Table II.1, namely the mixing equipment used in each batch manufacturing as well as the room conditions during cast and the drying temperatures used. The stability time points considered are also present in Table II.1. The qualitative and quantitative composition of each batch was kept essentially the same across the period under analysis.

Table II.1. Mixing equipment, ODFs' drying conditions and stability time points.

Batch	Sub-batch	Room temperature °C	Room relative humidity %	Drying temperature °C	Mixing equipment	Stability time points Months
A	A.1	17,5	58,5	40	Disperser	0; 0,5; 1; 2 and 5
	A.2	20,2	58,7	60	Disperser	0; 0,5; 1; 2 and 5
	A.4	18,7	48,5	40	Disperser	0; 0,5; 1; 2 and 5
	A.5	24,3	35,7	40	Disperser	0; 0,5; 1; 2 and 5
	A.6	25,7	31	60	Disperser	0; 0,5; 1; 2 and 5
	A.3	24,6	50,3	40	Disperser	0; 0,5; 1; 2 and 5
B	B.1	17,4	61,68	40	Disperser	0 and 0,5
	B.2	23,9	41,35	40	Disperser	0; 0,5 and 1
C	C.1	21,35	42,2	40	Mixer	0; 0,5; 1; 2 and 3
	C.2	21,75	41,7	50	Mixer	0; 0,5; 1; 2 and 3
	C.3	22,4	41,8	60	Mixer	0; 0,5; 1; 2 and 3
D	D.1	23,2	43,75	40	Mixer	0, 0.5, 1, 2 and 3
	D.2	23,9	41,5	50	Mixer	0, 0.5, 1, 2 and 3
	D.3	24,3	40,55	60	Mixer	0, 0.5, 1, 2 and 3
E	E.1	23,7	35,67	40	Disperser	0; 0,5; 1; 2; 3 and 6
F	F.1	22	30	40	Mixer	0; 0,5; 1; 3 and 6

2.4.2 Drug release profile during storage

Figure II.2 shows the drug release profiles immediately after manufacturing and at different time points (0,5; 1; 2 and 5 months). Each time point shown in Figure II.2 corresponds to the average of three independent determinations. From the analysis of this figure, it is possible to observe that the

drug release profiles in each sub-batch (ODFs obtained from the same liquid mixture batch and casted at different conditions as depicted in Table II.1) present a small initial variation, and that there is a reduction of drug release rate over time. It is also evident from the analysis of Figure II.2 that the rate of drug release varies significantly from sub-batch to sub-batch, as well as across stability time points.

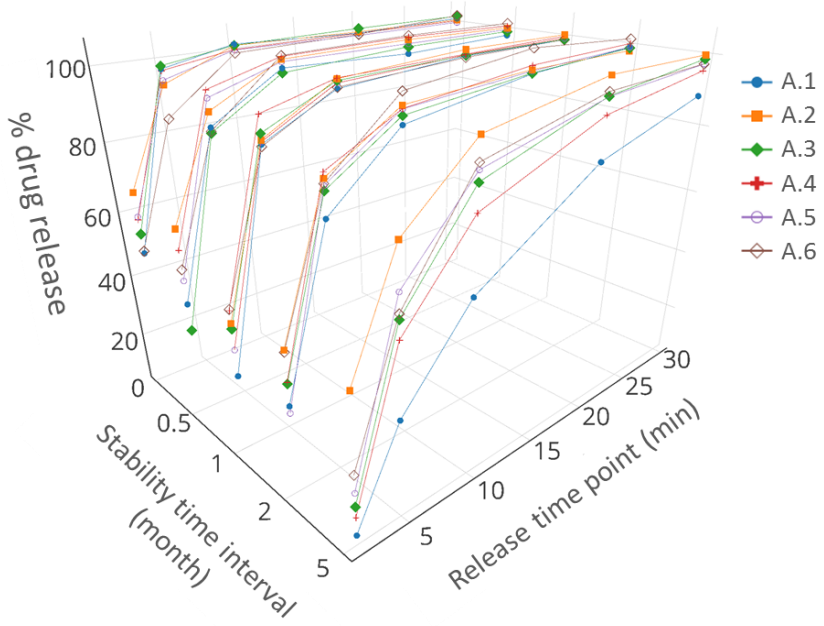


Figure II.2. Drug release profiles (% release vs min) of sub-batches A.1, A.2, A.3, A.4, A.5 and A.6 at different times for stability analysis (initial, 0,5, 1, 2 and 5 months). Storage conditions: 25°C / 60% RH.

2.4.3 Residual water content over time during storage

As mentioned before, the amount of residual water content in ODFs' expected to influence drug release due to incomplete film formation. Figure II.3 A to F show the residual water content of each sub-batch at different stability time points. From the analysis of these plots, it is possible to verify that ODFs dried at 60°C (A.2, A.6, C.3 and D.3) exhibit in general lower percentage of residual water content when compared with the other ODFs casted at lower drying temperature (40 and 50°C). An exception is observed for C.3 samples, where the initial average residual water content is higher than the initial average residual water content of C.1 and C.2 (see Figure II.3 C), although the

difference is not statistically significant. In most samples, it is observed a decreasing tendency for residual water content with storage time. However, such the decrease was only found to be statistically significant in the following cases: A.2 initial vs. 5 months (**p≤0,01), A.5 initial vs 5 months (***p≤0,001), D.3 initial vs 5 months (*p≤0,05).

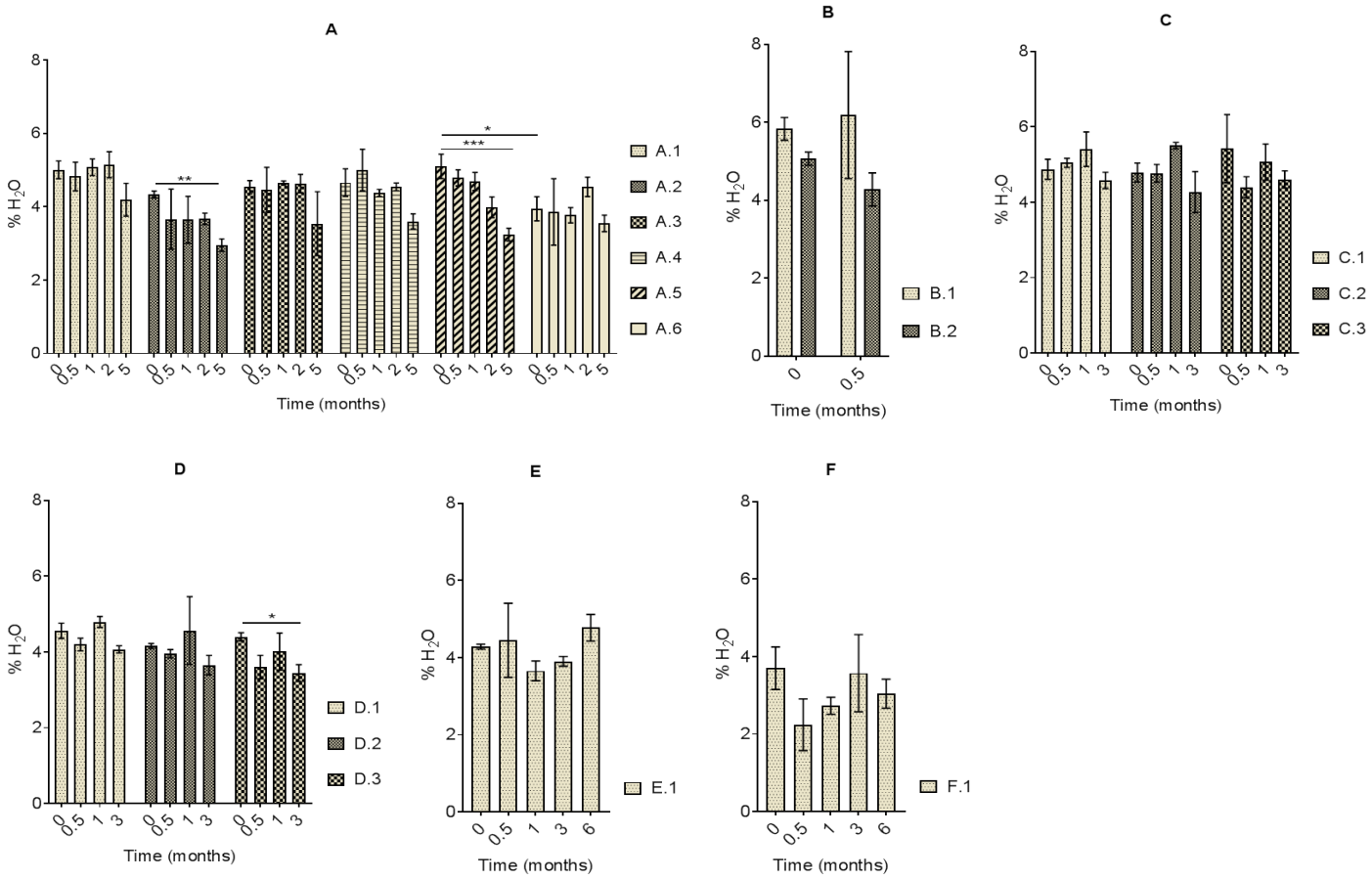


*Figure II.3. Residual water of initial, 0,5, 1, 2 and 5 months stability samples (storage at 25°C / 60% RH). A- data of sub-batches A.1 to A.6; B- data of sub-batches B.1 and B.2; C- data of sub-batches C.1, C.2 and C.3; D- data of sub-batches D.1, D.2 and D.3; E- data of sub-batch E.1; F- data of sub-batch F.1. The results are represented as average±SD (ns p>0,5, *p≤0,05, **p≤0,01, ***p≤0,001 by one-way ANOVA; post hoc Tukey's test)*

2.4.4 Preliminary risk assessment

In order to establish the set of potential cause-effect relationships, a cause-and-effect diagram for the ODFs was constructed (Figure II.4) based on previous experience [14] and as described elsewhere [12,68]. This diagram allowed for categorizing environmental, man, raw materials and process and product variables according to their *a priori* importance for explaining the CQAs variation. From all these variables, the ones that were not changed in the manufacturing of the different batches were not considered for further analysis. The remaining four CPPs (type of mixing equipment, drying temperature, room temperature and room RH) used in the following evaluation were in accordance with the information available in the literature [12,14,68,69] .

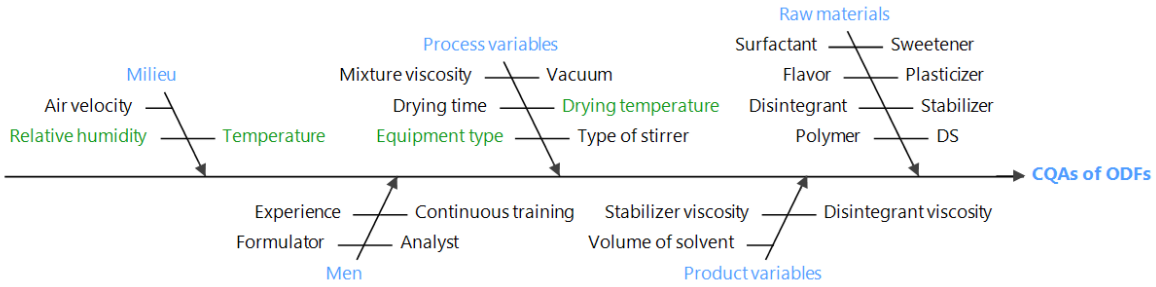


Figure II.4. Cause-and-effect diagram representing the possible interference of different factors (raw materials, product variables, process variables, men and milieu) with ODFs critical quality attributes (CQAs). The factors that changed during the manufacturing of different batches and that were considered for further analysis are represented in green.

Once the process parameters that may influence the CQAs variability were identified, the next step was to rank them according to their potential criticality in terms of risk. For that purpose, a REM was constructed to access the impact of process parameters that changed in the historical data, namely mixing equipment, drying temperature, room temperature and room RH (Table II.2) in the CQAs. The qualitative analysis for each parameter was performed by ranking them as: high, medium or low-risk(s) level considering the probability of the risk and severity of the associated impact.

Table II.2. Initial risk assessment of ODFs based on the Risk Estimation Matrix (REM). Each CPP was qualitatively ranked as high, medium or low-risk(s) level considering the probability of the risk and severity of the associated impact on the CQAs

ODFs CQAs	Critical Process Parameters (CPP)			
	Mixing equipment type	Drying temperature	Room temperature	Room relative humidity
Assay	Low	High	Low	Low
Content uniformity	Low	Low	Low	Low
Drug release	Medium	High	Medium	High
Related Substances	Low	High	Low	Low
Disintegration time	Medium	High	Low	Medium
Residual water content	High	High	Medium	High
Mechanical properties	Medium	High	Low	High

The type of mixing equipment impacts the separation of the polymer molecules in the solvent which influences the mixture viscosity and the water retention properties [74]. Therefore, the type of mixing equipment is a high risk CPP for residual water content. Drying temperature is associated with a high risk for all CQAs with the exception of content uniformity. High drying temperature can promote drug substances degradation resulting in lower assay as well as in increased amount of related substances/impurities. Additionally, the film formation is influenced by the drying process which impacts on the residual water content, mechanical properties and drug release. Room temperature represents a low to medium risk to all CQAs. The ODFs are not dried in an oven; therefore the room temperature together with the room RH may influence the residual water content and drug release. ODFs can easily absorb water that may influence ODFs' CQAs such as drug release, residual water content and mechanical properties; thus, room RH constitutes a high risk for these CQAs.

2.4.5 Effect of CPPs on the CQAs residual water content and drug release

The quality control analysis of the different batches showed that all CQAs were within the specification limits (data not shown) with the exception of the stability of drug release. As film formation from aqueous polymeric dispersions are known to be associated with changes in drug release rate due to water retention and incomplete film formation [65,66] the influence of high risk CPPs on drug release and residual water content was further assessed.

The model parameters were estimated using ordinary Standard Least Squares. In this context, models relating the CQA's (initial residual water content, initial drug release rate (τ) and stability trend (β)) with the identified CPPs were developed and analyzed. The CPPs analyzed included the mixing equipment, drying temperature, room temperature and room RH, as well as second order interaction effects that were possible to be reliably estimated from historical data, namely: drying temperature vs room RH and drying temperature vs room temperature. All models were thoroughly assessed from the standpoint of their fitting quality and statistical significance by means of the coefficient of determination (R^2) and several statistical hypothesis tests, such as ANOVA and individual tests to the significance of the regression parameters (p-value) as well as the possible presence of collinearity (Variance Inflation Factors, VIF). Non-significant terms were removed from the model. Table II.3 and Table II.4 summarize the results obtained.

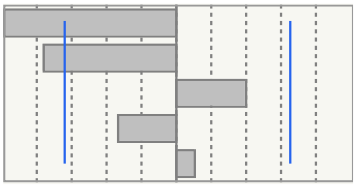
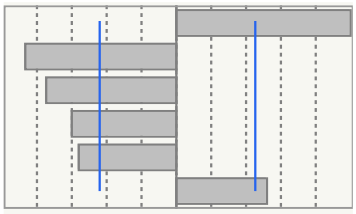
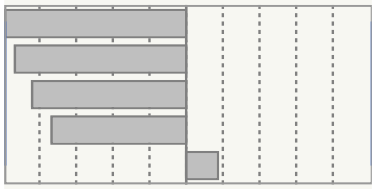
Table II.3. Summary of Least Squares Fit for each response.

	Summary of Fit		ANOVA
Responses	**RSquare**	**RSquare Adj**	**Prob>F**
Initial residual water content	0,695	0,543	0,0200
Initial drug release rate (τ)	0,798	0,663	0,0094
Stability trend (β)	0,447	0,171	0,2418

Table II.4 presents the CPPs coefficient estimates in the models developed for CQAs: initial residual water content, initial drug release rate (τ) and stability trend (β). The initial residual water content is mostly influenced by the interaction between room RH vs drying temperature and room

temperature vs drying temperature, while these parameters individually show no significant effect as denoted by the p-value >0,05. In the case of initial drug release rate (τ), all parameters were found to have a significant impact. The mixing equipment Disperser and the drying temperature have a positive effect on initial drug release rate (τ), whereas room RH, room temperature and the studied interactions all have negative effects. None of the studied parameters present a significant effect on stability trend (β). Nevertheless, room temperature, room RH and the interactions between RH versus drying temperature and room temperature versus drying temperature appear to have a negative effect, while drying temperature has a positive effect, which could became more evident if more data was available, increasing the power of the methods. These quantitative findings are consistent with the data reported elsewhere [66,67,75], where the drug release from pellets coated with aqueous polymeric dispersion was observed to depend on temperature and relative air humidity during manufacturing. The inclusion of stability trend (β) in the following analysis would not be strictly required considering that no significant parameters were found to impact this CQA. However, given the importance of this property to evaluate ODFs stability, it was decided to proceed with its inclusion as the trends predicted by the model are in good agreement with the existing background knowledge.

Table II.4. Sorted effect estimates for factors/ Critical Process Parameters (CPPs) used in the model.

Sorted Parameter Estimates		**Response: Initial residual water content**			
Term	**Estimate**	**Std Error**	**t Ratio**		**Prob>|t|**
Room relative humidity*Drying temperature	-0,00971	0,002796	-3,47		0,0060*
Room temperature*Drying temperature	-0,03447	0,012976	-2,66		0,0240*
Drying temperature	0,020236	0,014306	1,41		0,1876
Room temperature	-0,10212	0,086671	-1,18		0,266
Room relative humidity	0,0071	0,019749	0,36		0,7267
		Response: Initial drug release rate (τ)			
Mixing equipment [Disperser]	0,444819	0,08749	5,08		0,0007*
Room relative humidity %	-0,07841	0,017725	-4,42		0,0017*
Room temperature	-0,2815	0,073988	-3,8		0,0042*
Room relative humidity*Drying temperature	-0,00709	0,002315	-3,06		0,0136*
Room temperature*Drying temperature	-0,03094	0,010837	-2,85		0,0189*
Drying temperature	0,033439	0,01243	2,69		0,0248*
		Response: Stability trend (β)			
Room temperature	-0,33681	0,152402	-2,21		0,0516
Room relative humidity*Drying temperature	-0,0102	0,004917	-2,07		0,0648
Room temperature*Drying temperature	-0,04277	0,022816	-1,87		0,0904
Room relative humidity	-0,05639	0,034727	-1,62		0,1355
Drying temperature	0,009665	0,025156	0,38		0,7089

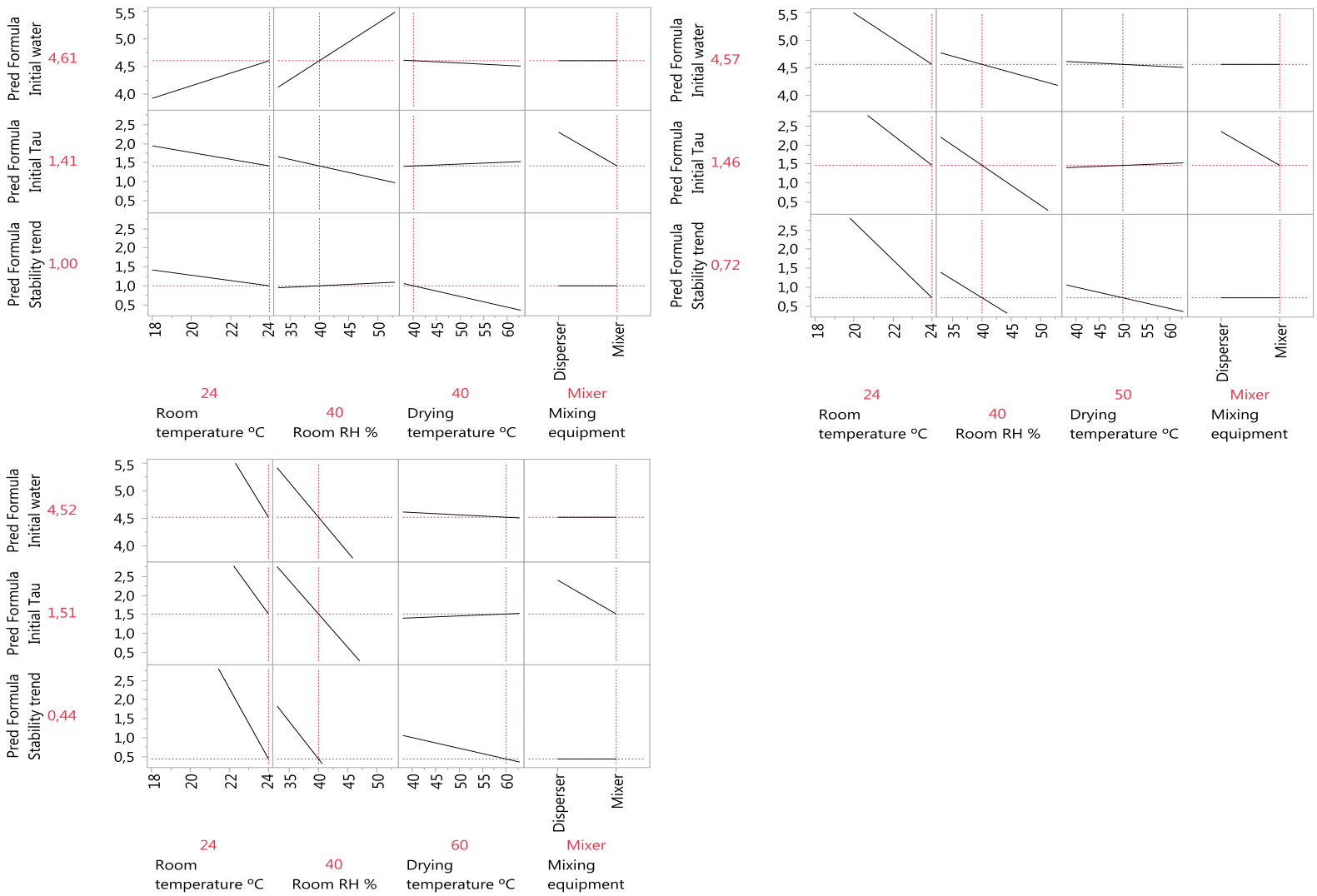


Figure II.5. Prediction profilers for initial residual water content, initial drug release rate (τ) and stability trend (β) considering different drying temperatures 40°C (top left), 50°C (top right) and 60°C (bottom left). The black lines represent the prediction trace, the vertical red lines correspond to the current value of the factor and, the red value on the vertical axis is the predicted response based on the current values of the factors.

2.4.6 Development of the design space

The prediction formulas developed for each CQA were used to construct the design space for the ODF's. Stability trend (β) is a very important factor to determine the stability of the drug release profile over time, and therefore it was also included in the predictive analysis despite the reduced variation explaining power of the model.

Figure II.5 displays the local dependencies of the different models regarding all the CPPs. More specifically, it illustrates the case where the drying temperature varies while the remaining CPPs are kept at constant levels (room temperature, room RH and type of mixing equipment). Small variations are observed for initial residual water content and initial drug release rate (τ) models, but a large variation can be observed in the case of the model for stability trend (β). Also, the black line slope differs depending on the drying temperature. A steep slope indicates that minor variations on the factor results in major changes in the response, whereas a negative slope denotes a negative effect and a positive slope implies a positive effect. For example, at 40°C of drying temperature there is a positive effect of room temperature and room RH on initial residual water content, whereas these effects are negative at 50°C and 60°C of drying temperature. This result is a consequence of the presence of strong interaction terms in the model, involving these CPPs and is indicative of the complexity of film formation phenomena, where the influence of parameters should be studied simultaneously and not in an independent fashion [66,67]. Based on the prediction profilers the initial risk assessment was reviewed where, for initial residual water content the mixing equipment should now be considered a low risk parameter and the room temperature a high risk parameter and, for the drug release all the CPPs should now be considered as high risk parameters.

In order to define the design space (i.e., the feasible working region from the standpoint of all relevant product properties), the limits for the initial residual water content, initial drug release rate (τ) and stability trend (β) were defined in order to meet the desired QTPP. The residual water content should be between 3% and 4,5%, initial tau should be below 2 and stability trend should be less than 0,5. Figure II.6 presents the design space for each tested drying temperature when a Mixer is used. The feasible working region corresponds to the white zone. In theory, 40°C would be the preferred drying temperature, due to potential drug substance degradation and impurities formation, but it was not possible to determine the feasible working region at this condition (Figure II.6). For drying temperatures of 50°C and 60°C, a narrow design space can already be established

(Figure II.6) and the quality data showed that no increase on the impurities content occurred on ODFs dried at these temperatures, which is indicative that 50°C and 60°C can be used as drying temperatures without compromising the ODFs quality. At a drying temperature of 50°C, it is feasible to work at room temperature of 23°C as long as the room RH is above 50%. If lower room RH is set, then a higher level of the room temperature is required (Figure II.6). At a drying temperature of 60°C, it is possible to work at room temperatures lower than the verified at drying temperature of 50°C (Figure II.6).

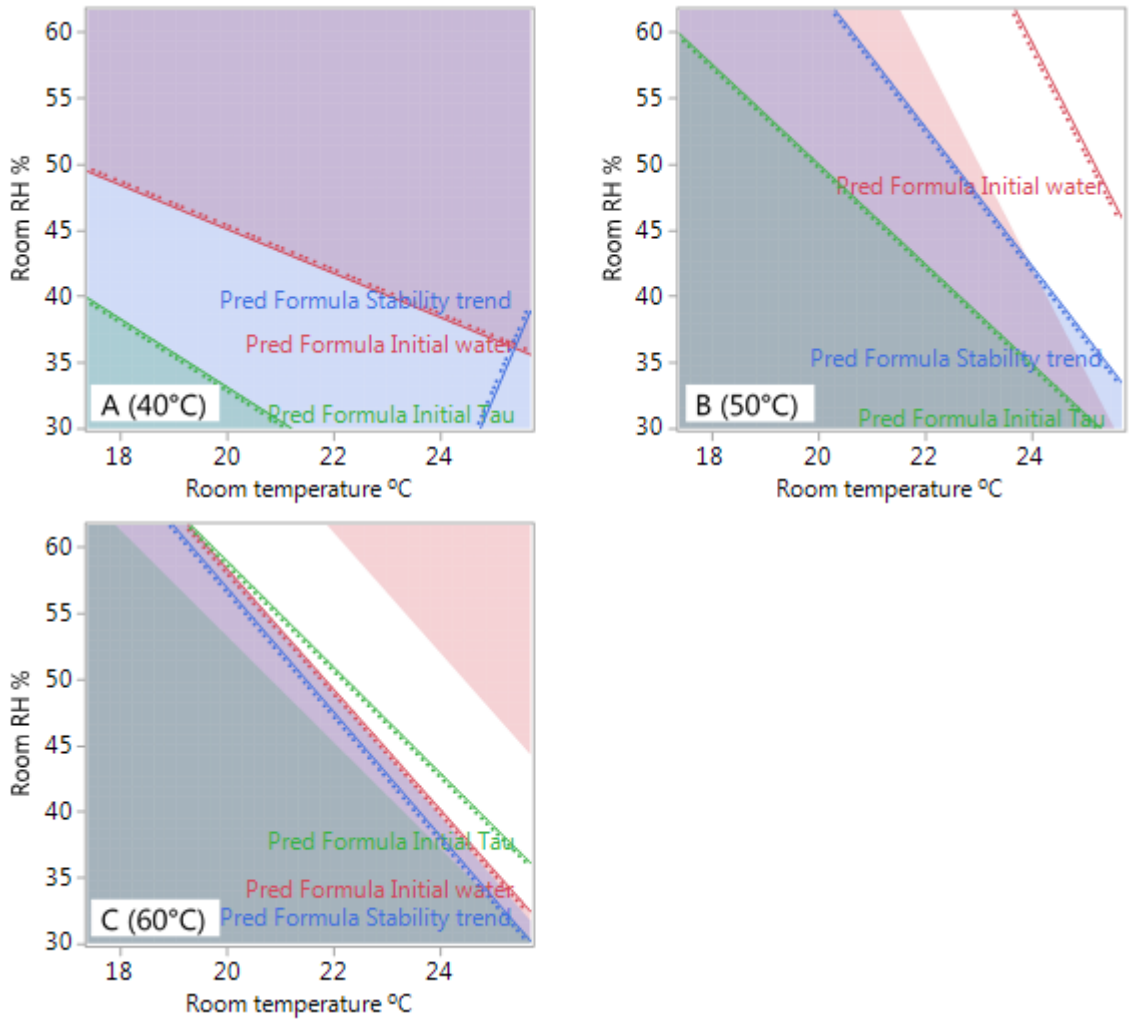


Figure II.6. Design space for different drying temperature when Mixer is used: 40 ℃ (A), 50 ℃ (B) and 60 ℃ (C). The unshaded white area represents the feasible working region and the dotted lines show the direction of increasing response values. Each response is represented by a color, initial residual water content is red, initial drug release rate (τ) is green and stability trend (β) is blue.

2.5 Conclusions

Despite the limitations, the application of advanced statistical tools such as visualization, predictive modeling (linear and non-linear), profilers and contour graphs, enabled the assessment of the significance of CPPs and to establish a design space. Another interesting aspect of this work dealt with the nature of one of the response variables (the drug release profile curve), which was not a scalar quantity, but a profile (a one-dimensional tensor), which implied the application of an innovative procedure in order to carry on with the rQbD approach. This procedure consisted in parametrically modeling the curves and using the parameters as the new CQAs. The work performed in this chapter exemplifies the application of QbD principles using retrospective data (rQbD) and illustrates its added value for increasing the knowledge of ODFs manufacturing process. Additional work should include now the adoption of conventional QbD and the usual DoE trials in order to extend the knowledge space and to validate the findings presented here. Nevertheless, this study established that some of the principles applied to polymeric coating of dosage forms can indeed be extended to ODFs.

III. Orodispersible films to treat a neurodegenerative disorder- clinical trial

A proof-of-concept clinical trial was conducted in order to validate, in the clinical setting, the performance and acceptability of investigational ODFs to treat a neurodegenerative disorder, that is often associated with physical disabilities and swallowing difficulties. In this section the results of the clinical trial conducted to evaluate the comparative bioavailability of ODFs, developed and manufactured according to the BlueOS® technology, with a commercially available product containing the same dose of drug substance in the form of capsules (RP) are presented. The clinical study evidenced that the test and RP exhibited comparable pharmacokinetics, safety and tolerability and that the ODFs had a fast disintegration in the mouth and a favorable taste acceptability.

3.1 Introduction

Several dosage forms have been purposed to overcome the swallowing difficulties of tablets and capsules such as gels, liquids, effervescent and orodispersible dosage forms [76–79]. Liquid formulations have some limitations like the need of measuring, dose inaccuracy, risk of chocking and aspiration, physical, chemical and microbial stability. Effervescent dosage forms require water availability and a waiting period to obtain complete dissolution [76,80]. Orodispersible films (ODFs) along with other orodispersible dosage forms (tablets, minitablets, granules, powders) offer several advantages such as ease of administration, no need for water intake and dose accuracy. Orodispersible films are preferred over other orodispersible formulations due to their faster disintegration, ease of handling, decreased risk of particles aspiration and better patient compliance [10,22,77]. These characteristics are particularly relevant for patients with neurodegenerative disorders frequently affected by dysphagia because the drug substances used in the treatment of such diseases are usually administered in the form of injectables, tablets or capsules. Therefore, ODFs containing the same dose of drug substance as in the commercially available capsules, were developed and their pharmacokinetics, safety and tolerability were assessed in healthy subjects.

Clinical trials are conducted to answer specific research questions regarding the product under development. They should be designed, conducted and analyzed according to sound scientific principles and the study objectives should be clearly defined. Therefore, a step-wise approach should be followed in which information from previous studies are used to support and plan the new studies [81]. The model DS undergoes phosphorylation to produce phosphate metabolite, the active moiety. The pharmacokinetic profiles of DS and its phosphate metabolite have been extensively investigated in healthy subjects, renal transplant patients and in special populations. These studies demonstrated that the DS has an oral bioavailability of more than 90% and its absorption is not affected by food intake. Single-dose studies showed that DS blood levels increase slowly to reach a broad plateau region for 6 to 48 hours, with a median t_{max} between 12 and 28 hours post-dosing. Both DS and phosphate metabolite have a half-life of 6 to 9 days and the steady-state is reached after 1 to 2 months of daily dosing. Furthermore, a low to moderate intersubject pharmacokinetic variability was observed in these studies. This information is essential to choose the appropriate study design, to select the subjects, to determine the sample size and, to define the measurement plan and the statistical analysis plan [81,82]. The quality and reliability of the results is highly dependent on the representativeness of the sample population, the standardization of the

measurement conditions and the sample size. For example, if the sample size is small, real differences will not be identified. On the other hand, too large sample size unnecessarily exposes subjects to stress and possible adverse events [81,82].

3.2 Materials and Methods

3.2.1 Study design

This study was a single-center (Blueclinical Ltd, Porto, Portugal), open-label, single-dose, laboratory-blinded, randomized, parallel-group study in healthy subjects under fasting conditions. Twenty-four healthy male and female volunteers were selected and twelve subjects were randomly assigned to each treatment group: test group received a single dose of ODF and the reference group received a single dose of reference product (RP) commercially available. The product administration occurred following at least 10 h of fasting. The subjects were confined to the study site from at least 12 h before dosing until at least 48 h post-dosing and, returned for the 72 h post-dose assessments. Follow-up visit occurred about 14 (±3) days after dosing. The study protocol and informed consent were approved by the CEIC – Ethics Committee for Clinical Research (Avenida do Brasil, Lisboa, Portugal), Infarmed, I.P. (Portuguese National Competent Authority) and CNPD (National Data Protection Committee). The clinical trial was conducted in compliance with the Declaration of Helsinki, GCP, the EMA regulations and the applicable Portuguese laws and regulations.

3.2.2 Study participants

Subjects were eligible for study participation if they fulfill all the inclusion criteria: able to understand and willing to adhere to all the requirements of the study as confirmed by giving voluntary written informed consent for participation; male or female gender; age between 18 and 40 years; Body Mass Index (BMI) within 18,5 – 30,0 kg/m^2; non-smoker or ex-smoker (stopped at least 6 months ago); healthy as determined by pre-study medical history, physical examination, vital signs, and 12-lead ECG; clinical laboratory test results within the normal range, at screening (if not within the normal range, abnormalities must be without clinical significance); negative tests for HBsAg, anti-HCVAb, anti-HIV1Ab and anti-HIV2Ab; negative result in an ethanol breath test; negative results in a drugs-of-abuse test in urine; if woman, she is not of childbearing potential or she agrees to use an acceptable contraceptive measure for the entire duration of the study and for at least 60 days after study completion.

Subjects were excluded from the study if they fulfill any of the exclusion criteria: history of severe hypersensitivity reactions to any medicines; known hypersensitivity to the drug substance or any of the investigational products excipients; history of gastrointestinal (GI), renal or hepatic disease, or surgery that may affect drug bioavailability; history of clinical significant peptic ulceration or active gastrointestinal bleeding, or of any other clinical significant GI disease or disorder; presence of any significant respiratory, gastrointestinal, renal, hepatic, hematological, lymphatic, neurological, cardiovascular, psychiatric, musculoskeletal, genitourinary, immunological, dermatological, endocrine, or connective tissue disease or disorder; history of any cardiac disease; presence of any abnormality in ECG morphology or ECG parameters, as assessed by a Cardiologist; family history of sudden death or relevant arrhythmia; presence of hypertension or hypotension with clinical significance; resting heart rate lower than 55 beats per minute; past or recent history of myocardial infarction, unstable angina, stroke, transient ischemic attack, decompensated heart failure requiring hospitalization, or Class III/IV heart failure; history of Mobitz Type II 2nd degree or 3rd degree atrioventricular (AV) block or sick sinus syndrome; baseline QTc interval ≥450 msec; history of symptomatic bradycardia or recurrent syncope; known immunodeficiency syndrome; with increased risk for opportunistic infections, including immunocompromised patients; history of chronic infection (e.g., hepatitis, tuberculosis); administration of live or attenuated vaccines scheduled to occur during the study and at two months after the end of the study; history of uveitis; history of any malignancies; any degree of liver impairment; history of alcoholism or drug abuse; history or presence of piercings in the mouth (e.g. tongue) or wearing braces or dentures; consumption of more than 14 units of ethanol a week; difficulty in collecting blood; any significant illness in the previous 28 days before admission to study period; use of any prescribed drugs in the previous 28 days before admission to study period, excepting for birth control medications; use of any Over-The-Counter (OTC) medicinal products (including food supplements, herbal supplements or vitamins) within 7 days before admission to study period, excepting for topical products without systemic absorption; use of a depot injection or an implant of any drug within 3 months prior to screening, excepting for birth control medications; used any investigational drug or participated in any clinical trial within 3 months prior to screening; participated in more than 1 clinical trial within the 12 months prior to screening; donated blood or had plasmapheresis in the previous 3 months prior to screening or had history of significant blood loss (≥ 350 mL) due to any reason; has been on a significantly abnormal diet during the 4 weeks preceding the first dose of study medication; any difficulty fasting or has any dietary restrictions such as lactose intolerance, vegan, low-fat, etc. that

may interfere with the diet served during the study; if woman, she has a positive pregnancy test; if woman, she is currently breast-feeding; if woman, her menstruation day(s) coincide(s) with the dosing day of the study; consumed pomelo pomegranate, starfruit or grapefruit products (fresh, canned, or frozen) from 7 days prior to admission to study period; if any surgical or medical condition exist that in the judgment of the investigator might interfere with the absorption, distribution, metabolism or elimination of the study drug, or, is likely to compromise the safety of subject; difficulties in swallowing tablets/capsules; intake of unusual diet (e.g. low sodium) for two weeks prior to admission to study period and not willing to avoid consumption of such diet until study completion; regular consumption of beverage or food containing methylxanthines (e.g. coffee, tea, cola, sodas, chocolate) equivalent to more than 500 mg methylxanthines per day; cannot communicate reliably with the investigator; not willing to co-operate with the requirements of the study.

3.2.3 Pharmacokinetic sampling

Several blood samples were collected prior to the administration of the test or the RP and over 72 h after dosing. Blood levels of the drug substance and its phosphate metabolite were determined by a LC-MS/MS validated method at Algorithme Pharma Inc. (Laval, Quebec, Canada). The validated concentration ranges were 20,0 to 2000,0 pg/mL for drug substance and 100 to 3000 pg/mL for metabolite. The coefficient of variation (% CV) was ≤15,0% for both. Samples with “no peak” or with calculated concentration lower than the limit of quantitation were reported as Below the Limit of Quantitation (BLQ).

3.2.4 Pharmacokinetic analysis

Pharmacokinetic parameters were determined using non-compartmental analyses with Phoenix™ WinNonlin™ 6.3 (Pharsight Corporation, Mountain View, California, USA). Pharmacokinetic parameters were estimated from the blood drug concentration versus time profile and included maximum observed blood concentration post dose (C_{max}); time to C_{max} (t_{max}); area under the blood concentration versus time curve (AUC) from time zero to the last sampling time at which concentrations were at or above the lower limit of quantification, calculated by the linear trapezoidal rule (AUC_{0-72}); apparent terminal elimination rate constant (λ_z) and apparent terminal elimination half-life ($t_{1/2}$), calculated from ln 2/ λ_z.

3.2.5 Relative bioavailability analysis

The relative bioavailability evaluation was determined by one-way analysis of variance (ANOVA). For the drug substance, ANOVA was performed on the body-weight adjusted ln-transformed C_{max} and AUC_{0-72} and for the metabolite it was performed on the non-adjusted ln-transformed C_{max} and AUC_{0-72}. The geometric least-square means and the 90% confidence intervals (CI) were calculated for the pharmacokinetic comparability between ODFs and RP. Pharmacokinetic comparability of test and reference products was concluded if the 90% CI fell within the 80,00-125,00% limits for C_{max} and AUC_{0-72} [83,84].

3.2.6 ODFs disintegration time and Taste acceptability

The taste acceptability assessment of ODFs was based in a numerical scale that was recorded immediately after ODF dosing as well as 30 seconds, 1, 2 and 5 minutes later. The scale consisted in 5 different levels: 0 – tasteless; 1 - acceptable bitterness; 2 – slight bitterness; 3 – moderately bitterness; 4 – strong bitterness. Additionally, the time until complete disintegration of the ODF was also evaluated.

3.2.7 Safety assessments

Only healthy subjects were eligible for the study as determined by pre-study medical history, physical examination, vital signs, ECG, and clinical laboratory tests. Subjects' safety was assessed throughout the study by monitoring adverse events (AEs), vital signs, 12-lead ECG, and hematology and plasma biochemistry. Clinically significant abnormalities in clinical laboratory, physical examination (including oral cavity examination), vital signs and ECG are reported as AEs. All AEs were tabulated and summarized according to the Medical Dictionary for Regulatory Activities (MedDRA version 19.0) and classified by system organ class (SOC) and preferred term (PT).

3.2.8 Statistical analysis

Statistical analysis, ANOVA and calculation of the 90% CI for the Test-to-Reference GMR were performed using SAS® 9.3 (SAS Institute Inc, Cary, NC, USA). Due to the noticeable between-group difference in mean body weight, drug substance pharmacokinetic parameters were normalized to body weight before statistical analysis. A normalization factor (NF) was obtained by dividing the dose (in mg) by the body weight (in kg) and the individual pharmacokinetic parameter was then divided by the determined NF.

3.3 Results and Discussion

ODFs are a very convenient dosage form to patients who have difficulties in swallowing, which was the main motivation for the development of an ODF formulation for these type of patients [43,44]. To validate this assumption, a proof-of-concept clinical trial was conducted in order to investigate, in the clinical setting, the performance and acceptability of investigational ODFs to treat a neurodegenerative disorder. The pharmacokinetic parameters of the investigational ODFs and the commercially available product containing the same dose of drug substance in the form of capsules as well as the acceptability of the ODFs, as assessed by the taste evaluation, and the disintegration time, were determined in healthy volunteers. The data was statistically compared to evaluate their relative bioavailability.

3.3.1 Subjects demographics

A total of 24 healthy male and female subjects were enrolled in this study, 12 subjects received a single dose of capsules (RP) and the other 12 received a single dose of ODFs (test). No early withdraws occurred in any of the treatment groups. Demographic data is summarized in Table III.1.

Table III.1. Baseline demographics of study participants.

	Test group (n=12)	**Reference group (n=12)**
Age (years) A ± SD	29±6	26±5
Weight (kg) A ± SD	71±14	61±12
Height (cm), A ± SD	169±8	166±7
Sex [n (%)]	7 males (58,3), 5 females (41,7)	4 males (33,3), 8 females (66,7)

n number of subjects, *A* average, *SD* standard deviation

3.3.2 Pharmacokinetics of the drug substance

The blood concentrations obtained with the RP and investigational ODFs and the estimated pharmacokinetic parameters are given in Figure III.1 (linear and log-linear scales) and Table III.2. Drug substance blood concentrations increased slowly and reached peak levels at approximately 11 h and 13 h after ODFs dosing and capsules dosing, respectively. The C_{max} was about 329,6 pg/mL for ODF group and 377,1 pg/mL for reference and it decreased slowly over time (λ_z of 0,01 h^{-1} in both groups) with a $t_{1/2}$ of approximately 62,38 h and 89,06 h respectively. Overall, the mean blood concentration-time profiles were similar for test and reference groups.

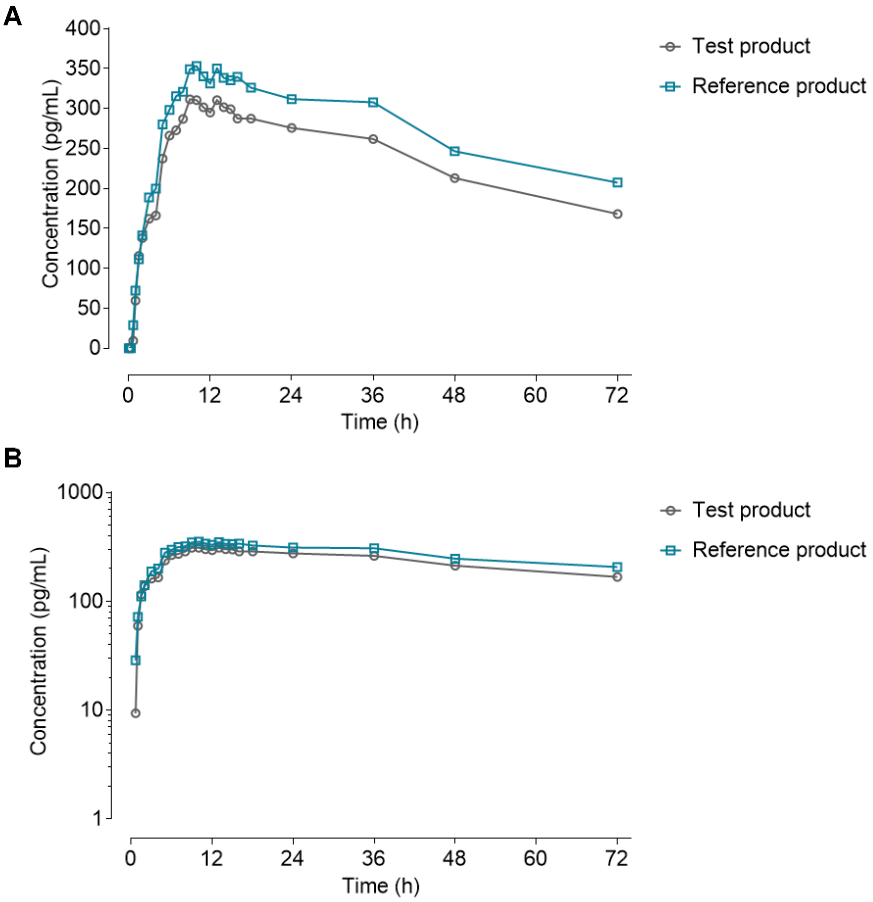


Figure III.1. Drug substance mean blood concentration versus time profile following single oral administration of ODFs (test product) and capsules (RP) to healthy subjects (linear (A) and log-linear (B) scales).

Table III.2. Pharmacokinetic parameters (average ± SD) of drug substance following oral administration of ODFs and capsules to healthy subjects.

	Test group (n=12)	**Reference group (n=12)**
C_{max}, pg/mL	329,6 ± 63,0	377,1 ± 95,2
t_{max}, h	11,00 ± 2,52	12,50 ± 4,70
AUC_{0-72}, pg.h/mL	16798,4 ± 3693,8	19474,8 ± 4721,1
λ_z, 1/h	0,01 ± 0,00	0,01 ± 0,00
$t_{½}$, h	62,38 ± 18,62	89,06 ± 60,15
NF mg/kg	0,00726 ± 0,00139	0,00839 ± 0,00144
C_{max} /NF pg/mL/(mg/kg)	46193,2 ± 9311,9	45165,0 ± 9112,4
AUC_{0-72} /NF pg.h/mL/(mg/kg)	2344466,9 ± 476934,2	2343631,0 ± 501682,0

SD standard deviation, *n* number of subjects, C_{max} maximum observed blood concentration post dose, t_{max} time to C_{max}, AUC_{0-72} area under the blood concentration versus time curve from time zero to 72 h, λ_z apparent terminal elimination rate constant, $t_{½}$ apparent terminal elimination half-life, *NF* normalization factor

No statistically significant difference (p>0,05), between subjects taking ODFs and subjects taking capsules, was observed in both regular and body weight-adjusted C_{max} and AUC_{0-72} (Table III.3). Additionally, with the body weight-adjustment the test-to-reference geometric means ratio (GMR) of C_{max} /NF, and AUC_{0-72} /NF and the 90% confidence interval were contained within the standard bioequivalence range of 80,00 to 125,00% (Table III.3).

Table III.3. Drug substance least square means, test-to-reference geometric means ratio and 90% confidence intervals for C_{max}, AUC_{0-72}, C_{max} /NF, and AUC_{0-72} /NF.

Parameter	ANOVA p-value	Geometric LSmeans		Test/Reference GMR (%)	90% CI
		Test	Reference		
C_{max}	0,210	324,0	365,8	88,58	75,38-104,08
C_{max} /NF	0,766	45395,0	44284,5	102,51	89,02-118,04
AUC_{0-72}	0,158	16434,9	18925,7	86,84	73,57-102,50
AUC_{0-72} /NF	0,955	2302485,6	2291149,2	100,49	86,59-116,63

C_{max} maximum observed blood concentration post dose, AUC_{0-72} area under the blood concentration versus time curve from time zero to 72 h, *NF* normalization factor , *LSmeans* least square means, *GMR* geometric means ration, *CI* confidence interval

3.3.3 Pharmacokinetics of the phosphate metabolite

The pharmacokinetic parameters of the phosphate metabolite, after single-dose of capsules and ODFs are given in Figure III.2 and Table III.4. Phosphate metabolite blood concentrations peaked earlier than those of drug substance (t_{max} 7 vs 11-12 hours) and the C_{max} was higher. Phosphate metabolite C_{max} was 558 pg/mL in test group (ODFs) and 522 pg/mL in reference group. The blood concentration decreased slowly over time with $t_{1/2}$ of 40,79 h for ODFs and 44,98 h for capsules. Phosphate metabolite blood concentration profile, over 72 h, was not as flat as the drug substance profile: drug substance had a broad plateau region while metabolite had a narrow shape (Figure III.1 and Figure III.2).

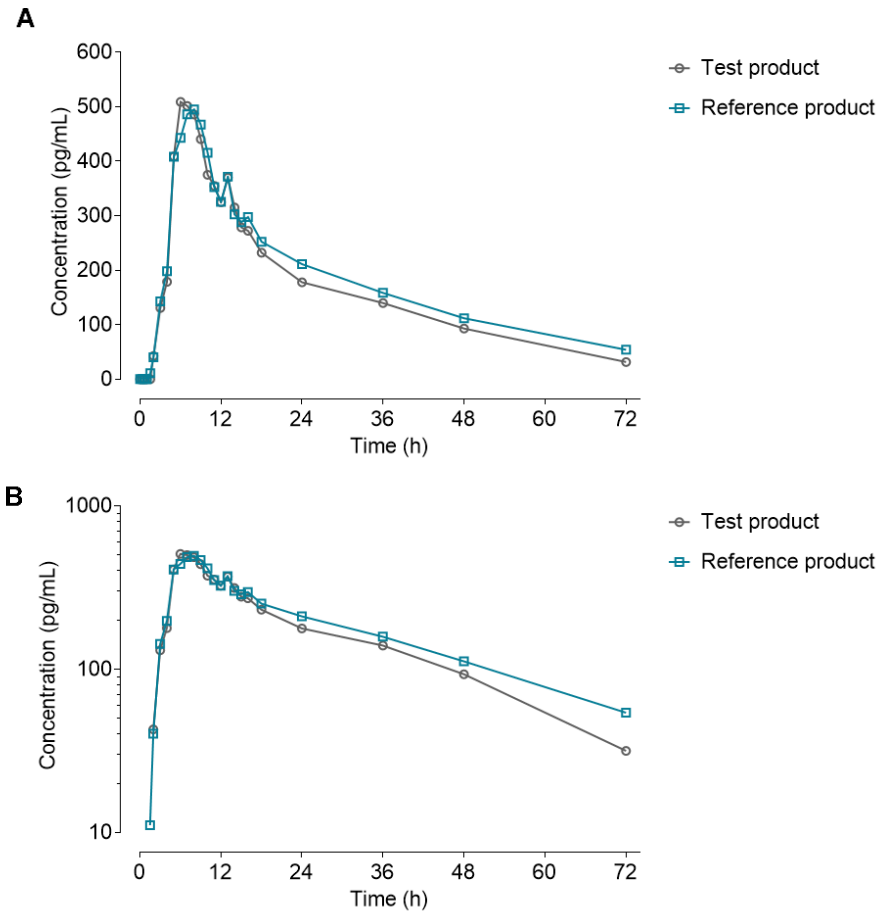


Figure III.2. Phosphate metabolite mean blood concentration versus time profile following single oral administration of ODFs (test product) and capsules (RP) to healthy subjects (linear (A) and log-linear (B) scales).

There was no statistically significant difference (p>0,05) between the C_{max} and AUC_{0-72} of the test and reference groups (Table III.5). Test-to-reference geometric means ratio of C_{max}, and AUC_{0-72} of phosphate metabolite were contained within the range of 80,00 to 125,00%. However, the lower limit of the 90% CI of AUC_{0-72} was 70,05, below the specified range (Table III.5). This result suggest that the extent of exposure to phosphate metabolite may be lower in some subjects taking ODFs when compared with the RP.

Table III.4. Pharmacokinetic parameters (average ± SD) of phosphate metabolite following oral administration of ODFs and capsules to healthy subjects.

	Test group (n=12)	**Reference group (n=12)**
C_{max}, pg/mL	558 ± 111	522 ± 107
t_{max}, h	7,33 ± 2,43	7,42 ± 1,08
AUC_{0-72}, pg.h/mL	10747 ± 3240	12166 ± 4213
λ_z, 1/h	0,03 ± 0,02	0,02 ± 0,01
$t_{½}$, h	40,79 ± 35,62	44,98 ± 19,86

SD standard deviation, *n* number of subjects, C_{max} maximum observed blood concentration post dose, t_{max} time to C_{max}, AUC_{0-72} area under the blood concentration versus time curve from time zero to 72 h, λ_z apparent terminal elimination rate constant, $t_{½}$ apparent terminal elimination half-life

Table III.5. Phosphate metabolite least square means, test-to-reference geometric means ratio and 90% confidence intervals for C_{max} and AUC_{0-72}.

		Geometric LSmeans			
Parameter	**ANOVA p-value**	**Test**	**Reference**	**Test/Reference GMR (%)**	**90% CI**
C_{max}	0,444	548	511	107,12	92,07-124,62
AUC_{0-72}	0,479	10278	11417	90,02	70,05-115,69

C_{max} maximum observed blood concentration post dose, AUC_{0-72} area under the blood concentration versus time curve from time zero to 72 h, *LSmeans* least square means, *GMR* geometric means ration, *CI* confidence interval

The pharmacokinetic parameters of the drug substance determined in this clinical trial were in accordance with the results found in literature. Pharmacokinetic parameters and statistical analysis of the results indicated that the rate (as assessed by C_{max}) and extend (as assessed by AUC_{0-72}) of systemic exposure from ODFs were not significantly different from the RP. Based on the accepted criteria for bioequivalence, this exploratory study provides evidence that the test product is bioequivalent to the RP. It is possible to find few publications describing the results of bioequivalence studies where ODFs were compared with tablets or orally disintegration tablets [39–42] and in those studies the bioequivalence was also demonstrated. Considering the satisfactory

results of this exploratory study, there are very good chances of success for the product to reach the market and thus it can be considered that the concept is proved in humans.

3.3.4 ODFs disintegration time and Taste acceptability

ODFs are designed to disintegrate in the mouth within seconds after being placed in the oral cavity due to the contact with saliva. Patient compliance and acceptability to the prescribed medication is highly dependent on drug substances and drug product tastes.

The elapsed time between administration and swallowing of the completely disintegrated ODFs was recorded for each subject. Additionally, the subjects were requested to fill in a form to assess the ODFs taste acceptability. The determined disintegration time was fast, confirming the in vitro results but there as a high inter-subject variability with an average of 115 seconds (Figure III.3 A). The high variability may be in part related to the differences in perception and sensory sensibility of each subject. Similarly to the observed in ODFs formulations developed by others [40–42], BlueOS® technology includes in its composition flavors and sweeteners for taste masking. ODFs' taste was well accepted by the volunteers of the study as depicted on Figure III.3 B. The worst score achieved was 2-slight bitterness, in three subjects, 2 minutes post-dosing (Figure III.3 B). The remaining volunteers indicated the scores 0- tasteless or 1- acceptable bitterness. These are promising results considering that it is well known that taste is a critical factor for patient compliance in dosage forms that are designed to disintegrate in the mouth. Therefore, ODF taste acceptability has also been evaluated by others in humans during product development [40,85]. In these reported studies, the subjects also showed a favorable acceptance of the oral films in terms of flavor and ease of use.

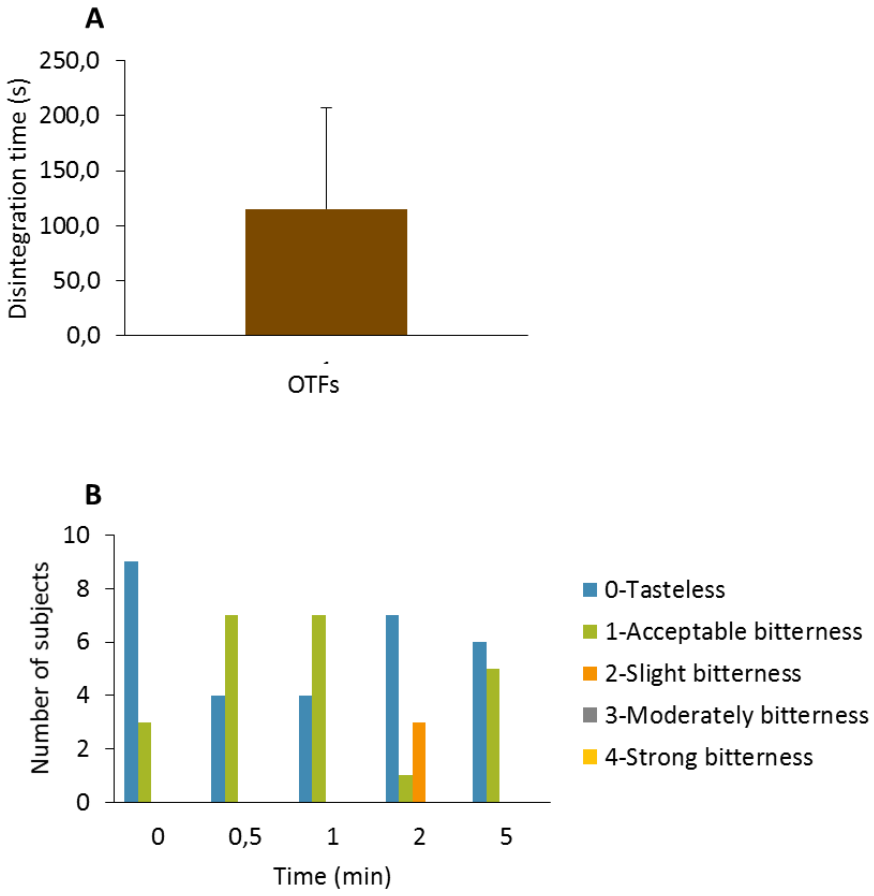


Figure III.3. Disintegration time (A, average ±SD) and taste acceptability (B) of ODFs.

3.3.5 Safety and tolerability

The safety and tolerability profile was comparable between the two study groups. There were no deaths or other serious adverse events and no Treatment Emergent Adverse Events (TEAEs) lead to subject withdrawal from the study. Overall, total TEAEs were reported by 92% of the participants in both groups (Table III.6). The most common TEAE was “sinus bradycardia” (cardiac disorders) and it was reported by 8 subjects in test group and 9 subjects in reference group.

Table III.6. Summary of treatment emergent adverse events by MeDRA system organ class (SOC).

System Organ Class	Test group (n=12)		Reference product (n=12)	
	All TEAEs	Drug-related TEAEs	All TEAEs	Drug-related TEAEs
Number of subjects (% of subjects)	11 (92%)	9 (75%)	11 (92%)	9 (75%)
Blood and lymphatic system disorders	3 (25%)	1 (8,3%)	1 (8,3%)	0
Cardiac disorders	8 (67%)	8 (67%)	9 (75%)	8 (67%)
Gastrointestinal disorders	1 (8,3%)	1 (8,3%)	0	0
General disorders and administration site conditions	0	0	1 (8,3%)	0
Infections and infestations	0	0	1 (8,3%)	0
Investigations	1 (8,3%)	0	0	0
Musculoskeletal and connective tissue disorders	1 (8,3%)	0	0	0
Nervous system disorders	4 (33%)	2 (17%)	2 (17%)	1 (8,3%)
Reproductive system and breast disorders	0	0	1 (8,3%)	0
Respiratory, thoracic and mediastinal disorders	0	0	1 (8,3%)	0
Skin and subcutaneous tissues disorders	1 (8,3%)	0	0	0

TEAEs Treatment emergent adverse events

The assessment of the local tolerability in subjects who received ODFs was performed by examining the oral cavity for signs of local irritation as recommended by the FDA guidance [86] and no subjects showed signs of irritation.

3.4 Conclusions

The pharmacokinetic parameters of the investigational ODFs and the commercially available product containing the same dose of drug substance in the form of capsules, as well as the acceptability of the ODFs, as assessed by the taste evaluation, and the disintegration time, were determined in healthy volunteers. The ODFs were well-tolerated in this population of healthy subjects either in terms of adverse events or in terms of taste acceptability. The systemic exposure to the drug substance was similar after the administration of the reference product and ODFs demonstrating that both formulations are bioequivalent. This proof of concept clinical trial demonstrates the potential to improve patient's compliance because it addresses an unmet medical

need of having an easy to swallow formulation able to facilitate the administration of DS to dysphagic patients. The next steps would be the technological transfer and scale-up of the product to a manufacturing facility with commercial scale capacity, to conduct a pivotal clinical trial and to request of a marketing authorization of the new medicinal product.

IV. Sublingual films to treat opioid dependence- formulation development part A

The main aim of this part of the work was to investigate the feasibility of developing a sublingual film formulation by fine-tuning of the orodispersible technology developed (BlueOS® technology) and described in Chapter II. The new formulation is intended to favor the sublingual absorption of the drugs embedded in the dosage form rather than gastrointestinal absorption. Although BlueOS® technology already has on its composition a mucoadhesive polymer, another polymer with mucoadhesive properties was included in the composition of the SIFs (carbopol). Also, different excipients were tested in order to investigate their ability to modulate the disintegration rate of the sublingual films, the drug release from the formulation and the pH achieved in the local of application (local pH). The most promising prototypes were fully characterized and their stability over time was assessed.

4.1 Introduction

Sublingual films (SlFs) are usually developed to deliver the drug substances through the sublingual mucosa either for local or systemic action [10,18,19,22]. Therefore, for the development of this dosage form it is important to consider some specificities of the oral cavity that have a direct impact in the administration and absorption of the drug substances [10,18,19,22]. The continuous saliva secretion may result in a loss of mucoadhesion and displacement of the SlFs, and in the wash-out of the drug substance prior to its absorption. Additionally, water and food intake during administration may result in the dosage form displacement or involuntary swallowing [1,8,10,18,19,22]. Therefore, the formulation of SlFs should include polymers that exhibit good adhesive properties to the mucosal membrane in order to be retained in place enough time to ensure drug absorption [1,19,22]. Besides this, the drug substances characteristics such as molecular size, lipophilicity, water solubility and acid/base properties may also impact the permeability and absorption. High lipophilicity (LogP (octanol/water)>2.0), unionized form, good water solubility and small molecular size (<800Da) are the preferred characteristics. The lipophilicity and the acid/base properties are dependent on the pH of the mucosal membrane and the pKa of the drug substance [1,18,22]. Thus, in order to have a versatile sublingual film technology in terms of number of drug substances that can be delivered, it is desirable to develop a formulation able to temporarily modify the local pH (interface saliva/mucosa) and evaluate if the use of solubilizers and permeation enhancers contribute to achieve the desired performance [1,19,22].

Buprenorphine and the combination buprenorphine/ naloxone are widely accepted as first-line treatment for opioid dependence [87–89]. Like other opioids, buprenorphine has an abuse potential and a significant risk of misuse and diversion [90,91]. These issues were expected to be mitigated by the development of sublingual tablets with a combination of buprenorphine/naloxone. However, the tablets can be crushed for snorting or intravenous injection and, were associated with higher risk of exposure in children [85]. To reduce the aforementioned risks, an alternative dosage form was developed, SlFs, that have a child resistant packaging and are difficult to crush and snort (Suboxone® sublingual films) [92].

A secondary goal of this part of the work was to use this new SIF formulation for the development of a pharmaceutical equivalent of a commercially available product, Suboxone® sublingual films, containing the same dose of drug substance (RP). The RP is a sublingual film containing buprenorphine hydrochloride and naloxone hydrochloride dihydrate. RP is supplied in four different

strengths combinations: 2 mg/ 0,5mg; 4 mg/ 1mg; 8 mg/2 mg and 12mg/ 3mg of buprenorphine/naloxone [93]. Buprenorphine is a μ opioid receptor partial agonist and κ opioid receptor antagonist, and naloxone is an antagonist at μ opioid receptors [51,93]. Naloxone is present in the SlFs to deter abuse of these products when modified to be injected. Lower doses of sublingual naloxone can be administered without precipitating withdrawal symptoms; however, when injected it produces marked opiate antagonist effects and withdrawal symptoms in individuals physically dependent on full opioid agonists [51,93].

4.2 Problem elicitation

The development of the BlueOS® technology was directed to the investigation of polymers and other excipients to obtain ODFs. The goal of this part of the work was to determine the feasibility of using the same base composition to develop SlFs. This required the incorporation of mucoadhesive polymers, solubilizers and pH modulators in order to meet the expected performance of sublingual films. Figure IV.1 summarizes the overall workflow adopted for problem identification, determination of QTPP and CQAs, compatibility assessment and, adjustment and modulation of mucoadhesion, absorption and drug release.

Figure IV.1. Flow chart with the main steps followed to obtain sublingual films.

4.3 Materials and Methods

4.3.1 Materials

Polyvinyl acetate dispersion (Kollicoat SR 30D, BASF-SE, Ludwigshafen, Germany); Polyvinyl alcohol 4-88 (Merck KGaA, Darmstadt, Germany); Carboxymethylcellulose Sodium (Blanose 7LF, Aqualon France BV, Alizay, France); Carbopol 971NF (Lubrizol Advanced Materials, Waalwijk, Netherlands); Tryethyl citrate (Merck KGaA, Darmstadt, Germany); Lemon flavor (IFF, Hilversum, Netherlands); Sucralose (Merck KGaA, Darmstadt, Germany); Polyvinylpyrrolidone 30 (Kollidon® 30, BASF-SE, Ludwigshafen, Germany); Polyvinylpyrrolidone 25 (Kollidon® 25, BASF-SE, Ludwigshafen, Germany) Vitamin E Polyethylene Glycol Succinate (Kolliphor™ TPGS, BASF-SE, Ludwigshafen, Germany); Sodium lauryl sulfate (Kolliphor® SLS, BASF-SE, Ludwigshafen, Germany); polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft co-polymer (Soluplus®, BASF-SE, Ludwigshafen, Germany); polyoxyl castor oil (Kolliphor™ EL, BASF-SE, Ludwigshafen, Germany); buprenorphine

hydrochloride (Arevipharma GmbH, Germany); naloxone hydrochloride (Aspen Oss B.V., The Netherlands).

4.3.2 Defining the QTPP and the CQAs

The formulation development started with the QTPP definition (Table IV.1) based on the study objectives, the properties of both drug substances and RP performance. After defining the desired performance of the product (QTPP) under development, the CQAs identification was based on the risk patients' injury due to failure to meet the quality target (Table IV.2).

Table IV.1. Quality target product profile (QTPP) for sublingual films. BUP- buprenorphine; NLX- naloxone; AV- acceptance value; NLT- Not less than; NMT- not more than; TAMC- Total Aerobic Microbial Count; TYMC- Total Yeast/ Mold Count

QTPP elements	Target	Justification
Dosage form	Sublingual film	Pharmaceutical equivalence requirements: same dosage form as RP [94]
Route of administration	Sublingual	Pharmaceutical equivalence requirements: same route of administration as RP [94]
Dosage strengths	Buprenorphine/Naloxone: 2mg/0,5mg; 4mg/1mg; 8mg/2mg; 12mg/3mg	Pharmaceutical equivalence requirements: same strengths as RP [93,94]
Pharmacokinetics	BUP: T_{max} approx 1h; C_{max} from approx 1 ng/mL to 3 ng/mL NLX: T_{max} approx 1h; C_{max} approx 54 pg/mL to 193 pg/mL	Bioequivalence requirement: meet the RP pharmacokinetics (80,00%-125,00%) [84,95,96]
Stability	At least 12 months at 25°C	Meet the RP stability [97]
Packaging	Child resistant pouches	It must ensure moisture and oxygen protection and avoid children accidental intake [98]
Appearance	Homogeneous films without lumps or air bubbles	Homogeneity is important to ensure good mechanical properties and uniformity of drug substances content
Size	2 mg/0,5 mg: 22,0 mm x 12,8 mm 4 mg/1 mg: 22,0 mm x 25,6 mm 8 mg/2 mg: 22,0 mm x 12,8 mm 12 mg/3 mg: 22,0 mm x 19,2 mm	Same as RP [93]
Mechanical properties	Puncture strength (N/mm^2): 0,3-0,5 Elongation to break (%): 3-15	The films must resist handling and manufacturing process without breaking [99]
DS identity	Positive	Ensure safety and efficacy
Assay	90-110%	Meet pharmacopeia requirements (USP40 - NF35)
Impurities	Reporting threshold 0,1%	Meet ICH Q3B(R2) requirements
Content uniformity	Uniformity of dosage units AV<15	Meet pharmacopeia requirements (USP40 - NF35)
Drug release	BUP NLT 75% (Q) NLX NLT 80% (Q)	Meet RP performance
Disintegration	NMT 3 min	Meet RP performance
Residual water	NMT 5%	The residual water content should guarantee good mechanical properties and avoid stickiness of the films [14,100]
pH	> 4	Circumvent RP patents (US 8017150, US 8603514 and US 8475832)
Residual solvents	To be defined	Meet pharmacopeia requirements (USP40 - NF35)and ICH Q3C(R5) requirements
Microbiology	TAMC NMT 102CFU/g TYMC NMT 101 CFU/g Staphylococcus aureus absent/g Pseudomonas aeruginosa absent/g	Meet pharmacopeia requirements (USP40-NF35)

Table IV.2. Sublingual films critical quality attributes (CQAs) and their justifications.

CQAs	Justification
Appearance	The presence of lumps may be indicative of heterogeneous SlFs.
Mechanical properties	The films must resist handling and manufacturing process without breaking. Formulation composition, CMAs and CPPS may influence the mechanical properties of the final dosage form.
Assay	Formulation composition, CMAs and CPPs may affect the content of the drug in the final product. It is mandatory to ensure a drug content within the acceptable range to guarantee safety and efficacy of the drug product.
Impurities	Patients' safety may be compromised by degradation products. These should be controlled according to pharmacopeia and ICH requirements.
Content uniformity	Formulation composition, CMAs and CPPs may impact content uniformity. Heterogeneity in the drug substances content will affect safety and efficacy of the final product.
Dissolution	Formulation composition, CMAs and CPPs may determine the DS release profile and affect the pharmacokinetic.
Disintegration	The drug release is affected by the disintegration time. Formulation composition, CMAs and CPPs may affect the film disintegration time.
Residual water	Water content may influence DS stability, disintegration time and drug release profile.
Local pH	pH may influence the DS solubility and absorption through the oral mucosa.

4.3.3 Patent landscape

A major concern in the development of innovative drug products is the possible infringement of intellectual property [101–103]. Therefore, the present study included an assessment of the patents of other oral films as well as the patents of the commercially available product containing the same dose of drug substances (RP). Three patents were identified as the most relevant regarding the protection of the RP: US 8017150, US 8603514 and US 8475832 [104].

Briefly, the US 8017150 patent covers the use of polyethylene oxide (low and high molecular weight) in combination with hydrophilic cellulosic polymers to obtain oral films that are uniform and disintegrate in water [105]. The US 8603514 covers formulations in which the drug substances may be coated with a taste masking agent, and the individual oral films have a specified uniformity. This important characteristic was achieved through the control of the matrix viscosity, and the control of the drying process [106]. The US 8475832 protects certain oral films that contain buffer systems

capable of produce a local pH in the range of 3-3,5 in the presence of saliva [107]. This pH is expected to modulate the absorption of buprenorphine and naloxone in order to achieve a product that is bioequivalent to Suboxone® tablets [107]. In summary, to avoid any patent infringement, polyethylene oxide in combination with hydrophilic cellulosic polymers should not be part of the formulation composition. Also, if there is the need of using water soluble or water swellable polymers, the drug substances should not be in close contact with taste masking agents. Finally, the use of buffer systems that renders a pH in the range of 3-3,5 should also be avoided.

Although these initial patent landscape and freedom to operate studies are essential for understanding the direct constraints to the formulation development, new patents that can pose a risk to the new product are published every day. Therefore, this work continued during the entire development to make sure that any potential patent infringement would be avoided [101].

4.3.4 Formulation development

The literature analysis and the RP characterization revealed that the sublingual films' strength does not increase proportionately with the size of the sublingual films [93,108]. If the dose increased proportionately with the size, the lowest dose would be too small for handling and the highest dose would be too large for applying comfortably. For this reason, two different formulations were developed: a low strength (LS) formulation (2 mg/ 0,5 mg and 4 mg/ 1 mg of buprenorphine/ naloxone) and a high strength (HS) formulation (8 mg/ 2 mg and 12 mg/ 3 mg of buprenorphine/ naloxone) were developed. Preliminary experiments were performed with the LS formulation in order to gain knowledge regarding the compatibility of the drug substances with the BlueOS® technology, pH modulation systems and drug release modulation.

4.3.5 Manufacturing process development

The SlFs were prepared by solvent-casting. Initially the manufacturing process followed was carried out in accordance with the described in BlueOS® patent [52]. However, the liquid mixture presented phase segregation and/or clumps formation. Different orders of excipients addition and different process parameters (magnetic stirring, mechanical stirring, mixing time and mixing speed) were tested to overcome the referred issues. At the end, the general manufacturing process selected consisted in the addition of the excipients to an aqueous suspension of the two drug substances. The mixture was casted and dried at 40°C on a heated table Coatmaster 510 (Erichsen, Germany).

The resulting film was cut into pieces of 300 mm^2, packaged and storage at 25°C / 60% RH according to the pre-stability program.

4.3.6 Characterization of sublingual films

4.3.6.1 Disintegration

The disintegration time of the SlFs was determined using the Petri dish method. Briefly, 4 mL of a phosphate buffer pH=6,8 (artificial saliva) at 37°C was placed in a Petri dish and then, the samples were placed in the center of the dish. The time at which it started to disintegrate was recorded.

4.3.6.2 Residual Water content

Residual water content was determined according to the described in Section 2.3.4.

4.3.6.3 Local pH

The pH at the local of application was determined using a surface electrode. Briefly, the SlFs were placed in a Petri dish with artificial saliva at ambient temperature and the final pH was recorded.

4.3.6.4 Mechanical tests

The Texture analyzer TA-XT Plus (Stable Microsystems, Godalming, UK) was used to determine the puncture strength of the SlFs, similarly to the described by Preis and colleagues [99]. A cylindrical probe with flat surface (diameter 6 mm) was moved with a velocity of 2 mm/s, once the probe contacted with the SlFs' surface the probe moved at a constant force (1 mm/s) until the breaking of the SlF. A plot of the applied force versus the displacement was recorded.

4.3.6.5 Assay

The drug substances content was determined by HPLC using external standards. The SlFs were dissolved in the appropriate solvent, diluted and filtered before analyzing. Separation was achieved with a X-Terra RP 18 (100 mm x 3mm, 3,5 µm). The elution was isocratic and gradient with the mobile phases A (Sodium perchlorate buffer 60mM), B (methanol) and C (acetonitrile) and a flow rate of 0,4 mL/min. The column temperature was maintained at 25°C, the detection was monitored at 210 nm using a PDA detector and the injection volume was 60 µL.

4.3.6.6 Related substances

The related substances were quantified by HPLC against external standards. SlFs were solubilized in an appropriate solvent, the suspension was centrifuged and a solid phase extraction was performed

to remove the excipients. The samples were analyzed with two methods, one for each drug substance.

Method for Buprenorphine

A Kromasil 100 C18 (250 × 4.6 mm, 5µm) was used for separation. The elution was isocratic and gradient using a flow rate of 1,5 mL/min and three mobile phases: A- Acetonitrile: THF: octanosulfonate, 2:4:94 v/v; B- Acetonitrile: THF: octanosulfonate, 17:4:79 v/v and C- Acetonitrile: THF: octanosulfonate, 50:4:46 v/v. The column was maintained at 40°C, the injection volume was 20 µL and the detection was monitored at 230 nm using a PDA detector.

Method for Naloxone

A Kromasil 100 C18 (250 × 4,6 mm, 5µm) was used for separation. The elution was isocratic and gradient using a flow rate of 1,0 mL/min and three mobile phases: A- ammonium acetate (10g/L), B- methanol, C- acetonitrile. The column was maintained at 40°C, the injection volume was 20 µL and the detection was monitored at 240 nm using a PDA detector.

4.3.6.7 Drug release

The drug release profile was investigated using an USP apparatus 5, paddle over disk Sotax XTend (Sotax, Switzerland) and the drug substances were quantified by HPLC. The drug release studies were carried out using 900 mL of acetate buffer pH 4,0 at 37°C±0,5°C with a rotation rate of 100 rpm. At each sampling interval (1, 2, 3, 5, 7 and 10 minutes) an aliquot of the dissolution medium was withdrawn and analyzed by HPLC. Separation was achieved with a X-Terra RP 18 (100 mm x 3mm, 3,5 µm). The elution was isocratic and gradient with the mobile phases A (sodium perchlorate buffer 60mM), B (methanol) and C (acetonitrile) and a flow rate of 0,4 mL/min. The column temperature was maintained at 25°C, the detection was monitored at 210 nm using a PDA detector and the injection volume was 60 µL.

4.3.6.8 Data analysis

GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California US (www.graphpad.com), was used for statistical data analysis of residual water content, disintegration time, local pH results and drug release profiles comparison. The results of the drug release profiles were analyzed by statistical comparison of the area under the curve (AUC) and dissolution efficiency

(DE) [109,110]. The AUC was calculated by the method of trapezoids for each sample and time point, and the DE was calculated according to the following equation:

$$DE = \left[\frac{AUC_{T1-Tf}}{\%D_{max} \times Tf}\right] \times 100$$

where $\%D_{max}$ is the maximum dissolved at the final time Tf, and $AUC_{T1\text{-}Tf}$ is the area under the curve from 1 min to 10 min. The AUC and the DE values were statistically compared by calculating the ANOVA post hoc Bonferroni's test (parametric data) or the Kruskal-Wallis test post hoc Dunn's test (nonparametric data) with a confidence level of 0,05. The null hypothesis establishes that there were no significant differences between the reference product and the test samples, thus the drug release profile are considered similar if p> 0,05.

4.4 Results and Discussion

The objective of this study was to develop a suitable SIFs formulation for the lowest dosage strengths (2 mg / 0,5mg and 4 mg/ 1mg, Buprenorphine/ Naloxone). This means that for these two strengths, the composition of the formulation will be exactly the same and the different strengths will be obtained by changing the dimensions of the SIFs.

The quantitative and qualitative formulation was defined based on the previous knowledge obtained during the development of BlueOS® technology [52]. Considering the nature of the project, the incorporation of two drug substances in a pre-existing technology, and the change from ODFs to SIFs, the development of the formulation was performed in a stage by stage approach. This approach allowed to identify possible interactions between the drug substances and the excipients of BlueOS® technology and to exploit different solutions to the challenges faced during the development. As mentioned in section 4.1, for the development of SIFs it is important to have mucoadhesive polymers and to have a pH that provides the most suitable environment for the absorption of the drug substances. Carboxymethylcellulose sodium is a mucoadhesive polymer and is one of the base components of the BlueOS® technology. Since carbopol is also a mucoadhesive polymer and it may confer an acidic pH to the formulations, it was included in the composition of SIFs formulations. Also, different excipients were tested in order to investigate their ability to modulate the disintegration rate of the SIFs and the drug release from the formulation.

4.4.1 DS and BlueOS® technology compatibility

In the first attempt to mixture the drug substances and the components of the BlueOS® technology it was observed the formation of clumps. This phenomenon may be attributed to ionic interactions between carboxymethylcellulose sodium (negatively charged, Figure IV.2) with both drug substances (positively charged, Figure IV.4 and Figure IV.5), and / or to the possible formation of hydrogen-bonds between naloxone and tryethyl citrate and / or polyvinyl acetate. In Figure IV.2 it is possible to observe that the mentioned excipients have hydrogen donor groups (-OH) and hydrogen acceptor groups (=O, O^-) that can interact with the drug substances hydrogen donor and acceptor groups (Figure IV.4 and Figure IV.5).

Since the addition of fine powder particles to the polyvinyl acetate dispersion has been reported to result in coagulate formation [111,112] and in this work buprenorphine was added as micronized powder, the observed formation of clumps may also be a result of this coagulate formation. To overcome this problem, different orders of excipients addition and different process parameters (e.g. magnetic stirring, mechanical stirring, mixing time and speed) were tested until it was ensured an uniform liquid mixture without clumps or phase segregation.

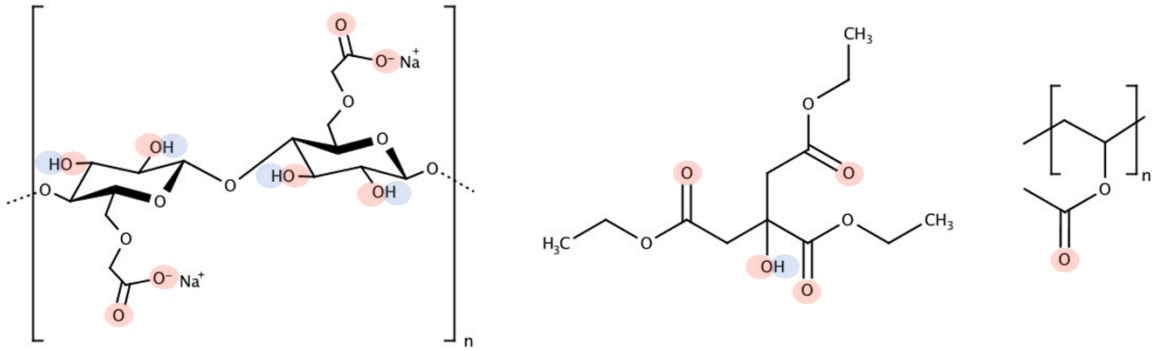


Figure IV.2. Molecular structures of carboxymethylcellulose sodium (1), tryethyl citrate (2) and polyvinyl acetate (3) obtained with MarvinSketch software. Hydrogen donor groups are represented in blue and hydrogen acceptor groups are represented in red.

4.4.2 pH modulation

As referred before, the absorption of drugs substances through the oral mucosa is dependent on their lipophilicity, water solubility, acid/ base properties and molecular size [1]. Both buprenorphine and naloxone are weak bases (high pKa) that at physiologic pHs can exist in ionized or unionized forms (Figure IV.3, Figure IV.4 and Figure IV.5). Naloxone is highly water soluble while buprenorphine is only slightly soluble in water [108].

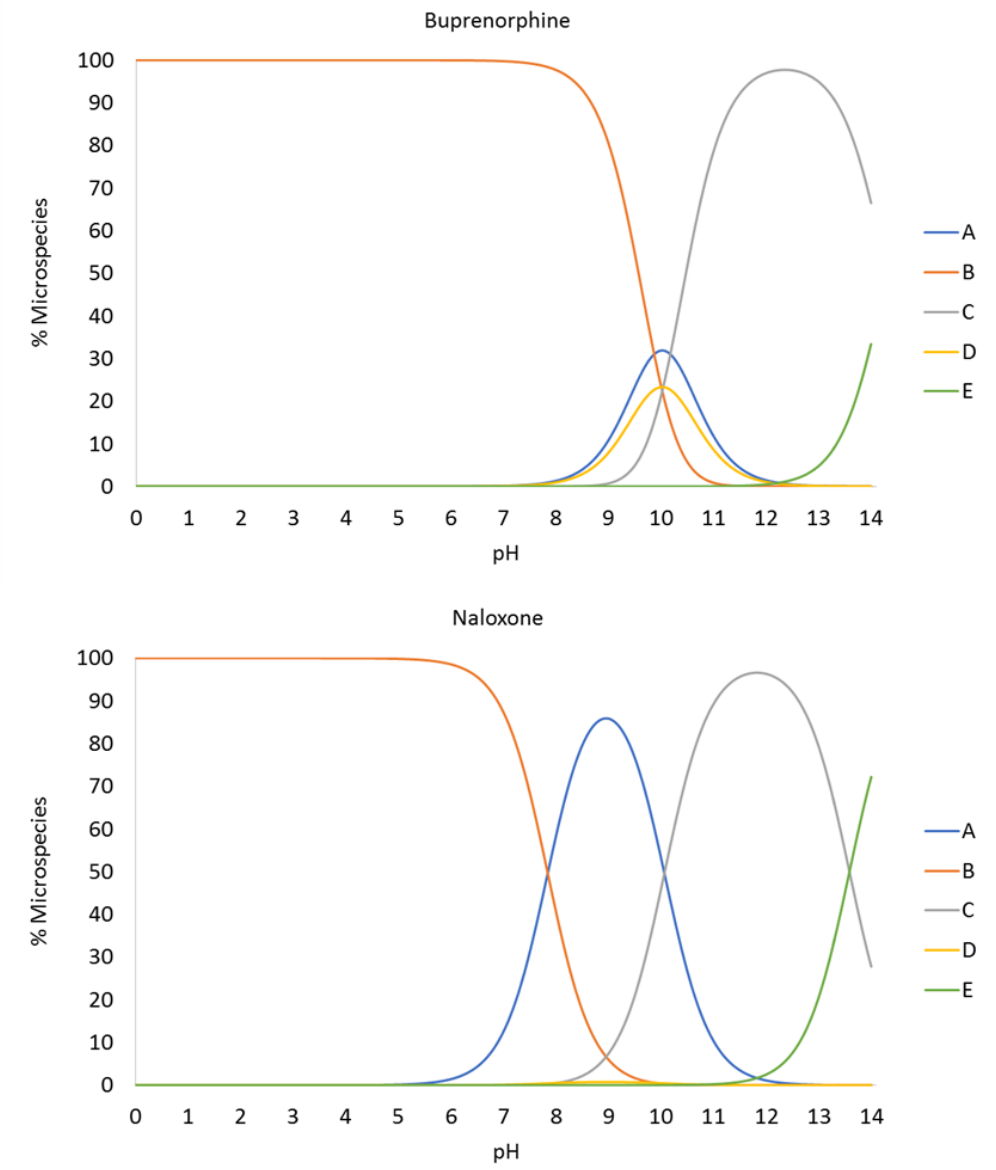


Figure IV.3. Buprenorphine (top image) and naloxone (bottom image) microspecies distribution depending on the pH. A is the unionized form while B, C, D and E represent the ionized forms of each drug substance as illustrated in Figure IV.4 and Figure IV.5.

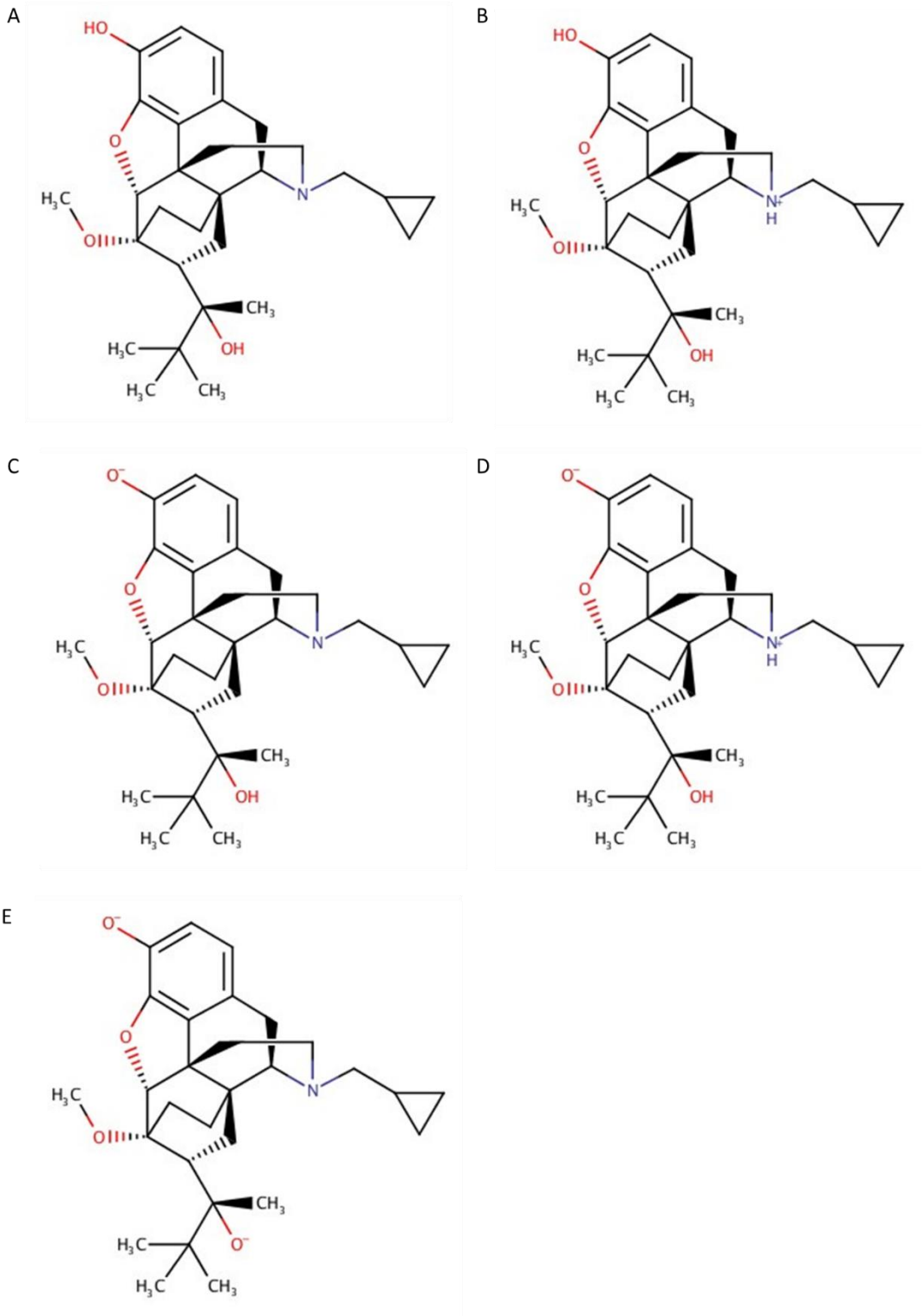


Figure IV.4. Buprenorphine microspecies determined using MarvinSketch software.

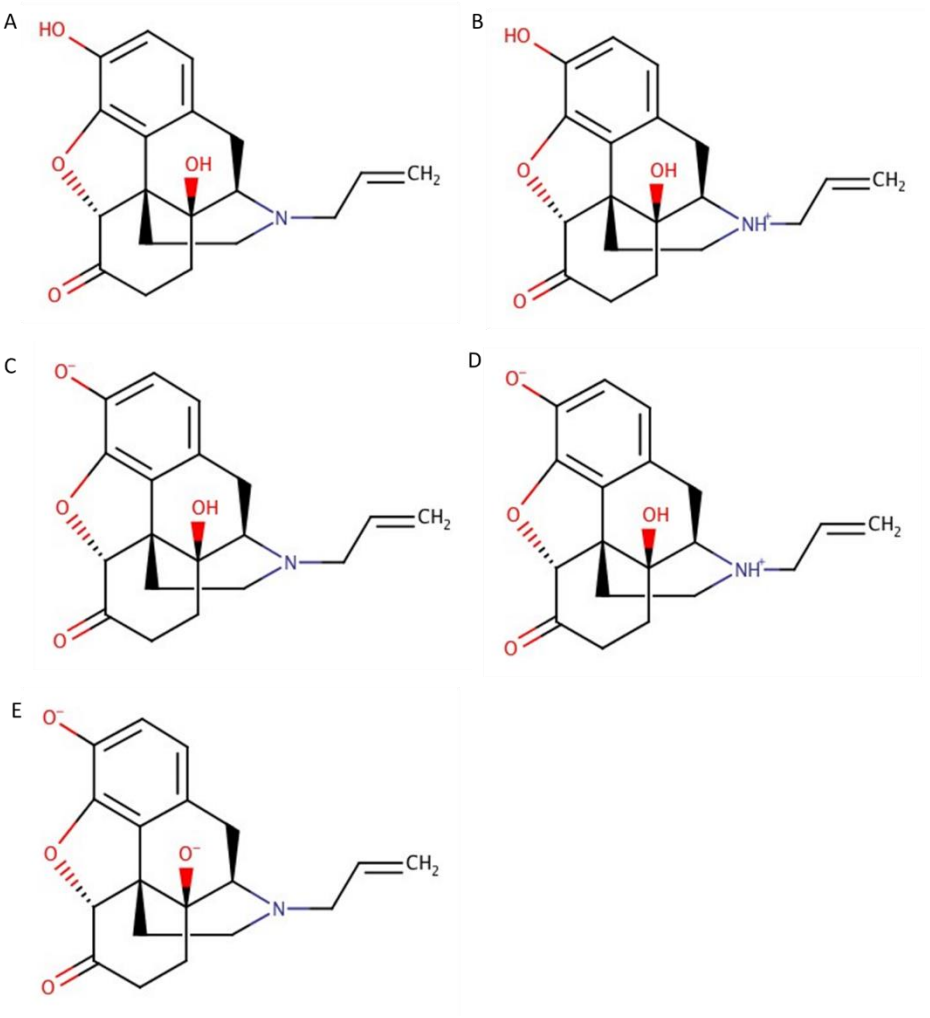


Figure IV.5. Naloxone microspecies determined using MarvinSketch software.

According to the microspecies distribution depicted in Figure IV.3, between pH 6 and 7 about 99% of buprenorphine is in the ionized form (microspecies B, Figure IV.4) while about 98% to about 87% of naloxone is in the ionized form (microspecies B, Figure IV.5) and about 2% to 13% is in the unionized form (microspecies A, Figure IV.5). Therefore, at pH between 6 and 7 an amount of naloxone is present in the more permeable form, the unionized. This is in accordance with the described in the patent US 8475832 [107] where formulations with a pH of about 6,5 had higher absorption of naloxone when compared to formulations with pH of 5 – 5,5 and pH of 3 - 3,5. In fact, the inventors of the patent showed that only at pH of 3 – 3,5 the SlFs were bioequivalent to

Suboxone sublingual tablets because they were able to decrease the absorption of naloxone without compromising the absorption of buprenorphine. At this pH both buprenorphine and naloxone are 100% present in the ionized form (microspecies B, Figure IV.3, Figure IV.4 and Figure IV.5).

In this work different strategies were followed to modify the local pH, in order to modulate the permeation and absorption of the drug substances, without using a buffer system as described in the US 8475832 patent [107]. The different approaches performed avoid the use of a combination of carboxylic acids with their carboxylate salts (e.g. citric acid/ sodium citrate), that is protected by the aforementioned patent.

Three systems derived from polymers used in the formulation (e.g. acid polymer and its "conjugated base") were investigated for that purpose:

1. Polymeric acid/conjugated base system based on carboxymethylcellulose sodium (NaCMC).

Acetic acid was used to convert a percentage of NaCMC in its conjugated base.

2. Polymeric acid/conjugated base system based on carbopol.

Sodium hydroxide was used to convert a percentage of carbopol in its conjugated base (sodium acrylate).

3. Strong acid effect based on carbopol.

Different organic acids (ascorbic acid, malic acid, tartaric acid and citric acid) were added to carbopol in an attempt to obtain a synergistic effect in lowering the local pH.

As presented on Table IV.3 none of the strategies followed provided a pH between 3 and 3,5, that corresponds to the local pH of the RP. Additionally, some of the SlFs exhibited lumps and the disintegration time was compromised. An increase in the disintegration time was expected, considering that low substituted sodium carboxymethylcellulose (acting as disintegrant) is converted to the less soluble form at low pH (≤ 4) [74,112,113]. Koo and colleagues [114] observed a negative impact on the disintegration of PVA films in the presence of acids, and attributed this phenomena to an increase in PVA crystallinity. This may explain why a change in pH add a deleterious effect on the stability of the SIFs because they also contain PVA [112,114]. Further studies should be performed in order to have a deeper understanding of the mechanisms behind the increase in the disintegration times in these experiments. Nevertheless, based on the available references and to circumvent the patent US 8475832 [107] it was decided to maintain the pH of the films under development at around 4,5 to 5. According to the microspecies distribution illustrated

in Figure IV.3, both drug substances at pH of about 4,5 - 5 should be in the same form as they are in pH of about 3 – 3,5. The initially obtained SlFs had a pH of about 5 and the inclusion of carbopol alone in their composition was enough to decrease the pH to about 4,5.

Table IV.3. Sublingual films resulting local pH of formulations LS.1 to LS.20.

SlFs code	Resulting local pH	Comments
Polymeric acid/conjugated base system based on NaCMC		
LS.1	4,185	The SlFs obtained do not disintegrate and had a heterogeneous appearance.
LS.2	5,360	
Polymeric acid/conjugated base system based on carbopol		
LS.3	5,342	Heterogeneous SlFs with small to big lumps were obtained. After storage for a short period of time, at room temperature, in LPDE bags the disintegration time increased and some SlFs stop to disintegrate.
LS.4	6,144	
LS.5	4,990	
LS.6	4,789	
LS.7	4,399	
LS.8	4,794	
LS.9	4,610	
LS.10	4,476	
LS.11	4,331	
LS.12	4,516	
Strong acid effect based on carbopol		
LS.13	6,421	Increasing amounts of ascorbic acid were added to carbopol. After storage for a short period of time, at room temperature, in LPDE bags, the SlFs stop disintegrating.
LS.14	5,222	
LS.15	4,415	
LS.16	4,302	
LS.17	4,213	
LS.18	4,380	Malic acid. After storage for a short period of time, at room temperature, in LPDE bags, the SlFs stop disintegrating.
LS.19	4,924	Tartaric acid. After storage for a short period of time, at room temperature, in LPDE bags, the SlFs stop disintegrating.
LS.20	4,747	Citric acid. After storage for a short period of time, at room temperature, in LPDE bags, the SlFs stop disintegrating.

4.4.3 Drug release profile modulation

An appropriate drug release is critical to obtain the desired absorption and bioavailability of the drug substances and this is even more important when working with drug substances with low solubility [115]. In this work there was the need to select a solubilizer to ensure the homogeneity of the liquid mixture in terms of buprenorphine content that has a low water solubility, improving the manufacturing process and to achieve similar drug release profile observed in the RP. Six different solubilizers were evaluated: sodium lauryl sulfate (SLS), polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft co-polymer (Soluplus®), polyvinylpyrrolidone 25 (PVP 25), polyvinylpyrrolidone 30 (PVP 30), polyoxyl castor oil (Kolliphor™ EL) and Vitamin E Polyethylene Glycol Succinate (Kolliphor™ TPGS) [112,115].

The effect of including SLS in the composition of SlFs was investigated using two different concentrations (LS.22 and LS.23) while the remaining solubilizers were only investigated using one concentration (#LS.24 to #LS.29). Formulation LS.21 corresponds to the sublingual film without any solubilizer in its composition (see Table IV.3).

Table IV.4- Qualitative and quantitative composition of the formulations prepared to study the effect of the solubilizers: sodium lauryl sulfate (SLS), polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft co-polymer (Soluplus), polyvinylpyrrolidone 25 (PVP 25), polyvinylpyrrolidone 30 (PVP 30), polyoxyl castor oil (Kolliphor EL) and Vitamin E Polyethylene Glycol Succinate TPGS) in the solubility of buprenorphine in the liquid mixture and in its drug release profile.

	% (w/w)								
Raw material	**LS.21**	**LS.22**	**LS.23**	**LS.24**	**LS.25**	**LS.26**	**LS.27**	**LS.28**	**LS.29**
Buprenorphine HCl	8,81	8,81	8,81	8,81	8,81	8,81	8,81	8,81	8,81
Naloxone HCl $2H_2O$	2,39	2,39	2,39	2,39	2,39	2,39	2,39	2,39	2,39
Other excipients	93,80	85,30	87,80	87,80	87,80	87,80	87,80	87,80	87,80
SLS		3,50	1,00						
Soluplus				1,00					
PVP 25					1,00				
PVP 30						1,00			
Kolliphor EL							1,00		
TPGS								1,00	1,00

The following responses were investigated: pH of the liquid mixtures, SlFs average weight, drug substances content and disintegration time (Table IV.5). Mixtures' pHs were above 4, which was essential to prevent the conversion of the disintegrant into its insoluble form. Despite the similar composition of all formulations, a variation in SlFs weight and drug substances content was

observed. In most of the cases, it was not possible to achieve the required drug content (90,0%-110,0%). This fact can be attributed to changes in the liquid mixture viscosity due to the direct influence of the solubilizers and also to small variations in the mixing speed and time required to ensure the complete dispersion of buprenorphine. The QTPP (Table IV.1) defines a disintegration time not more than 3 minutes that should meet the RP performance. The RP composition is completely hydrophilic and the disintegration of the RP SlFs results in fine fragments of smaller dimensions than the SlFs obtained with these experiments. Therefore, for the purpose of this work the disintegration time specification will only be closed when a drug release profile similar to the RP is reached. Nevertheless, SlFs were considered to have a fast disintegration if they completely disintegrate into small pieces forming a homogeneous dispersion and, a slow disintegration when they disintegrate into fragments of larger sizes. SlFs LS.28 and LS.29 were the ones that exhibited a faster disintegration (Table IV.5).

Table IV.5- Characterization of formulations LS.22 to LS.29 regarding, mixture pH and sublingual films (SlFs) drug substances content, average weight and disintegration time. Fast disintegration- SlFs completely disintegrate into small pieces forming a homogeneous dispersion; Slow disintegration- SlFs disintegrate into fragments of larger sizes.

		Buprenorphine		Naloxone			Disintegration	
Formulation	**Mixture pH**	**Assay (%)**	**RSD**	**Assay (%)**	**RSD**	**Weight (mg)**	**Time (s)**	**Observations**
LS.22	4,512	88,33	0,28	97,94	0,19	23,2	60	Slow dissolution of some components of the SlFs resulting in a transparent matrix.
LS.23	4,539	77,07	0,87	84,56	0,24	23,12	54	Slow disintegration.
LS.24	4,56	82,56	0,71	83,65	0,34	22,62	58	Slow disintegration
LS.25	4,544	93,52	0,07	94,71	0,16	26,02	46	Initial fast disintegration that slows down during the remaining disintegration time.
LS.26	4,539	87,09	0,25	88,53	0,3	23,72	43	Slow disintegration.
LS.27	4,529	89,36	0,99	90,05	1,09	24,49	120	Slow disintegration.
LS.28	4,511	86,26	0,11	84,82	0,33	22,78	30	Fast disintegration.
LS.29	4,463	89,85	0,05	87,64	0,29	24,14	31	Fast disintegration.

The drug release profiles of formulations LS.21 to LS.29 and RP are present in Table IV.6, and in Figure IV.6 top and bottom for buprenorphine and naloxone, respectively. Visually, none of the formulations exhibited a similar drug release profile to the RP and a higher difference was observed

for buprenorphine. There are several methods to compare drug release profiles and the model-independent method similarity factor (f2) is the most preferred method by FDA and EMA [116–118]. However, the application of the f2 method is subjected to several principles that were not fulfilled by these samples, namely a coefficient of variation lower than 20% in the first minutes and not more than one mean value superior to 85% [116]. In these situations other methods are appropriate namely the f2 bootstrapping for drug release points with high variability and the multivariate statistical difference (MSD) when the criteria of f2 methods are not met. The MSD can be applied to the original drug release data or to derived model parameters [109,110,117–119]. The fast drug release profile of OFs difficult the use of model-dependent methods and the MSD of the original data is insensitive to the shape and the location of the curves. Therefore, in this work was adopted the statistical comparison of AUC and DE because it provides a good correlation to the in vivo absorbed amount of drug substances [109,110]. An average of 12 individual values for each time point and for each formulation should be used irrespective of the comparison method [116,117]. Nevertheless, for the purpose of this work an initial assessment was performed with the available data (less than 12 individual values). Additionally, a standardization of data was implemented in the cases were the last measured time was different from 100% due to the uncertainty of the measuring method or low drug substances content. When the last measurement was different from 100% due to incomplete drug release, no standardization was performed [109].

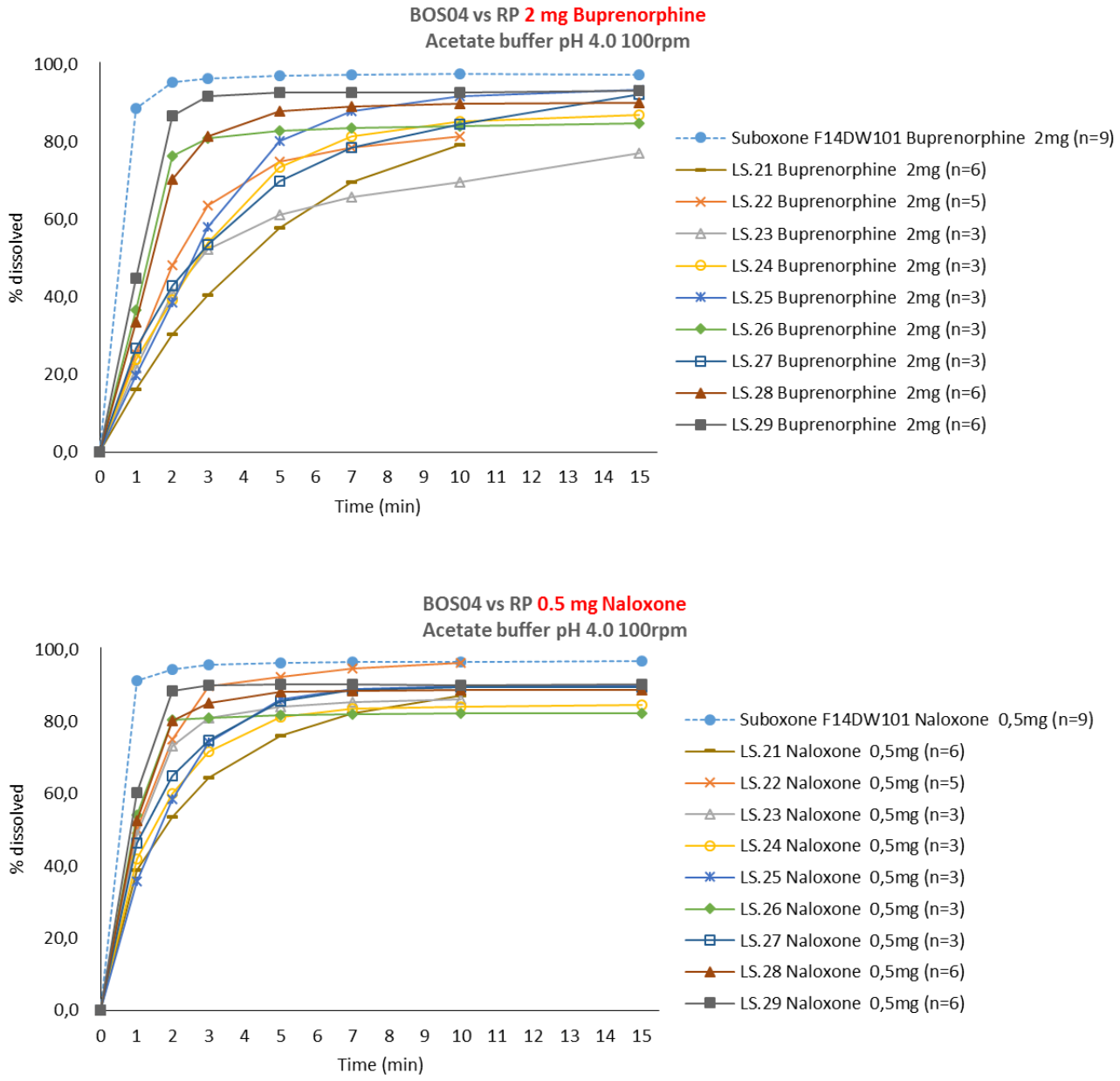


Figure IV.6. Drug release profile of Buprenorphine (top) and Naloxone (bottom) of formulations LS.21 to LS.29 (solid lines) and RP (dashed lines).

Table IV.6. Buprenorphine and Naloxone drug release profile of reference product and formulations LS.21 to LS.29 (average ± SD).

	Buprenorphine									
Time (min)	**Suboxone F14DW101**	**LS.21**	**LS.22**	**LS.23**	**LS.24**	**LS.25**	**LS.26**	**LS.27**	**LS.28**	**LS.29**
1	88,7±8,7	16,3±6,5	25,4±10,5	21,7±2,3	23,9±7,5	19,7±3,5	36,7±5,3	26,8±8,1	33,5±7,3	44,9±13,9
2	95,4±5,0	30,4±10,1	48,3±17,2	41,0±1,0	39,4±9,2	38,7±9,2	76,4±3,3	42,9±12,2	70,3±12,0	86,8±5,5
3	96,5±3,4	40,5±12,4	63,6±13,2	52,4±0,3	54,1±7,2	58,2±12,6	81,0±3,1	53,5±12,0	81,5±8,9	91,8±3,7
5	97,2±1,9	57,9±15,3	74,9±6,1	61,3±0,9	73,4±5,4	80,3±8,5	82,8±2,0	69,8±8,1	87,9±3,7	92,8±3,4
7	97,4±1,3	69,6±14,6	78,7±4,2	65,7±0,9	81,5±3,8	87,9±4,5	83,6±1,2	78,5±4,6	89,2±3,0	92,9±3,4
10	97,5±1,1	79,4±12,6	81,4±3,0	69,7±1,0	85,3±2,5	91,8±2,1	84,2±0,6	84,6±3,3	89,9±2,7	92,8±3,2
15	97,4±1,0			77,0±3,4	87,1±1,5	93,4±1,7	84,8±0,5	92,4±3,8	90,2±2,7	93,2±3,5
	Naloxone									
Time (min)	**Suboxone F14DW101**	**LS.21**	**LS.22**	**LS.23**	**LS.24**	**LS.25**	**LS.26**	**LS.27**	**LS.28**	**LS.29**
1	91,3±7,7	38,9±12,5	50,3±16,4	48,6±6,4	41,8±11,4	35,7±5,9	54,2±5,2	46,3±11,7	52,4±9,5	60,4±11,7
2	94,5±4,1	53,6±14,8	75,0±17,6	73,3±3,0	60,0±10,4	58,4±10,0	80,5±2,9	64,9±12,0	80,2±9,1	88,4±3,7
3	95,6±2,8	64,3±15,4	89,8±5,8	81,0±2,7	71,7±6,6	74,3±9,9	81,0±2,1	74,7±9,0	85,0±5,9	90,0±3,4
5	96,2±1,6	76,2±13,6	92,4±3,9	84,1±3,7	81,2±3,4	86,1±3,8	81,6±1,0	85,6±4,2	88,1±2,9	90,2±3,4
7	96,5±1,5	82,3±10,6	94,8±2,6	85,3±4,3	83,5±2,4	88,9±1,9	81,9±0,4	88,6±3,8	88,5±2,7	90,2±3,4
10	96,6±1,3	87,3±7,0	96,3±1,7	86,3±5,2	84,1±1,8	89,8±1,9	82,3±0,4	89,4±4,0	88,7±2,6	90,1±3,3
15	96,6±1,3			87,0±5,8	84,6±1,7	90,0±1,9	82,4±0,4	89,6±4,0	88,7±2,6	90,3±3,4

According to this method the formulations LS.26, LS.28 and LS.29 could be considered similar to the reference product (p>0,05 for AUc and DE for both drug substances, Table IV.7). The solubilizers used in these formulations were PVP 30 for LS.26 and TPGS for LS.28 and LS.29. For this reason solubilizers PVP 30 and TPGS were selected for further studies.

Table IV.7. Drug release profile comparison through statistical comparison of area under the curve (AUC) and dissolution efficiency (DE). The drug release profile are considered similar to the reference product if p> 0,05.

		Buprenorphine		**Naloxone**	
	Test	**p-value**	**Conclusion**	**p-value**	**Conclusion**
LS.21	AUC	0,003	Not similar	0,01	Not similar
	DE	<0,001	Not similar	<0,001	Not similar
LS.22	AUC	0,25	Similar	0,34	Similar
	DE	0,06	Similar	0,04	Not similar
LS.23	AUC	0,03	Not similar	0,89	Similar
	DE	0,10	Similar	0,30	Similar
LS.24	AUC	0,05	Not similar	0,17	Similar
	DE	0,04	Not similar	0,04	Not similar
LS.25	AUC	0,04	Not similar	0,07	Similar
	DE	0,03	Not similar	0,01	Not similar
LS.26	AUC	>0,99	Similar	>0,99	Similar
	DE	>0,99	Similar	>0,99	Similar
LS.27	AUC	0,009	Not similar	0,19	Similar
	DE	0,02	Not similar	0,05	Not similar
LS.28	AUC	0,28	Similar	0,87	Similar
	DE	0,19	Similar	0,09	Similar
LS.29	AUC	>0,99	Similar	>0,99	Similar
	DE	0,72	Similar	0,75	Similar

New formulations with the same composition as in formulations LS.26 and LS.28 (Table IV.8) were prepared and a pre-stability study was initiated. Immediately after manufacturing, the SlFs were individually packaged and stored at 25°C / 60% RH.

Table IV.8. Qualitative and quantitative composition (percentage weight/ weight) of formulations LS.30 and LS.31.

	% (w/w)	
Raw material	**LS.30**	**LS.31**
Buprenorphine	8,81%	8,81%
Naloxone	2,39%	2,39%
Other excipients	87,80%	87,80%
TPGS	1,00%	0,00%
PVP 30	0,00%	1,00%

The drug substances content was about 89% in formulation LS.30 and was stable during 1 month of storage (Table IV.9). In formulation LS.31, the buprenorphine and naloxone content was within the specification range (90-110%) (Table IV.9).

The LS.30 SlFs had a fast disintegration but, after the storage period, the disintegration profile of these films was changed, (Table IV.9) which was reflected in the decrease in the percentage of drug release (Figure IV.7 and Figure IV.8). SlFs of LS.31 also had an initial fast disintegration time (28 s) that, with storage, slightly increased (36 s) and bigger fragments were observed in the end of the disintegration test. As already discussed in chapter II, the decrease in the drug release rate upon storage may be associated with the physical aging of the polymeric matrices. This phenomenon associated with the lower water solubility of buprenorphine may explain the more accentuated decrease in buprenorphine release when compared to naloxone.

Table IV.9. Characterization of formulations LS.30 and LS.31 regarding average weight, drug substances content and disintegration time. Initial data and after storage 1 month at 25°C / 60% RH. ND- not determined.

	Buprenorphine		**Naloxone**			
Formulation	**Assay (%)**	**RSD**	**Assay (%)**	**RSD**	**Weight (mg)**	**Disintegration Time (s)**
			T0M			
LS.30	88,32	0,83	89,38	1,2	22,68	35
LS.31	97,78	0,54	94,58	0,13	25,42	28
			T1M			
LS.30	89,52	1,14	89,26	0,05	23,04	No disintegration
LS.31	ND		ND		24,93	36

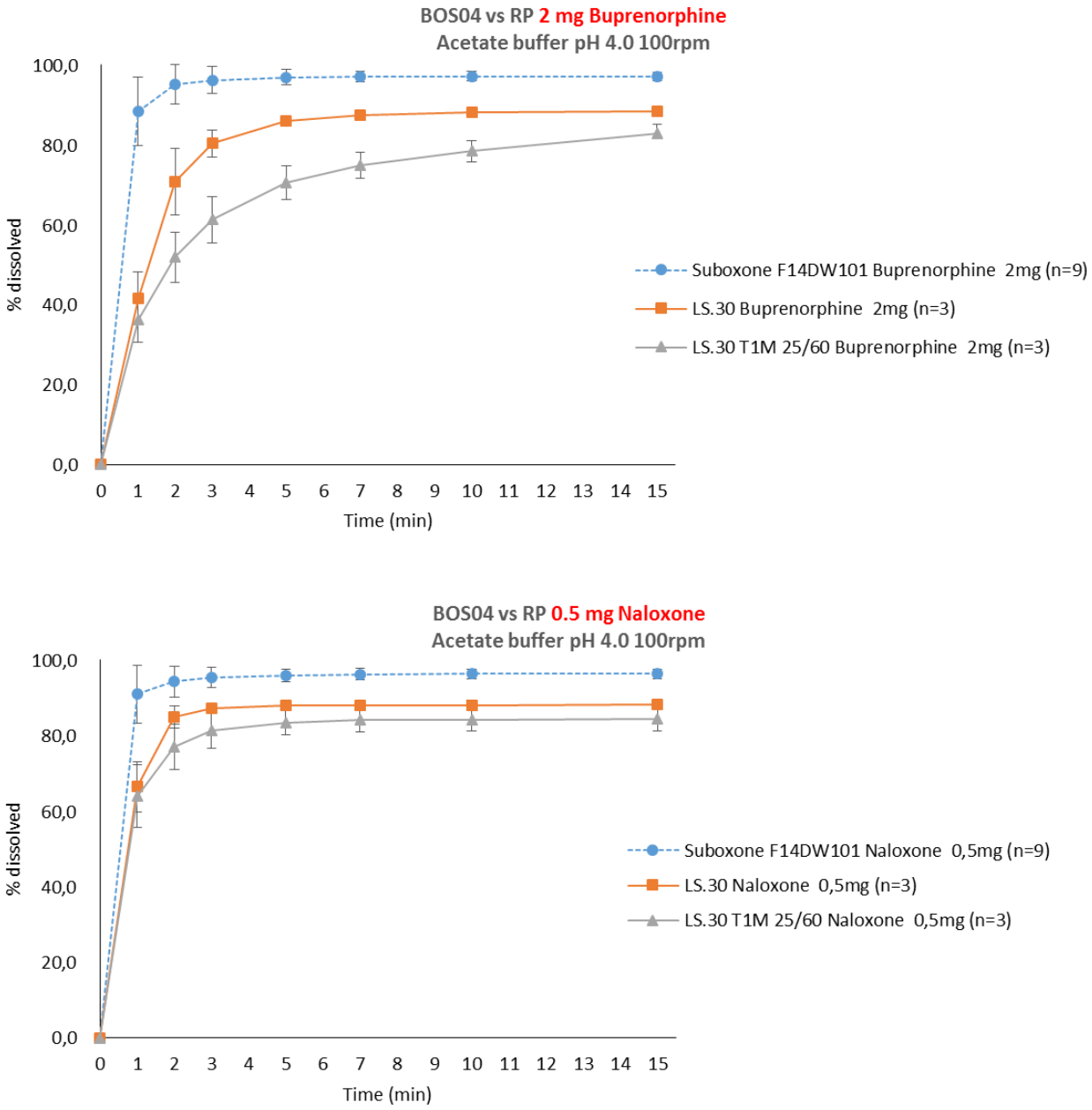


Figure IV.7. Drug release profile of Buprenorphine (top) and Naloxone (bottom) of formulations LS.30 (solid lines) and RP (dashed lines). Initial data (average ± SD) and after storage 1 month at 25°C / 60% RH.

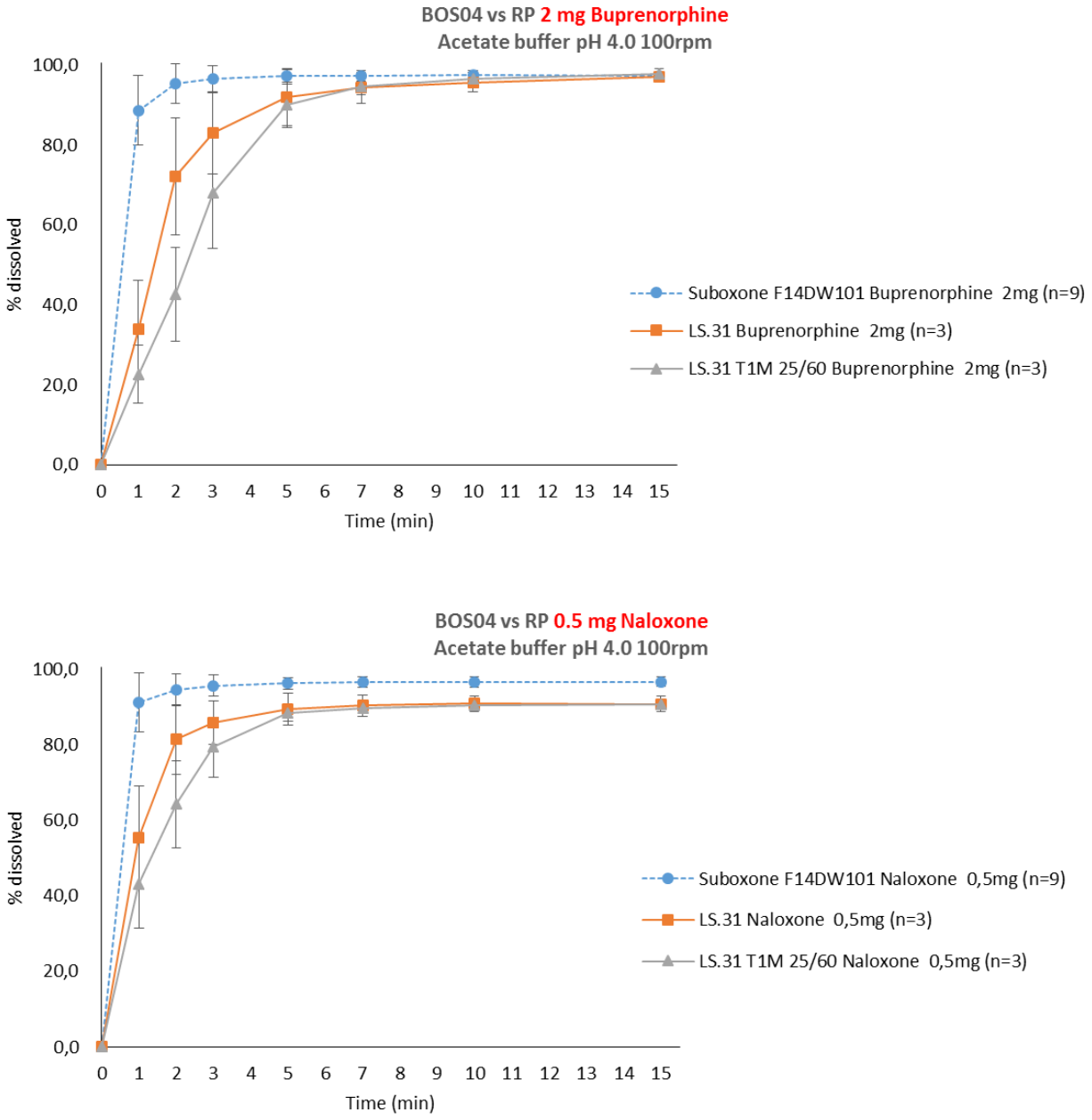


Figure IV.8. Drug release profile of Buprenorphine (top) and Naloxone (bottom) of formulations LS.31 (solid lines) and RP (dashed lines). Initial data (average ± SD) and after storage 1 month at 25°C / 60% RH.

From all the tested solubilizers, PVP 30 and TPGS exhibited the best performance (Figure IV.6, Figure IV.7 and Figure IV.8). TPGS is an amphiphilic molecule that has been approved by FDA and, it has been used as an emulsifier and surfactant to improve the solubility and absorption of poorly water

soluble drugs [120–122]. Similarly to the observed by others who worked with nanoparticles, nanomicelles and submicron suspensions [120–122], an improvement of drug release was obtained by using these solubilizers; however, PVP30 showed better results in terms of naloxone related substances formation after storage 1 month at 25°C / 60% RH (Table IV.10). Contrarily to the described by others, when studying amorphous solid suspensions [123–125] this study shows an increase in the drug release rate when higher molecular weight PVP was used (Figure IV.6). Ugaonkar and co-workers [123] and, Manchero and colleagues [124] pointed out that the slower drug release obtained when using high molecular weight PVP is due to an increase of the viscosity of the polymer. As a consequence, there is the formation of a viscous layer around the dissolving tablets and particles that leads to a decrease in the drug release rate [123,124]. On the other hand, the work of Knopp et al [126], that studied the drug-polymer solubility using several molecular weights of PVP, showed no significant influence of PVP molecular weight in the solubility of indomethacin in amorphous solid dispersions. A possible explanation for the different results of this work and the contributions of Ugaonkar and Manchero may be the dissimilarities of the dosage forms, the drug substances used and other components of the formulation. In our study, PVP was initially selected to improve the solubility of buprenorphine in the liquid mixture which in turn result in the formation of SlFs with a more homogenous appearance. An increase in the liquid mixture viscosity contributes to guarantee the content uniformity of the SlFs, because the sedimentation of the drug substance particles is minimized during the manufacturing process.

Table IV.10. Total amount and total amount to report of buprenorphine and naloxone related substances of sublingual films LS.30 and LS.31. Initial data and after storage 1 and 2 months at 25°C / 60% RH.

		LS.30			LS.31	
		T0	**T1**	**T2**	**T0**	**T1**
Naloxone	**Total**	1,22	1,26	1,88	0,57	0,71
	To report	0,66	0,62	1,05	0,25	0,40
Buprenorphine	**Total**	0,51	0,45	0,59	0,58	0,48
	To report	≤0,1	≤0,1	≤0,1	≤0,1	≤0,1

4.4.4 Content fine tuning and Scale-up

In order to obtain formulations with a drug content within the specification range (90%-110%) five new formulations (LS.32 to LS.36) were prepared with increasing amount of DS in order to achieve

the desired content (Table IV.12). The increase in the amount of DS was compensated equally with the other excipients to guarantee that the proportion between them was maintained.

Table IV.11. Qualitative and quantitative composition of formulations LS.32 to LS.36 designed to fine tune the drug substances content in the sublingual films.

	% (w/w)				
Raw material	**LS.32**	**LS.33**	**LS.34**	**LS.35**	**LS.36**
Buprenorphine HCl	8,81%	8,86%	9,00%	9,10%	9,80%
Naloxone HCl $2H_2O$	2,50%	2,48%	2,70%	2,70%	2,65%
Other excipients	87,69%	87,66%	87,20%	86,20%	85,42%
PVP 30	1,00%	1,00%	1,10%	2,00%	2,13%

The assay results are presented in Table IV.12. SlFs obtained from formulation LS.36 presented the most promising results in terms of drug substances content because it was within the specification range and it had the lowest difference between naloxone and buprenorphine results. At such small scale, it was difficult to obtain a good homogenization of the liquid mixture of all the components of the formulation, which can explain the differences in content in the different formulations, as well as the lower assay for buprenorphine.

Table IV.12. Characterization of LS.32 to LS.36 regarding average weight and drug substances content.

	Buprenorphine		**Naloxone**		
Formulation	**Assay (%)**	**RSD**	**Assay (%)**	**RSD**	**Weight (mg)**
LS.32	91,96	2,95	94,22	1,15	23,60
LS.33	92,18	0,58	95,04	0,05	23,14
LS.34	90,59	0,72	98,53	0,61	22,30
LS.35	93,81	0,01	102,50	0,19	22,41
LS.36	103,06	0,72	105,27	1,36	23,71

The next step was a first upscaling of about 10 times using the same composition of formulation LS.36. The scale-up required adjustments in the mixing speed, mixing time and stirrer type (4-bladed or anchor propeller). Also, half of the liquid mixture was casted in the same day of its production

(LS.37 A) and the other half was left under stirring (100 rpm) during 20h and after that the casting was performed (LS.37 B).

Table IV.13. Characterization of formulations LS.37 A and LS.37 B regarding liquid mixture uniformity, uniformity of dosage units (UDU), drug substances content/ assay, related substances (total to report) and residual water content.

	Assay in the liquid mixture (%)		UDU		Assay (%) ± RSD		Related substances (%)		Residual water content
Formulation	**BUP**	**NLX**	**BUP**	**NLX**	**BUP**	**NLX**	**BUP**	**NLX**	
LS.37 A	Top 89,70 Middle 110,45 Bottom 101,98	Top 102,16 Middle 110,45 Bottom 105,80	14	14,3	98,93± 0,74	97,08± 3,58	≤0,1	0,17	6,36
LS.37 B	Top 100,03 Middle 105,44 Bottom 104,91	Top 101,95 Middle 105,77 Bottom 105,70	10,9	13,8	95,66± 0,09	94,2± 0,02	≤0,1	≤0,1	5,51

The results of this experiment showed that it is feasible to perform the scale-up of this formulation, because it was possible to obtain a liquid mixture without clumps and SlFs with an acceptable appearance. However, some adjustments to the manufacturing process were required in order to obtain a homogenous mixture since buprenorphine and naloxone were not uniformly distributed in the liquid mixture (Table IV.13) in both casting days. The samples of the liquid mixture collected in the middle and bottom of the vessel exhibited a higher content of both drug substances than the samples collected in the top of the vessel, suggesting that the drug substances particles tend to sediment in the bottom of the vessel (buprenorphine) or were not properly dissolved (naloxone). Nevertheless, LS.37 B SlFs exhibited an uniform aspect and a good resistance to handling while LS.37 A SlFs displayed a sand like aspect. The drug substances content of LS.37 A was about 97% to 99% while in LS.37 B it was about 94% to 96% (Table IV.13), which can be a consequence of casting a non-homogeneous liquid mixture. The drug release profile of both DS is similar in the two formulations (LS.37 A and LS.37 B) but the drug release rate is slower than observed for the RP (Figure IV.9). No increase in related substances to report was observed for LS.37 B suggesting that the mixture can be used at least during 48h (Table IV.13). Though such information should be better accessed in an holding-time study including a microbiological evaluation, pH, viscosity and other

relevant attributes of the mixture as defined by the general guidance on “Holding-time” studies [127].

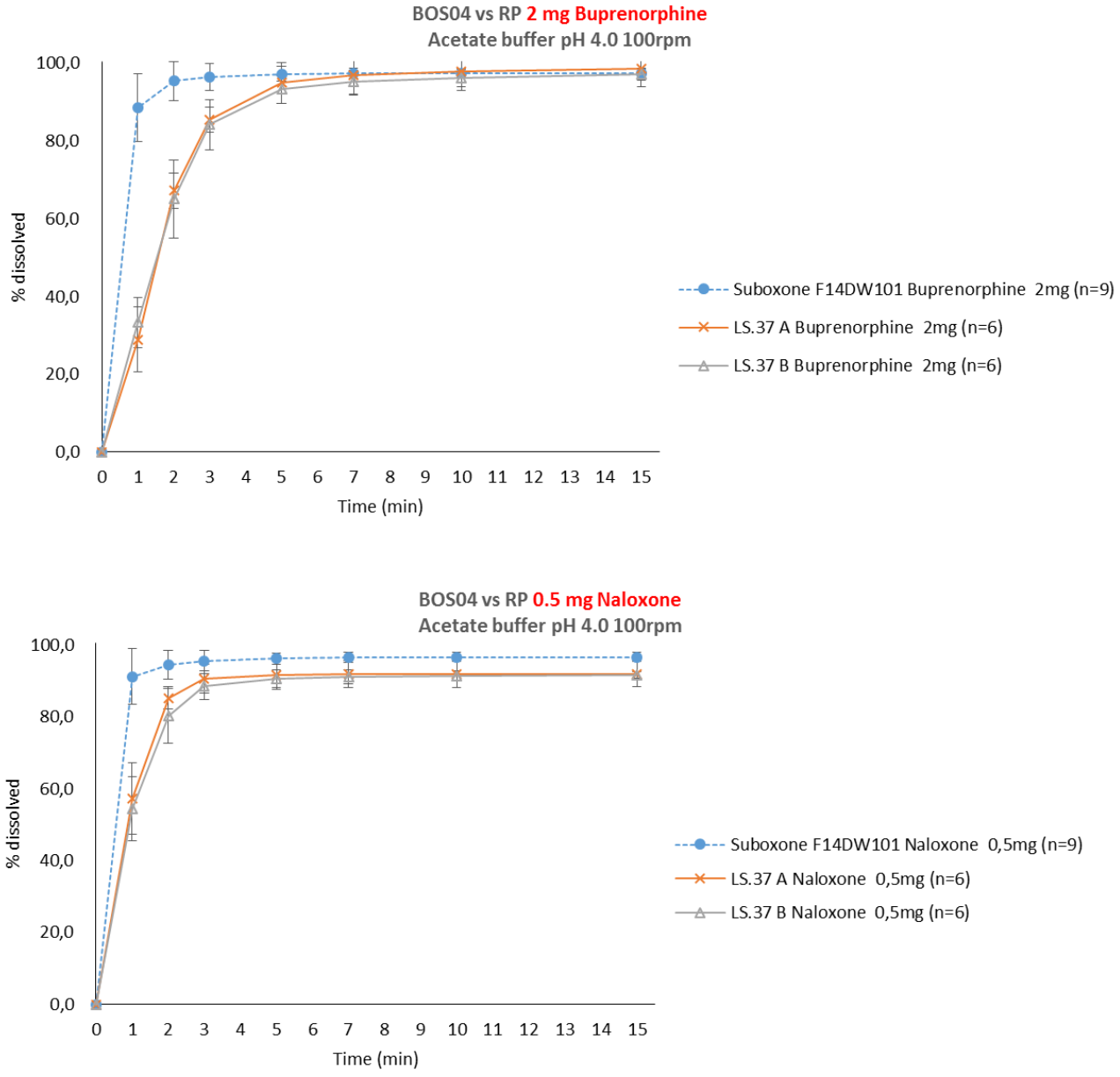


Figure IV.9. Drug release profile of Buprenorphine (top) and Naloxone (bottom) of formulations LS.37 A and LS.37 B (solid lines) and RP (dashed lines). Initial data (average ± SD).

A pre-stability study was initiated to investigate the stability of these SIFs upon storage in two different conditions 25°C / 60% RH and 40°C / 75% RH. Buprenorphine and naloxone content decreased slightly after 1 month for both conditions and remained unchanged in other stability time points (Table IV.14 and Table IV.15). Considering that there was a high variability on films' weight for both formulations (LS.37 A and LS.37 B), the decrease observed for the drugs assay may not be entirely associated with an increase on related substances content.

Table IV.14. Buprenorphine and naloxone content of formulation LS.37 A and LS.37 B content after 1, 3 and 6 months at 25°C / 60% RH.

	Assay (%)	LS.37 A				LS.37 B			
		T0	T1M	T3M	T6M	T0	T1M	T3M	T6M
Naloxone	Average	97,08	92,49	94,03	94,13	94,2	93,9	92,56	95,12
	RSD	3,58	0,71	0,44	0,15	0,02	0,99	4,39	0,48
Buprenorphine	Average	98,93	97,48	97,17	95,45	95,66	96,96	94,21	94,93
	RSD	0,74	0,43	0,51	0,90	0,09	0,50	3,99	1,54

Table IV.15. Buprenorphine and naloxone content of formulation LS.37 A and LS.37 B content after 1 and 3 months at 40°C / 75% RH.

	Assay (%)	LS.37 A			LS.37 B	
		T0	T1M	T3M	T0	T1M
Naloxone	Average	97,08	90,88	92,93	94,2	91,96
	RSD	3,58	1,88	0,23	0,02	0,28
Buprenorphine	Average	98,93	95,4	95,42	95,66	95,63
	RSD	0,74	1,36	0,03	0,09	0,05

The related substances content increased over time for both DS after 1, 3 and 6 months of storage at 25°C / 60% RH (Table IV.16). The total amount of related substances is similar in LS.37 A and LS.37 B. As expected, the samples stored at 40°C / 75% RH exhibited, in general, a higher amount of related substances when compared with the same time point at the condition 25°C / 60% RH (Table IV.17). Formulation LS.37 B is the exception, where the total amount of related substances after 1 month is higher in samples stored at 25°C / 60% RH than in samples stored at 40°C / 75% RH.

Table IV.16. Total amount and total amount to report of buprenorphine and naloxone related substances of sublingual films LS.37 A and LS.37 B. Initial data and after storage 1, 3 and 6 months at 25°C / 60% RH.

		LS.37 A				LS.37 B			
		T0	**T1M**	**T3M**	**T6M**	**T0**	**T1M**	**T3M**	**T6M**
Naloxone	**Total**	0,66	0,67	0,72	1,05	0,29	0,95	0,84	1,06
	To report	0,17	≤0,1	≤0,1	0,51	≤0,1	0,27	0,21	0,39
Buprenorphine	**Total**	0,24	0,30	0,53	0,37	0,13	0,31	0,50	0,31
	To report	≤0,1	≤0,1	≤0,1	≤0,1	≤0,1	≤0,1	≤0,1	≤0,1

Table IV.17. Total amount and total amount to report of buprenorphine and naloxone related substances of sublingual films LS.37 A and LS.37 B. Initial data and after storage 1 and 3 months at 40°C / 75% RH.

		LS.37 A			LS.37 B	
		T0	**T1M**	**T3M**	**T0**	**T1M**
Naloxone	**Total**	0,66	0,65	1,09	0,29	0,64
	To report	0,17	≤0,1	0,59	≤0,1	≤0,1
Buprenorphine	**Total**	0,24	0,54	0,60	0,13	0,45
	To report	≤0,1	≤0,1	0,21	≤0,1	≤0,1

The drug release of both DS decreased after 1 month at 25°C / 60%RH in the two formulations (Figure IV.10 and Figure IV.11). Formulation LS.37 B maintained a stable drug release in the other stability time points while the rate of drug release from formulation LS.37 A continue to decrease until the end of the study (Figure IV.10 and Figure IV.11), which may be related with changes in the polymeric matrix during storage as discussed in chapter II. The decrease on drug release was higher after storage at 40°C / 75%RH (data not shown).

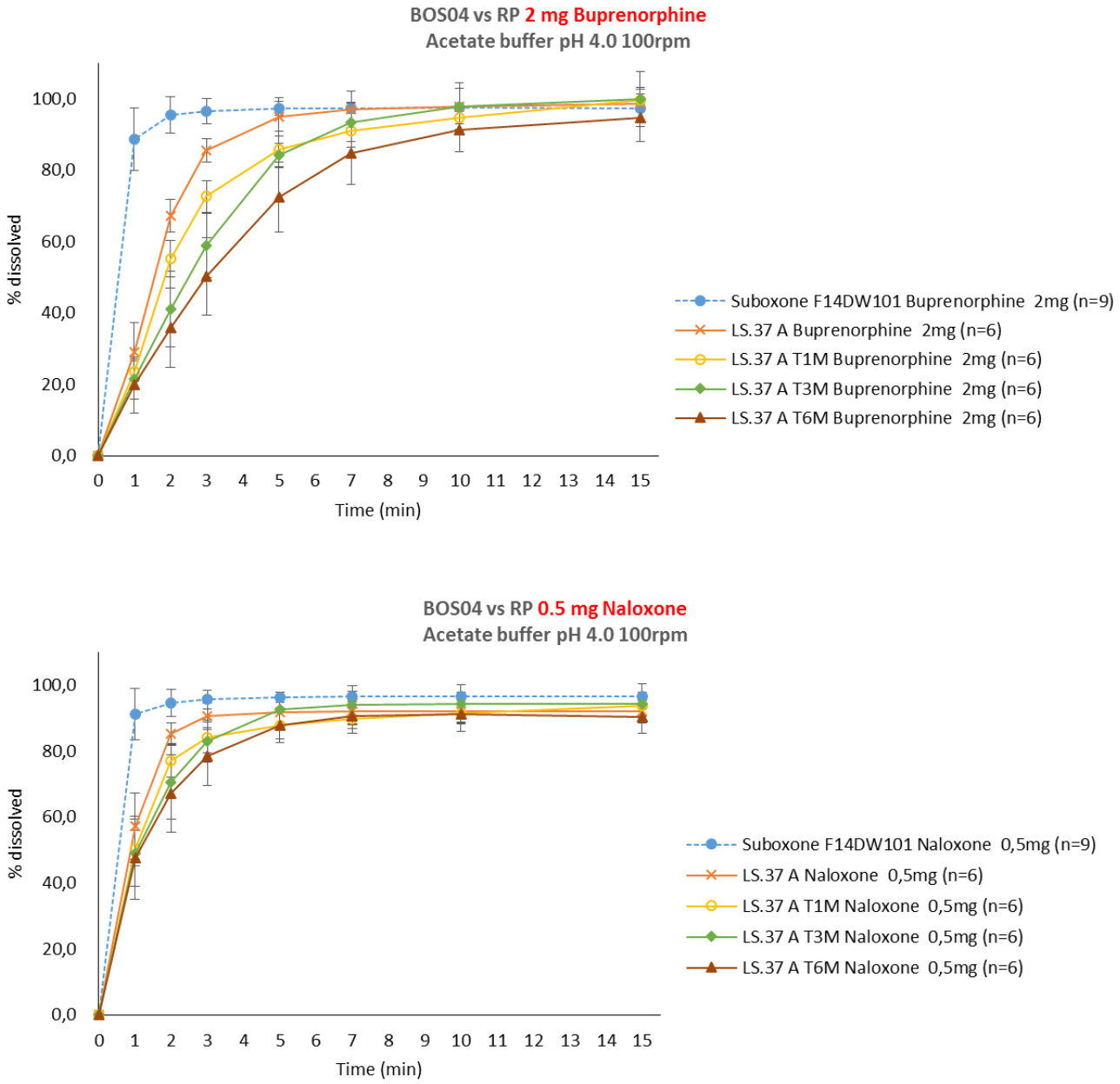


Figure IV.10. Drug release profile of Buprenorphine (top) and Naloxone (bottom) of formulations LS.37 A (solid lines) and RP (dashed lines). Initial data and after storage 1, 3 and 6 months at 25°C / 60% RH (average± SD).

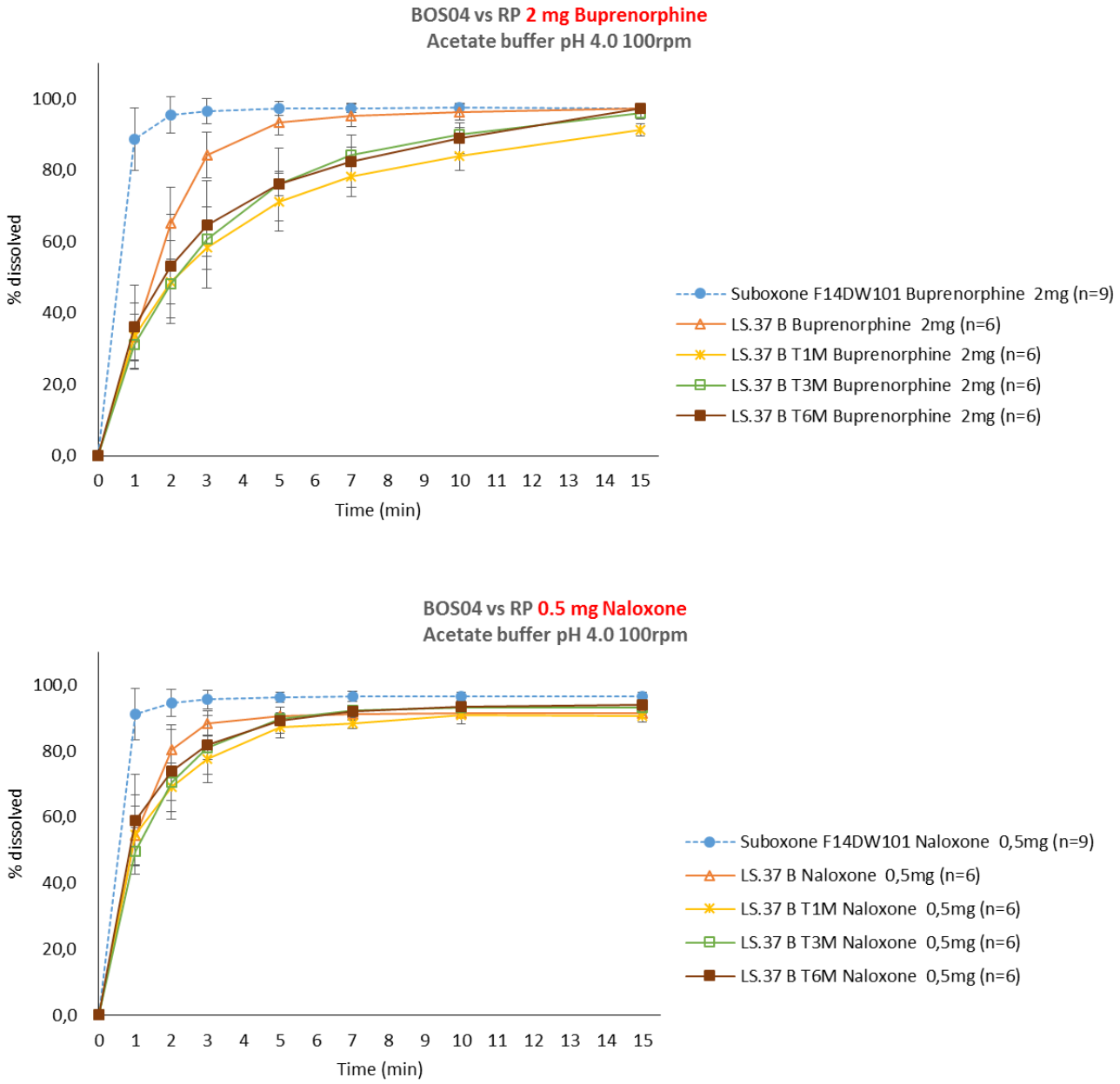


Figure IV.11. Drug release profile of Buprenorphine (top) and Naloxone (bottom) of formulations LS.37 B (solid lines) and RP (dashed lines). Initial data and after storage 1, 3 and 6 months at 25°C / 60% RH (average± SD).

4.5 Conclusions

The versatility of oral films' technology was evaluated in this chapter with the development of sublingual films to treat opioid dependence. This required the inclusion of mucoadhesive polymers

and solubilizers to improve the drug release of buprenorphine, a poorly water soluble molecule. A first scale-up of about 10 times was performed to identify possible CPPs and to assess the stability of the SIFs obtained in different casting days. The scale was still very small and no data was available regarding the characterization of the mixture. The next scale-up studies should include a holding time study of the liquid mixture to assess the viscosity, uniformity, related substances formation and microbiological attributes over a pre-determined time period [127].

V. Sublingual films to treat opioid dependence- formulation development part B

The goal of the present work was to apply the QbD principles such as risk assessment tools and DoE to investigate and develop sublingual films with higher dosage strengths than the described in the former chapter. This approach was based on the work performed in the previous chapter that contributed to improve the general understanding of oral films, as well as to the increase of the specific knowledge about the attributes that products for the oral mucosa should have. With the data modeling a greater understanding of how material attributes influence the quality attributes of the product was achieved. Additionally, the prototypes' stability over time was assed.

5.1 Introduction

The majority of the oral films (OFs) available on the market uses water-soluble drugs which are easy to formulate for fast disintegration / dissolution, uniform drug substance content and preservation of the mechanical properties [17,128–131]. Though, there is a growing number of poorly water-soluble drug candidates in the pharmaceutical industry that could also benefit with the OFs technology.

In recent studies different strategies have been employed to enable the incorporation of poorly water-soluble drug substances in OFs, namely hot-melt extrusion (HME), organic solvent casting and addition of solubilizers [17,27,128,132]. In HME the dissolution enhancement of the drug depends on the stability of the solid dispersion during storage, where too high drug loading can result in uncontrolled crystallization. Organic solvent casting faces the same stability problem as the HME method. The evaporation of the solvent in which the drug substance is dissolved may result in uncontrolled crystallization if the drug loading in the solvent is too high. These additional constraints in the drug loading of OFs, that already have a limited capacity, restrict the drug substances to be incorporated. Additionally, for products developed for the paediatric population the permitted amounts of residual solvents is very low when compared to the adult population and, it can be challenging to reach those levels [17,128–130].

The addition of the drug substances in the form of liquid or solid suspensions has been indicated as a promising approach to overcome the challenges mentioned above [128,131]. Woertz and Kleinebudde [128] assessed the physical and chemical stability of ODFs containing 2 mg /6 cm^2 of loperamide hydrochloride (poor water solubility) prepared by suspending the micronized powder in water. They were able to define a formulation that provided ODFs where no changes in the polymorphic form occurred. De Mohac and co-workers [131] used a mixed approach to improve the drug release rate of ODFs having olanzapine. They tested formulations containing the drug substance suspended in a polyvinylpyrrolidone solution and formulations with further addition of solubilizers. No changes in the polymorphic form of olanzapine were observed and the drug release increased in the presence of polyvinylpyrrolidone alone and in combination with solubilizers.

As mentioned in section 4.1 the combination of buprenorphine and naloxone is used as first-line treatment for opioid dependence [87–89] and it was firstly introduced on the market as sublingual tablets. However, sublingual tablets have been associated with misuse and abuse due to the ease

of crushing for snorting or intravenous injection [85]. Sublingual films (SlFs) were introduced as an alternative dosage form because they are difficult to crush and snort [92]. In the previous chapter, SlFs containing buprenorphine and naloxone were prepared by water based solvent casting. Buprenorphine has poor water solubility which required the addition of solubilizers to improve the dispersion in the mixture and to increase the drug release. The goal of this part of the work was to develop SlFs containing higher drug loading (8 mg/2 mg and 12 mg/ 3 mg of buprenorphine/ naloxone) than the SlFs prepared in chapter IV (2 mg/ 0,5 mg and 4 mg/ 1 mg of buprenorphine/ naloxone). To achieve this, a QbD approach was followed to better understand how material attributes influence the quality attributes of SlFs.

5.2 Problem elicitation

The development of the lowest strength (LS) formulation allowed to investigate and identify critical aspects of SlFs formulations and to define a qualitative and quantitative composition for SlFs of buprenorphine and naloxone. Therefore, for the development of the highest strength (HS) formulation it was possible to follow a more straightforward QbD approach with the application of risk assessment tools and design of experiments as illustrated in Figure V.1.

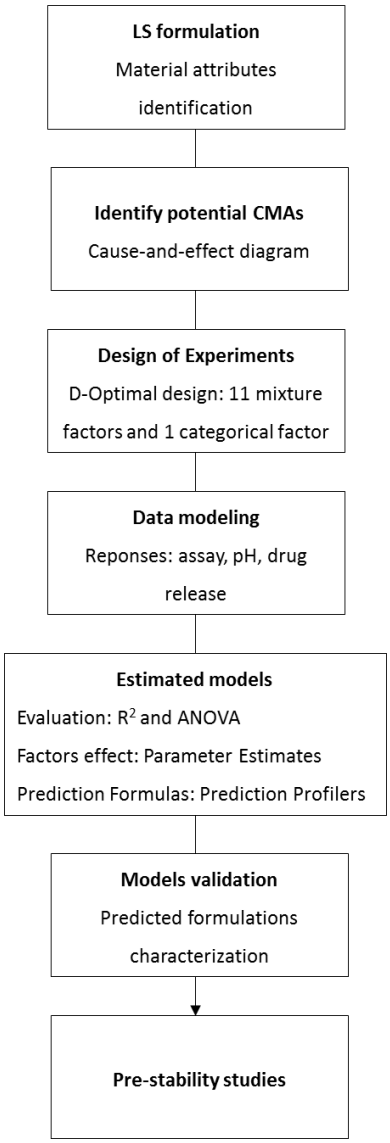


Figure V.1. Flow chart with the main steps followed to obtain sublingual films.

5.3 Materials and methods

5.3.1 Materials

The materials used in this work are described in section 4.3.1.

5.3.2 Risk assessment

A cause-and-effect diagram (Figure V.2) for the SlFs was constructed based on the knowledge acquired during the development of the low strength formulation and the work performed in Chapter II, in order to establish the set of potential cause-effect relationships. The investigation

during the development of the high strength formulation focused on the raw materials used, its concentration and the molecular weight of the solubilizer (green colored in Figure V.2). This choice was based in the need to validate the previous results obtained with PVP of different molecular weights and to define the quantitative composition of the SlFs. Therefore, a DoE was constructed where the concentrations of raw materials were explored as well as the molecular weight of PVP.

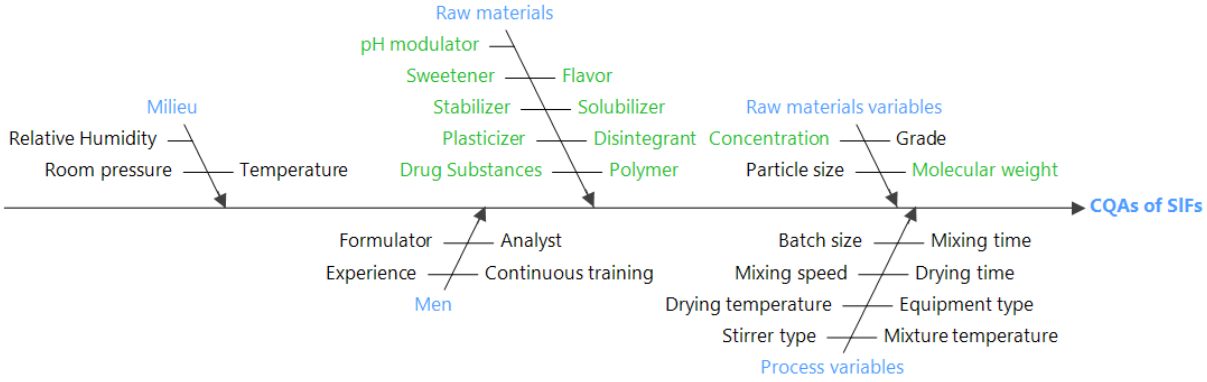


Figure V.2. Cause-and-effect diagram representing the possible interference of different factors (raw materials, raw materials variables, process variables, men and milieu) with sublingual films (SlFs) critical quality attributes (CQAs). The factors that were studied in this section are represented in green.

5.3.3 Formulation development

The knowledge acquired during the research and development of the LS formulation was used to generate a D-Optimal Design (JMP® software, versions 12 and 13, SAS Institute Inc., Cary, NC, 1989-2007) for the development of the HS formulation with 11 mixture factors and 1 categorical factor. The model was designed to study only the main effects and two center points were added originating a total of 66 experiments as default and a minimum of 14. The strategy followed in this study included an approach with the minimum number of runs required for the DoE.

5.3.4 Manufacturing process development

Refer to section 4.3.5 for more details.

5.3.5 Characterization of sublingual films

The characterization methods disintegration, residual water content, local pH, mechanical tests, assay, related substances, drug release and data analysis used in this chapter, are the same as described in the development of the LS formulation (section 4.3.6).

5.3.5.1 FTIR spectroscopy

Fourier transform infrared (FTIR) spectra were recorded with a FTIR – spectrometer (Perkin Elmer, Spectrum 2). The SlFs were directly measured using an attenuated total reflectance (ATR) module equipped with a diamond crystal. Data collection was performed with a 4 cm^{-1} resolution and 11 scans over the wavenumber region of 4000-500 cm^{-1}. The drug substances were measured by the KBr method with a 4 cm^{-1} resolution and 8 scans over the wavenumber region of 3800-650 cm^{-1}.

5.4 Results and Discussion

After developing the low strength formulation, the research work proceeded with the development of a suitable formulation to obtain SlFs for the highest dosage strengths (8 mg / 2 mg and 12 mg/ 3 mg, Buprenorphine/ Naloxone). The composition of the formulation of the two highest strengths is the same, the difference resides in the size of the final product.

5.4.1 Composition and characterization of sublingual films

In Table V.1 is listed the qualitative and quantitative composition of DoE set of formulations. The studied ranges were 20 to 30% for buprenorphine HCl, 5 to 8% for naloxone HCl $2H_2O$, 0 to 2% for carbopol, and 4 to 10% for solubilizer. These ranges were selected based on the results obtained in the research work of the low strength formulation.

The obtained SlFs were characterized regarding drug substances content, average weight, disintegration time, residual water content, mechanical properties, pH and drug release profile. The results show a high variability of the drug substances content and the films' weight (Table V.2) with only a few experiments within the assay specification range, namely HS.2, HS.4, HS.8, HS.11, HS.13 and HS.14 for naloxone, and HS.9 and HS.10 for buprenorphine. There was also a considerable variability in terms of disintegration time, residual water content, mechanical properties and local pH (Figure V.3). In Figure V.3, it is possible to observe that only for disintegration time and local pH all the SlFs were in accordance with the QTPP; for the others properties, some SlFs were within the

specification range and others were outside. Nevertheless, this result was expected considering the nature of the study where different ranges of the raw materials were tested in order to have a knowledge space large enough to perform the data modeling.

The SlFs presented poor mechanical properties with elongation values below 3% and puncture strength below 0,3 N/mm^2 (Figure V.3). The maintenance of proper mechanical properties for manufacture and handling is one of the greatest challenges of high drug loading in oral films. Usually, to account for higher drug loading the amount of other components (e.g. film-forming polymers and plasticizers) must be reduced [26,128,129]. In fact, several authors observed a decrease in the flexibility and an increase in the stiffness of OFs with increasing drug loadings [26,128,129]. In this work is hard to establish such direct correlation because all the components of the SlFs varied as a result of the DoE. Nevertheless, comparing the mechanical properties of SlFs containing buprenorphine and naloxone (Figure V.3 C and D) with placebo SlFs (data not shown) there was a clear decrease in the elongation to break and in the puncture strength. This indicates that drug loaded SlFs had less flexibility (assessed by lower values of elongation to break) and supported lower forces during manufacturing and handling (assessed by lower values of puncture strength) when compared to placebo SlFs.

Table V.1. Qualitative and quantitative composition of DoE set of formulations. The design, with 11 mixture factors and 1 categorical factor, was generated using JMP® software.

	% (w/w)													
Raw material	**HS.1**	**HS.2**	**HS.3**	**HS.4**	**HS.5**	**HS.6**	**HS.7**	**HS.8**	**HS.9**	**HS.10**	**HS.11**	**HS.12**	**HS.13**	**HS.14**
Buprenorphine HCl	20	30	22,23	20	30	20	20	20	22,23	30	30	30	30	20
Naloxone HCl $2H_2O$	5	8	8,55	8	8	5	8	8	8,55	5	5	8	5	5
Other excipients	63	58	58,12	62	50	68	68	66	58,12	55	59	50	59	65
Carbopol 971	2	0	4,08	0	2	2	0	2	4,08	0	2	2	2	0
Solubilizer	10	4	7,02	10	10	5	4	4	7,02	10	4	10	4	10
Type solubilizer	PVP 30	PVP 30	PVP 30	PVP 25	PVP 30	PVP 30	PVP 25	PVP 30	PVP 25	PVP 25	PVP 30	PVP 25	PVP 25	PVP 30

Table V.2. Characterization of formulations HS.1 to HS.14 regarding average weight and drug substances content. The values in bold indicate results within the specification range 90%-110%

	Buprenorphine		**Naloxone**		
Formulation	**Assay (%)**	**RSD**	**Assay (%)**	**RSD**	**Weight (mg)**
HS.1	63,64	2,53	56,31	0,70	42,80
HS.2	110,22	0,85	**107,16**	0,41	51,36
HS.3	85,88	1,27	123,71	0,53	54,60
HS.4	68,53	1,24	**99,03**	0,78	45,66
HS.5	166,46	6,17	177,81	2,42	78,57
HS.6	81,34	0,80	72,44	0,47	56,67
HS.7	116,5	3,83	166,04	0,91	75,66
HS.8	62,06	1,02	**93,37**	0,72	44,80
HS.9	**96,99**	1,95	137,69	0,29	56,47
HS.10	**105,92**	0,47	64,86	0,72	48,44
HS.11	159,3	1,38	**99,18**	0,29	73,48
HS.12	154,04	1,24	150,12	0,72	68,73
HS.13	160,44	0,75	**98,77**	0,32	73,52
HS.14	116,95	2,98	**106,35**	0,69	80,63

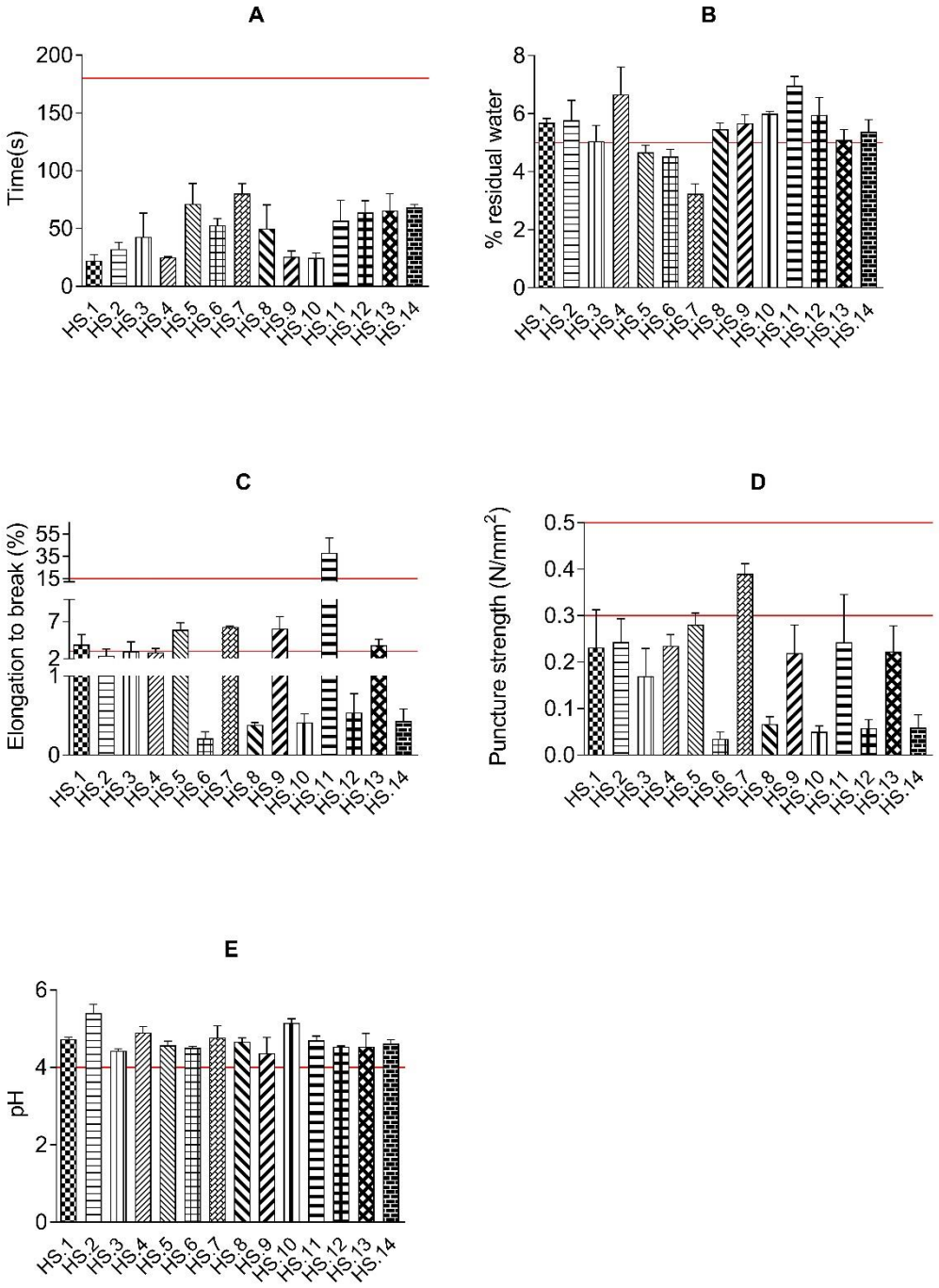


Figure V.3. Disintegration time (A), residual water (B), mechanical properties (C and D) and local pH (E) of sublingual films HS.1 to HS.14. Results are expressed as average ±SD (n=3). The horizontal red lines represent the limits defined in the QTPP. Disintegration time < 180s; residual water content < 5%; elongation to break 3-15%; puncture strength 0,3-0,5 N/mm²; pH >4.

5.4.2 Formulation selection - data modeling

The average values for the CQAs drug substances assay, drug release at one and two minutes, and local pH were used for data modeling. The model parameters were estimated using ordinary Standard Least Squares. All the models were evaluated for their fitting quality and statistical significance by means of the coefficient of determination (R^2) and several statistical hypothesis tests, such as ANOVA and individual tests to the significance of the regression parameters (p-value) as well as the possible presence of collinearity (Variance Inflation Factors, VIF). The prediction formula for each response was used to construct the prediction profiler illustrated in Figure V.4.

The model parameters estimates for buprenorphine, naloxone, carbopol, solubilizer and type of solubilizer are present in Table V.3 along with their significance level. A negative estimate means that the higher the parameter value the lower is the value of the response (CQA), and a positive estimate indicate that the higher the parameter value the higher is the value of the response (CQA). For example, the solubilizer seems to have a positive effect in buprenorphine release at 1 minute, which suggests that when increased amounts of solubilizer are used the drug release rate would be faster, but this effect is statistically non-significant ($p<0,05$). The prediction profiler (Figure V.4) shows, in a graphical way, how the parameters studied (concentration of raw materials and type of solubilizer) influence the CQAS. The lines' slopes indicate if the influence is positive or negative and higher slopes indicate higher impact on certain CQA.

The solubilizer type was included in this study to validate the results obtained in chapter IV. In Figure V.4 and Table V.3 it is possible to observe that the lower molecular weight PVP (PVP 25) has a negative effect in the drug release. This means that when PVP 25 is used as solubilizer the drug release of both DS would be slower than when PVP 30 is used. These results are in accordance with the data previously described in section 4.4.3, but are different from the results obtained by others where the increase in polymers' molecular weight resulted in the decrease of drug release [123–125]. No disturbances in the film-formation, that could compromise the disintegration and drug release, are expected to occur when the drug substances are uniformly distributed in the polymeric matrix [17]. As already discussed in section 4.4.3, the viscosity increase in high molecular weight polymers improved the homogeneity of SlFs either in terms of appearance and content uniformity.

*Table V.3. Sorted effect estimates for Critical Quality Attributes (CQAs) used in the model. Significance level (*p≤0,05).*

Sorted Parameter Estimates			CQA: local pH	
Term	**Estimate**	**Std Error**	**t Ratio**	**Prob>|t|**
Buprenorphine	5,6780446	0,339314	16,73	0,0036*
Carbopol 971	-13,4337	1,836938	-7,31	0,0182*
Naloxone	5,5819191	1,273583	4,38	0,0483*
Solubilizer	2,2030597	0,715199	3,08	0,0912
Type solubilizer (PVP 25)	-0,041637	0,019546	-2,13	0,1669
			CQA: Buprenorphine Assay	
Buprenorphine	621,5993	48,34628	12,86	0,0060*
Solubilizer	40,166044	101,9032	0,39	0,7315
Naloxone	-60,042	181,463	-0,33	0,7722
Carbopol 971	-67,13116	261,7312	-0,26	0,8215
Type solubilizer (PVP 25)	0,4858996	2,785018	0,17	0,8776
			CQA: Buprenorphine 1 min	
Buprenorphine	-77,04823	67,25151	-1,15	0,3705
Solubilizer	157,97216	141,7513	1,11	0,3811
Type solubilizer (PVP 25)	-3,709293	3,874065	-0,96	0,4394
Naloxone	-232,939	252,4218	-0,92	0,4535
Carbopol 971	-0,188718	364,0779	-0,00	0,9996
			CQA: Buprenorphine 2 min	
Carbopol 971	-453,1167	298,0639	-1,52	0,2678
Type solubilizer (PVP 25)	-4,435051	3,171625	-1,40	0,2969
Buprenorphine	-63,72577	55,05757	-1,16	0,3666
Solubilizer	100,41412	116,0491	0,87	0,4781
Naloxone	-7,905954	206,6531	-0,04	0,9730
			CQA: Naloxone Assay	
Naloxone	1583,2627	160,4973	9,86	0,0101*
Buprenorphine	211,57126	42,76051	4,95	0,0385*
Solubilizer	129,53332	90,12968	1,44	0,2872
Carbopol 971	-251,2168	231,4916	-1,09	0,3912
Type solubilizer (PVP 25)	0,2072855	2,463246	0,08	0,9406
			CQA: Naloxone 1 min	
Solubilizer	120,14707	169,9933	0,71	0,5530
Type solubilizer (PVP 25)	-1,782806	4,64592	-0,38	0,7381
Naloxone	64,32748	302,7135	0,21	0,8514
Buprenorphine	10,985947	80,65047	0,14	0,9041
Carbopol 971	2,8984329	436,6156	0,01	0,9953
			CQA: Naloxone 2 min	
Naloxone	215,07856	113,0267	1,90	0,1974
Solubilizer	116,44095	63,47185	1,83	0,2080
Buprenorphine	39,975143	30,11315	1,33	0,3156
Type solubilizer (PVP 25)	-2,039608	1,734687	-1,18	0,3607
Carbopol 971	-83,93966	163,0229	-0,51	0,6579

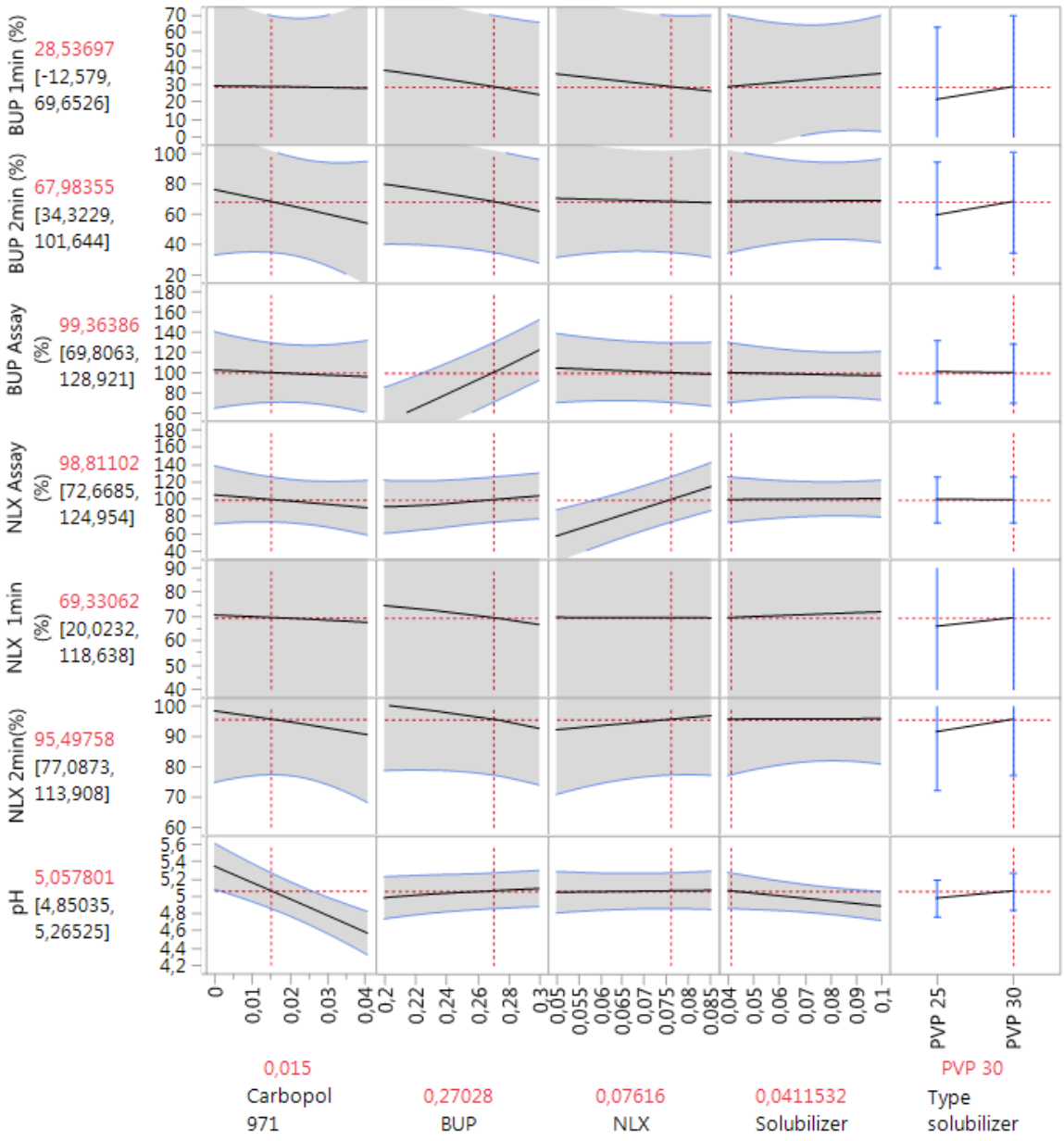


Figure V.4- Prediction profilers for buprenorphine (BUP) and naloxone (NLX) assay, drug release at 1 min and 2 min and, local pH. The black lines represent the prediction trace, the vertical red lines correspond to the current value of the factor, the red value on the vertical axis is the predicted response based on the current values of the factors, and the grey shaded zone represent the confidence interval of the mean predicted value.

Two target formulations (Table V.4) expected to match the QTPP and validate the data modeling performed, were generated using the prediction profiler. The obtained SlFs were characterized and

a pre-stability study was initiated. The drug substances content was higher than the specification in SlFs HS.16 (Table V.6), the drug release profile of HS.15 was similar to the RP for both DS (p>0,5) while HS.16 was only similar to RP in the drug release of naloxone, and the local pH of HS.15 (with carbopol) was lower than the pH of HS.16 (without carbopol). The comparison between the predicted responses (Table V.5) and the obtained values (Table V.6) show some differences. Nevertheless, they were contained within the 95% confidence interval (CI) with the exception of the local pH of HS.15 and HS.16, where the obtained value was below the lower limit of the CI. The results are satisfactory considering that some models used to construct the prediction profiles do not had statistical significance, which results in a less reliable prediction with broad CI. The models could be improved with more experiments or if a reduced number of parameters / factors were used to obtain the DoE.

Table V.4. Qualitative and quantitative composition of HS.15 and HS.16 determined through the prediction profiler.

	% (w/w)	
Raw material	**HS.15**	**HS.16**
Buprenorphine HCl	27,03	26,80
Naloxone HCl $2H_2O$	7,62	7,40
Other excipients	59,74	60,93
Carbopol 971	1,50	0,00
Solubilizer	4,11	4,86
Type solubilizer	PVP 30	PVP 30

Table V.5- Critical Quality Attributes (CQAs) predicted values and 95% confidence interval (CI). BUP- buprenorphine, NLX- Naloxone.

	HS.15		**HS.16**	
	Predicted CQAs	**95% CI**	**Predicted CQAs**	**95% CI**
BUP 1min	28,54	-12,58-69,65	27,19	-21,88-76,26
BUP 2min	67,98	34,32-101,64	70,73	30,55-110,90
BUP Assay	99,36	69,81-128,92	99,11	63,83-134,39
NLX Assay	98,81	72,67-124,95	99,23	68,03-130,43
NLX 1min	69,33	20,02-118,64	65,08	6,23-123,93
NLX 2min	95,50	77,09-113,91	93,37	71,40-115,34
Local pH	5,06	4,85-5,27	5,29	5,04-5,54

Table V.6- Characterization of sublingual films HS.15 and HS.16 regarding drug substances content, average weight, drug release and local pH. BUP- buprenorphine, NLX- Naloxone.

	Buprenorphine		Naloxone			Drug release				
Formulation	**Assay (%)**	**RSD**	**Assay (%)**	**RSD**	**Weight (mg)**	**BUP 1min**	**BUP 2min**	**NLX 1min**	**NLX 2min**	**Local pH**
HS.15	106,29	0,53	104,97	0,42	53,38	23,71	65,20	67,18	91,55	4,710
HS.16	115,53	1,42	113,20	0,92	59,37	28,62	63,75	62,33	91,48	4,891

The analysis of FTIR spectra is employed to evaluate possible interactions of drug substances - excipients and excipients - excipients. This is based in the identification of changes in the intensity or in the shape of the functional groups bands [16,26,133]. The FTIR spectra of buprenorphine, naloxone, placebo SlFs, HS.15 SlFs and HS.16 SlFs are displayed in Figure V.5. The buprenorphine and the naloxone IR spectra showed characteristic bands of: aromatic rings at wavenumbers 3150-3000 cm^{-1}, 1600-1500 cm^{-1}, hydroxyl groups at wavenumbers 3300-3400 cm^{-1} and cyclic ethers at 1050-1030 cm^{-1} [134]. Additionally, it is possible to identify the carbonyl group of naloxone at wavenumbers of 1700 cm^{-1}. The sublingual films spectra are dominated by the characteristic bands of carbonyl and alcohols groups (3300, 1730, 1420, 1230 and 1020 cm^{-1}) and, alkene groups (1650 cm^{-1}) present in the four main components of the formulation. It is interesting to note, that the SlFs spectrum do not exhibited differences in intensity and shape of the main bands by the incorporation of drug substances. The work of Zhao and colleagues [133] demonstrated that in oral films of PVA comprising meclizine, the typical IR bands of the drug substance and the main bands of PVA do not changed. This indicated that no interaction existed between the drug substance and the polymer. Also, in the work of Saoji et al [16] it was verified that the major bands of the drug substance were retained in ternary mixtures with different polymers. It may sound that the results of the present work are different from the ones just referred; however, it should be highlighted that the SlFs analyzed are a complex matrix of film-forming polymer, disintegrant, stabilizer, plasticizer, solubilizer, pH modulator, sweeteners, flavor and drug substances. Therefore, the characteristic bands of the drug substances would be masked by the placebo bands, which does not necessarily mean that there were interactions between the drug substances and the excipients.

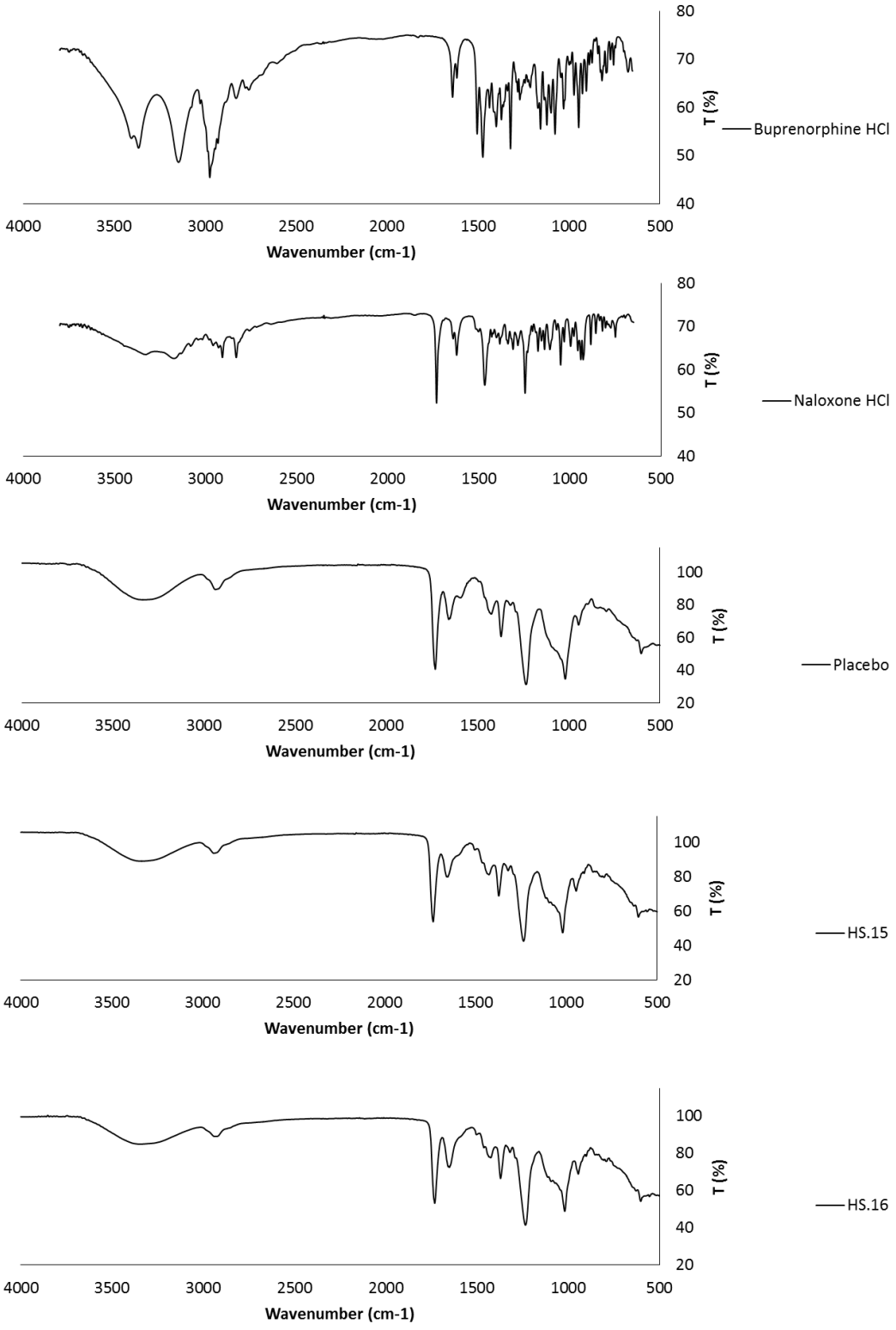


Figure V.5. Infrared spectrum of buprenorphine, naloxone, placebo SlFs and HS.15 and HS.16 SlFs.

5.4.3 Pre-stability studies

The performance of the SlFs of formulations HS.15 and HS.16 was evaluated after storage at 25°C / 60% RH.

Although the difference is not statistically significant (p>0,05), a decrease in the elongation to break and the puncture strength of HS.15 after 1 month was observed (Figure V.6 C and D). Also, the residual water content and the disintegration time are higher after 1 month for both formulations (Figure V.6 A and B). The increase in the disintegration time is accompained by an accentuated decrease in drug release rate of formulation HS.15 after 1 month and a less pronounced decrease after 5 months of storage (Figure V.7). For formulation HS.16 a marked reduction in drug release rate was observed after 5 months of storage (Figure V.8). These findings are in accordance to the described in section II: the room temperature and the room RH during manufacturing have an impact on the stability of the polymeric matrix that in turn influence on the DS release. The drug substances content was stable up to 5 months despite the increase in related substances content (Table V.7). These results indicated that the formulation and the packaging material ensures the stability of the drugs substances at least during this period of time.

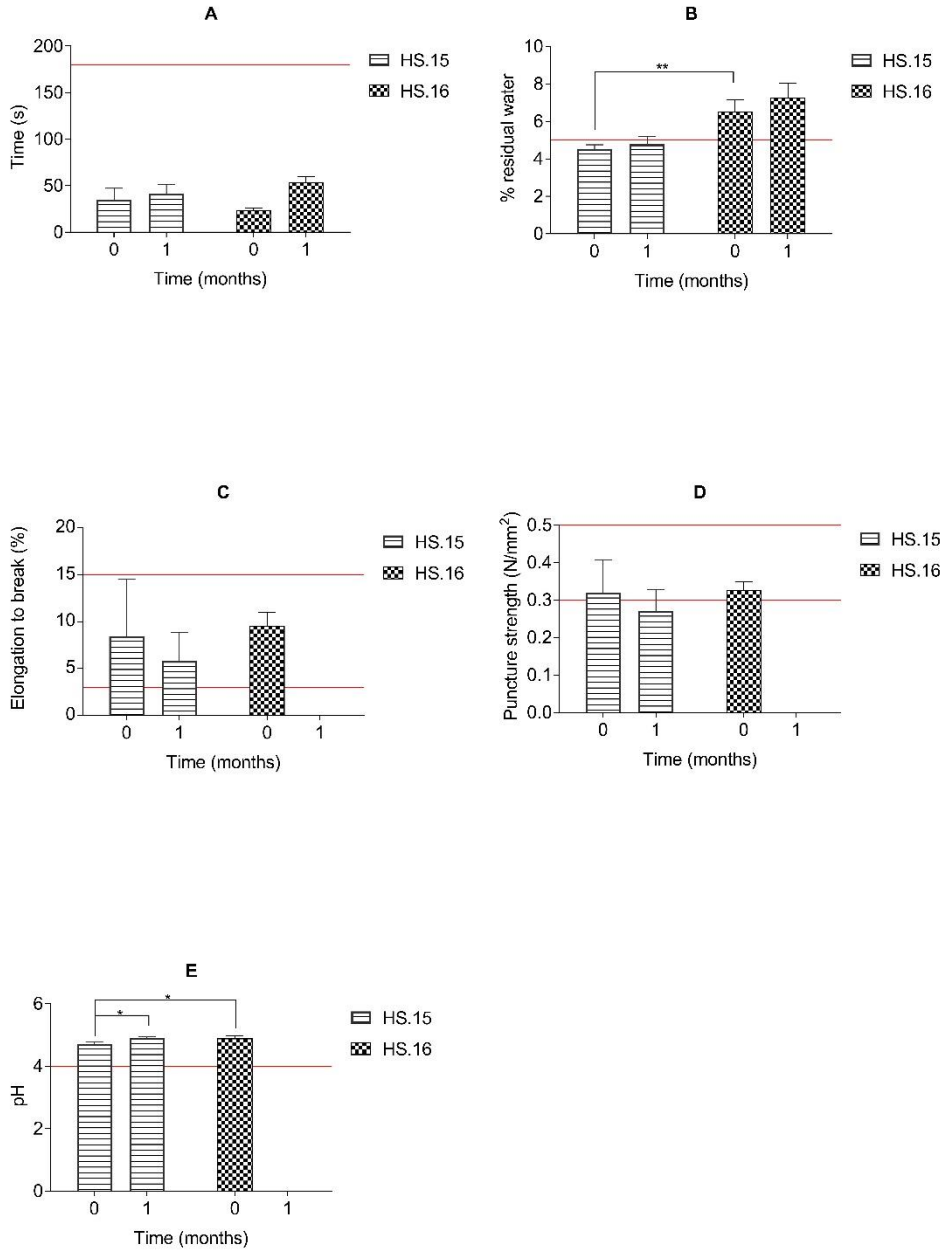


*Figure V.6- Disintegration time (A), residual water (B), mechanical properties (C and D) and local pH (E) of sublingual films HS.15 and HS.16 after storage 1 month at 25 °C / 60% RH. Results are expressed as average ±SD (n=3). The horizontal red lines represent the limits defined in the QTPP. Disintegration time < 180s; residual water content < 5%; elongation to break 3-15%; puncture strength 0,3-0,5 N/mm²; pH >4. (ns p> 0.05; *p < 0.05; ** p < 0.01; *** p < 0.001 by one-way ANOVA; post hoc Bonferroni's test).*

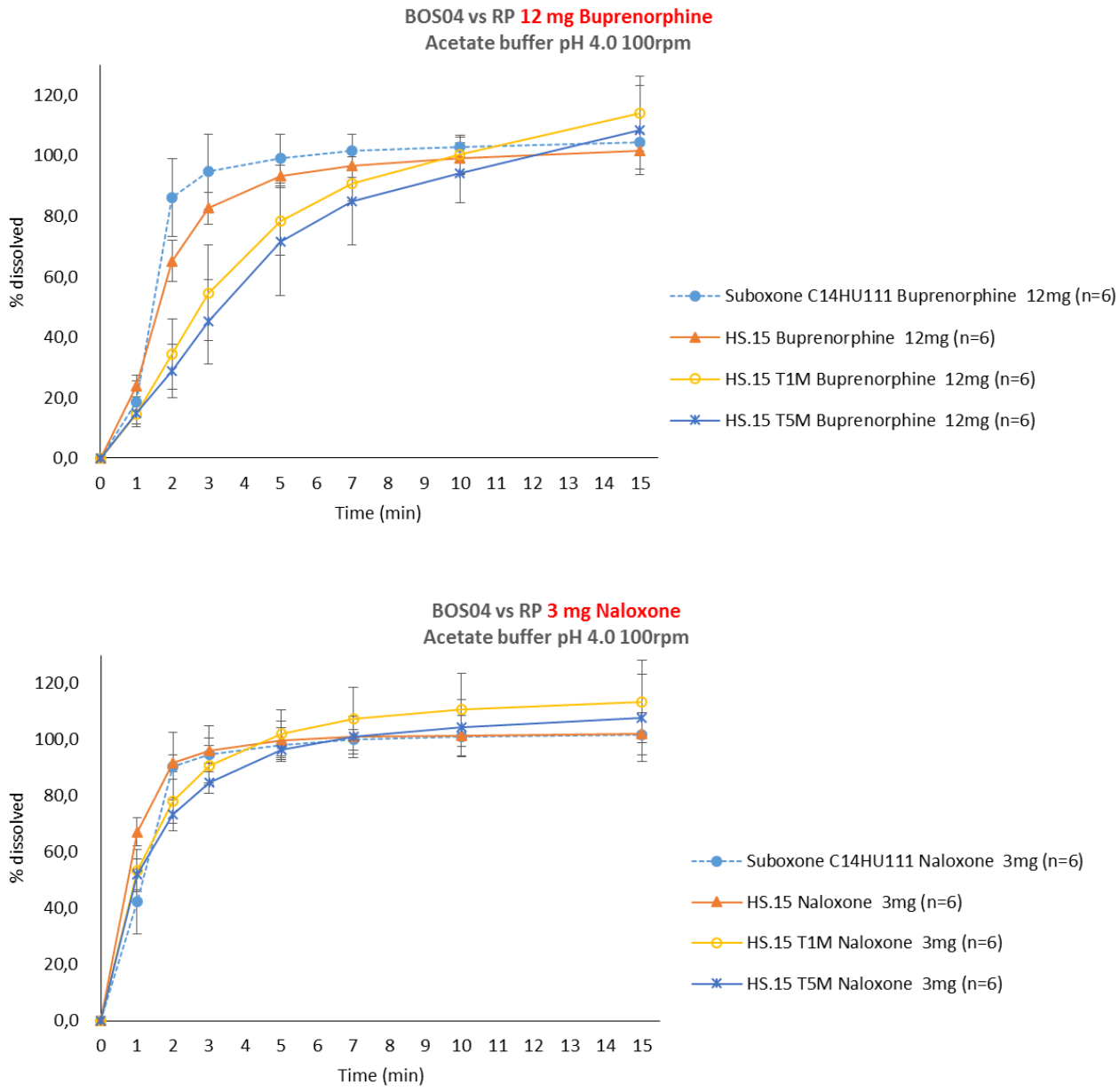


Figure V.7. Drug release profile of Buprenorphine (top) and Naloxone (bottom) of formulation HS.15 (solid lines) and RP (dashed lines). Initial data and after storage 1 and 5 months at 25°C / 60% RH (average ± SD).

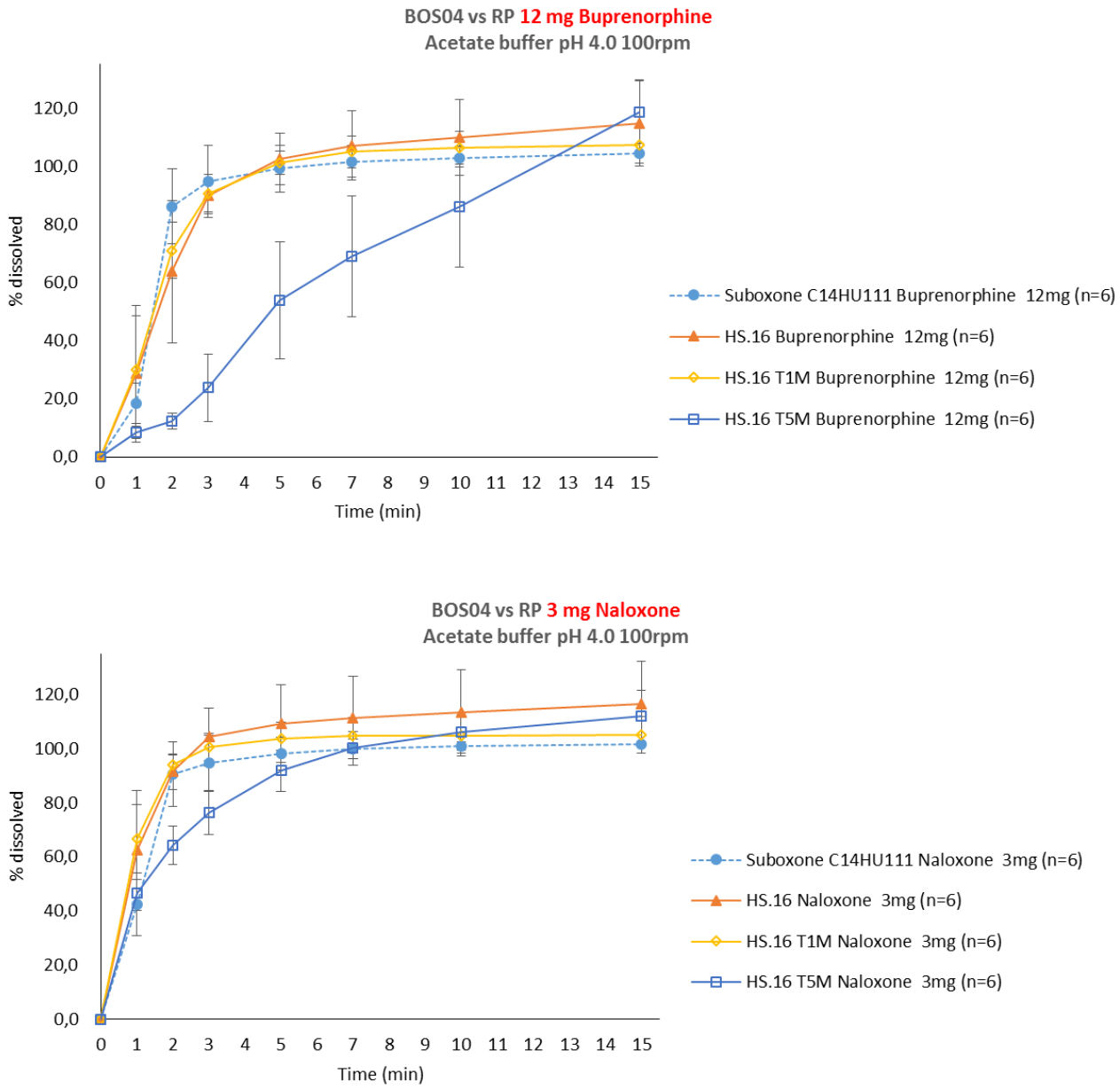


Figure V.8. Drug release profile of Buprenorphine (top) and Naloxone (bottom) of formulation HS.16 (solid lines) and RP (dashed lines). Initial data and after storage 1 and 5 months at 25°C / 60% RH (average ± SD).

Table V.7. Buprenorphine and naloxone content and, total amount and total amount to report of related substances of formulation HS.15 and HS.16 after 1 and 5 months at 25°C / 60% RH.

		HS.15			HS.16		
		T0	T1M	T5M	T0	T1M	T5M
		Assay (%)					
Naloxone	Average	104,97	112,18	112,27	113,23	120,21	114,95
	RSD	0,42	0,44	1,07	0,92	0,40	0,42
Buprenorphine	Average	106,29	111,08	109,23	115,53	121,61	112,70
	RSD	0,53	0,60	1,07	1,42	0,82	0,78
		Related substances (%)					
Naloxone	Total	0,22	0,51	1,40	0,57	0,80	1,86
	To report	≤ 0,1	≤ 0,1	0,60	≤ 0,1	≤ 0,1	0,98
Buprenorphine	Total	0,35	0,26	0,60	0,42	0,57	0,37
	To report	≤ 0,1	≤ 0,1	≤ 0,1	0,17	≤ 0,1	≤ 0,1

5.5 Conclusions

The application of QbD principles, namely cause-and-effect diagram and DoE, granted the understanding of the relationship between material attributes and drug product CQAs assay, drug release and pH. Two formulations with the potential to meet the QTPP were defined based in this approach and their characterization demonstrated promising results: drug release profiles and impurities profile similar to those observed for other products already available in the market. Additional work should include an augmenting design to extend the characterization range, to increase the robustness of the predictive models and to define the design space. After that, the scale-up should be performed taking into consideration that and increase in batch size requires the use of a larger mixing equipment with improved performance, which necessarily requires some adjustments in the CPPs values defined for the small scale equipment. Also, in order to reduce the risk of scaling-up it is highly recommended that laboratory, pilot and product scale equipment have the same operating principles [135,136].

VI. General Conclusions

Considering some performance limitations associated to conventional dosage forms as well as particular features of some diseases and characteristics of elderly and pediatric population, oral films emerge as an effective solution for some of the issues.

In this thesis, the manufacturing process of orodispersible films (ODFs) was investigated and optimized through the application of QbD principles using retrospective data (rQbD) enabling to achieve a higher-level of understanding of the manufacturing process of oral films. This is an approach that can be applied to other investigational medicinal products being developed (e.g. tablets and capsules) in order to increase the existing knowledge.

The positive application of rQbD enabled advancing to the GMP (Good Manufacturing Practice) production of an investigational medicinal product, to treat a neurodegenerative disorder. The clinical trial was conducted in order to validate, in the clinical setting, the performance and acceptability of the investigational ODFs to treat a neurodegenerative disorder when compared to other marketed product. The ODFs were well tolerated by the population of healthy subjects, and the systemic exposure to the drug substance was similar between the two groups which demonstrates that both capsules and ODFs are bioequivalent. Considering the positive results of this exploratory study, there are good chances of success for the product in reaching the market, and it can be considered that the concept is proved in humans.

Further work with the oral films technology intended to explore the formulation tailoring, by developing sublingual films to treat opioid dependence. Preliminary experiments were performed to investigate and select excipients that are known to promote the mucoadhesion and the solubilization of poorly soluble drug substances. This set of experiments were essential to employ the QbD principles for formulation development and optimization. The DoE approach helped to visualize how CMAs influence the CQAs drug substances assay, drug release and pH, and to select two promising formulations. The results showed that it was possible to develop sublingual films using BlueOS® technology by fine tuning the film composition.

VII. Final remarks and Future perspectives

The path from drug discovery to drug development ending at the regulatory approval of new drugs is costly and time consuming, which has led to an increasing interest in the research of advanced drug delivery technologies able to overcome the limitations of conventional dosage forms. The strategy involving the reformulation of existing drugs in order to improve their efficacy, safety and/or patient compliance is less expensive, faster, safer and, with higher probability of success because the efficacy and safety of the drug substances is already known. In the era of patient centric formulations, the oral films (OFs) technology came to light as patient-friendly and flexible dosage form that can address several limitations of conventional oral dosage forms.

For the manufacturing of oral films, solvent casting is the process of choice, where its application require the tight control of many variables that are only briefly described in the literature. It is possible to find references that indicate the film casting and the drying process as the critical steps for the scale-up of this technology. The film casting is influenced by the physicochemical properties of the liquid mixture and by the speed of casting. The drying process is also influenced by the physicochemical properties of the liquid mixture, the dryers' type, number of dryers and, air flow rate and relative humidity. Other parameters that should be considered during development, but mentioned in a lesser extent, are the chemical and physical stability of the liquid mixture, the ease of removal of the casted films from process liners, the wet coat thickness and, the drying time and temperature. Although the reference to these process parameters and materials attributes exists, it is not always clear how they influence the quality of the final product, especially in laboratory scale equipment. Despite the indication that solvent casting is simple to implement when compared to other manufacturing techniques, during the development of this work it was verified that the information is scarce, and similarities with other techniques and products should be sought. Indeed, in this thesis, it was demonstrated that some of the principles applied to polymeric coating of tablets and granules can be extended to oral films.

The application of QbD principles should be seen has an opportunity to gain greater understanding of product and manufacturing process performance, and not only as a requirement from the regulatory authorities. It is a common mistake to define the QTPP and the CQAs in the beginning of the project, and do not perform an update during the project execution. These are dynamic tools

that should be frequently reviewed to ensure that they reflect the changes in the project goals. When adequately applied, the QbD tools could be very useful to find general trends and significant predictors of the CQAs as demonstrated in this thesis. Nevertheless, prior knowledge and the execution of preliminary experiments is fundamental to proper planning the work, namely the DoE. This is even more critical when new dosage forms are being developed, because the knowledge about the technology and the interactions with the drug substances is limited at this development phase.

During the execution of this work two major challenges were encountered: 1) the lack of detailed characterization methods and acceptable limits from the regulatory authorities; and 2) the development of a hydrophobic matrix with the same performance of hydrophilic matrices. Regarding the first aspect, numerous works have been performed to develop and optimize characterization methods; however characterization and quality control should be standardized and, specifications for mechanical strength should be given. Only then it will be possible to consistently compare results. The second aspect was more demanding since the disintegration and drug release of hydrophobic matrices is inherently different. For example, the QTPP defined the product dimensions based in the commercially available product; however, when those dimensions were tested the SlFs had a slower drug release when compared to the marketed product. This was related with the thickness increase required to have appropriate drug substances content for the small SlFs size. If the matrix was hydrophilic such problem would not have occurred. In future developments, the QTPP should account with the intrinsic differences between hydrophobic and hydrophilic matrices and, a target for the SlFs weight should be defined. With that information the percentage of drug substances per SlF weight would be fixed, reducing the number of factors to be included in the DoE study. Additionally, the investigation and development of these novel technologies would greatly benefit with the use of advanced characterization techniques, such as polarized light microscopy, scanning electron microscopy, X-ray powder diffraction and differential scanning calorimetry. This represents an opportunity to strengthen partnerships aiming to contribute with sound and science based knowledge about oral films.

As mentioned, oral films represent an attractive option for life cycle management, which is why the majority of approvals of oral films were realized by bioequivalence studies. Despite the need of performing specific clinical trials, it would be valuable to develop oral films with improved bioavailability in the near future. This could improve the safety and compliance of the patients with

the treatment. The growing number of products under development using oral films technology, together with their advantages over conventional dosage forms, are evidence that this technology is here to stay.

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